19.001 Anti-tuberculosis evaluation, triterpenoidal and fatty acid constituents of Ximenia Americana (Olacaceae) stem bark methanol extract

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Purpose: The prevalence of multi-drug resistant tuberculosis infections is an increasing health challenge globally. Attention is now being shifted to the use of the rich forest biodiversity as applicable in ethno-medicine to combat this disease. Ximenia americana is used in ethno-medicine for the treatment of skin infections, poison, post-partum hemorrhage, anaemia, dysentery, and bacterial infections. This study investigated the triterpenoidal and fatty acid composition, and in vitro anti-tuberculosis activity of the stem bark of Ximenia americana.

Methods & Materials: The macerated methanol extract (XAME) of the stem bark was evaluated for antituberculosis activity by the Lowensten Jensen method against de-contaminated clinical strains of Mycobacterium tuberculosis. The XAME was fractionated by open column chromatography on a normal phase silica gel column with a 25 % stepwise gradient of chloroform-methanol as mobile phase. The constituents of the non-polar column fractions eluted with 100% chloroform were characterization using Gas Chromatography-Mass spectroscopic (GC-MS) techniques after comparison with reference NIST library compound.

Results: The XAME (5 mg/mL) showed bacteriostatic activity against the Mycobacterium tuberculosis. GC-MS analysis of the non-polar column fractions afforded two lupane-type triterpenoids: Lup-20-(29)-en-3-one and lupeol, two phytosteroids: campesterol, stigmasterol and β-sitosterol, one fridelane-type triterpenoid: Friedelan-3-one and the fatty acids: palmitic acid methyl ester, palmitic acid, 13-octadecenoic acid, heptadecanoic acid, oleic acid methyl ester, eicosanoic acid methyl ester, 10.12-octadecadiynoic acid, docosanoic acid, tetracosanoic acid methyl ester, and hexacosanoic acid methyl ester.

Conclusion: The presence of these bioactive triterpenoids and fatty acids could offer an explanation for the ethno-medicinal uses of this plant. Further work is on-going to isolate in pure form, and characterized the bioactive constituents in the XAME with the view of discovering lead compounds for the treatment of tuberculosis and associated opportunistic bacterial infections.


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Purpose: Streptococcus pneumoniae is one of the leading causes of death which disseminates through colonizers to cause serious infections. In Jordan, children are majority where colonizers with this bacteria can disseminate and cause infections. Determine pneumococcal carriage rate, resistance, serotype distribution and coverage of pneumococcal Conjugate Vaccines (PCVs) from children attending daily care centers in Jordan.

Methods & Materials: Nasopharyngeal swabs were taken from 279 healthy Jordanian children from the capital Amman (n=114) and from Madaba (n=165) with age from 2 to 144 months. Swabs were cultivated and the suspected alpha hemolytic isolates were tested for Optochin sensitivity and bile solubility for identification. Isolates were analyzed for antimicrobial susceptibility, serotyping using the Neufeld Quellung method.

Results: Total pneumococcal carriage in Amman and Madaba was 14.0% and 41.2%, respectively. Coverage of PCV7, PCV10 and PCV13 in Amman was 6.3%, 6.3% and 12.5%; whereas in Madaba was 48.5%, 48.5% and 58.8%, respectively. 55 (48.2%) cases taken from Amman were vaccinated between 1-3 PCV injections. Coverage of Multiresistant
isolates by PCV7 and PCV13 in Amman and Madaba was 14.3%, 28.6% and 59.5%, 78.4% for Madaba. Rate of carriage for cases below 4 months of age was 60% and can all be covered by the PCV. Carriage in Amman and Madaba was highest in age group 2-6M (40%) in Amman and 25-36M (57.1%) in Madaba. Resistance rates in Amman and Madaba was: Penicillin (87.5%; 98.5%), clarithromycin (62.5%; 58.7%), clindamycin (25%; 35.3%), trimethoprim-sulfamethoxazole (32.2%; 52.9%), tetracycline (44%; 55.9%). Multiresistance in Amman and Madaba was 43.8% and 54.4%. 52 isolates were macrolide resistant in both cities, where 63.6% and 36.4% were M-phenotype and cMLSB for Amman and 41.5% and 58.5% for Madaba. Predominant serotypes were 11A (12.5%) and 9N (12.5%) in Amman and for Madaba 23F (16.2%), 19F (11.8%) and 6B (11.8%).

**Conclusion:** There was a significant differences in carriage, but the resistance is high. Localizing specific serotypes is recommended for be better control with the available PCVs.

19.003 Appraisal of antimicrobial medicinal plants with the potential of improving health condition in developing tropical countries

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**Purpose:** To evaluate the efforts made so far in validating the use of common tropical climate herbs in providing solutions to illnesses prevailing in the tropics.

**Methods & Materials:** Main stream search using popular search engines such of google, Pubmed and some African journal websites including those specialized in publishing herbal alternatives in providing solutions to common illnesses. There is no end to claims by various societies on usefulness of all sorts of plants but those herbs listed below are among the Selected medicinal plants included: Allium cepa, Allium sativum, Aloe vera, Carica papaya, Colocasia esculenta, Garcinia kola, Gossypium hirsutum, Ipomoea batatas, Jatropha curcas, Jatropha tanjorensis, Mangifera indica, Mucuna pruriens, Musa paradisiaca, Ocimum gratissimum, Pismum guajava, Telfairia occidentalis, Spermocoe ocyoides, Vernonia amygdalina, Alchornea cordifolia, Afrononum albo violaceum, Anonna senegalensis, Cymbopogon densiflorus, Bridelia ferruginea, Ceiba pentandra, Morinda lucida, Hymenocardia acida, Coleus kilmamandcharis, Dacryodes edulis, and Vigna unguiculata. The research done so far used either leaf, stem, branch, root or back and maximum asepsis observed.

**Results:** From the result recorded so far so far, it appears majority of the researches published focused on bacterial and fungal infections with little attention paid to viral or parasitic infections. Again, gastro intestinal, soft tissue, blood and lymphatic infections received most of the attentions contrary to expected respiratory and genitourinary tract infections also very common in the tropics. Multidrug resistance and therapy with food supplementations using antioxidants were also sparingly addressed with non showing lethal effect on re-infecting organisms. Much room is still left for their use during outbreaks as confidence is still placed on imported drugs to save lives despite what we know about local herbs. It appears that research which tends towards bacteria and fungal and not parasites and viral solutions reflect lack of facilities including modern equipments and human resources for health for validation of claims. Stringent regulation in drug trial and development by various authorities may also have impacted on the advances noted.

**Conclusion:** Local Medicinal herbs can act as broad spectrum agents serving as antimicrobial, alternative if given multidisciplinary attention and funding.

19.004 Plasmid-mediated colistin-resistance in Escherichia coli isolated in poultry and broiler meat in Austria in 2016

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**Purpose:** Colistin is regarded as a last line defence against infections caused by multidrug-resistant Gram-negative bacteria. Plasmid-encoded colistin resistance mediated by the mobile colistin resistance-1 (mcr-1) gene has been identified in food animal, human, food, and environmental isolates on every continent. We report on results of a screening survey on colistin-resistant E. coli in retail broiler meat and poultry caecal samples collected in Austria in 2016.

**Methods & Materials:** A total of 164 caecal samples from poultry (110 broilers, 54 turkeys) and 115 meat samples from broilers were collected at slaughterhouses and at retail outlets in the framework of an EU surveillance programme on antimicrobial resistance between
February and June 2016 in Austria. Samples were screened for the presence of colistin-resistant *E. coli* using non-selective enrichment broth and MacConkey agar supplemented with 0.2 to 0.6 mg/L colistin. Antimicrobial susceptibility was determined by Sensititre microbroth dilution using EUVSEC and EUVSEC2 plates. Colistin-resistant isolates were sequenced using the Illumina MiSeq platform. The assembled genomes were analyzed with pipelines MLST, PlasmidFinder, and ResFinder available from the Center for Genomic Epidemiology. PCR amplification was performed to identify plasmid-encoded AmpC genes.

**Results:** Two colistin-resistant *E. coli* strains were isolated from broiler meat, one of domestic and one of Italian origin. Two additional isolates were found in caecal samples from broilers and turkeys, respectively. All isolates except the one from turkey carried the *mcr-1* gene. The *mcr-1*-positive isolates belonged to different MLST sequence types (ST-10, ST-616, ST-43) and showed a MIC value of 4 to 8 mg/L for colistin. The isolates harboured multiple plasmid replicons with IncFIC(FLII), IncFIB(AP001918) and IncX4 being present in all of them. Besides *mcr-1*, the isolates contained up to seven additional resistance genes including *blaTEM-1B* which was detected in all strains. The isolates were found to be negative for ESBLs, plasmid-mediated AmpC, and carbapenemases.

**Conclusion:** Using selective screening for colistin-resistant bacteria, 0.9% of caecal samples and 1.7% of meat samples from broilers were shown to be positive for *E. coli* carrying *mcr-1*. The study is the first documentation of plasmid-mediated colistin resistance in *E. coli* isolated from poultry and poultry meat in Austria.

![Image](image_url)

**Virulence factor profiles and genetic background of quinolone-resistant *Escherichia coli* isolated from hospital effluent**

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**Purpose:** The widespread species *Escherichia coli* includes a broad variety of different types, ranging from highly pathogenic strains causing worldwide outbreaks of severe disease to avirulent isolates which are part of the normal intestinal flora. Pathogenicity is mainly determined by specific virulence factors which include adhesins, invasins, toxins and capsule. Several studies on clinical *E. coli* have demonstrated relationship between antibiotic resistance and low prevalence of virulence factors and suggested that quinolone resistance may be directly associated with the loss of virulence. The objective of this study was to evaluate the potential virulence of quinolone-resistant *E. coli* strains recovered from wastewater hospital.

**Methods & Materials:** 24 isolates of *E. coli* were selected on Tergitol-7 agar supplemented with ciprofloxacin (2 mg/L) and identified by API 20E system. These strains were screened by PCR for 20 virulence genes: *papA*, *sfa/foc*, *afa/dra*, *fimH*, *kpsMII*, *entB*, *irp2*, *iutA*, *iroN*, *hlyF*, *iss*, *ompT*, *traT*, *eae*, *bfp*, *aggR*, *LT*, *ST*, *stx*, and *ibeA*. Phylogenetics groups and sequence-types were determined by PCR and sequencing. The clonal relationship between isolates was investigated by ERIC-PCR.

**Results:** Virulence genes detected were: *fimH* (n= 24, 100%), *entB* (n= 24, 100%), *traT* (n= 20, 83.3%), *hlyF* (n= 17, 70.8%), *ompT* (n= 16, 66.6%), *iss* (n= 10, 41.6%), *iroN* (n= 10, 41.6%), *irp2* (n= 7, 29.1%), and *iutA* (n= 6, 25%). The combination of virulence factors allowed to distinguish eight virulence profiles, of which five were predictive of APEC pathotype, they include *iss*, *ompT*, *iroN*, *iutA* and *hlyF* genes. Phylogenetic groups A, B1 and D and sequence types ST405, ST443, ST101, ST10 and ST347 were identified in these isolates. ERIC-PCR molecular typing of isolates showed 12 different genetic profiles.

**Conclusion:** This study highlighted the possible association between quinolone resistance and certain virulence factors and the potential role of hospital effluents in the dissemination of virulence genes in natural environments.

![Image](image_url)

**Extracellular biosynthesis of silver nanoparticles from *Bacillus brevis* (NCIM 2533) and their antibacterial activity against multi-drug resistance clinical isolates**

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**Purpose:** Extracellular synthesis of silver bionanoparticles (Ag-BNPs) from the bacterium *Bacillus brevis* (NCIM 2533) were done. These were characterized and confirmed to see the antimicrobial activity against multi-drug resistant clinical isolates, *Salmonella typhi* and *Staphylococcus aureus*.

**Methods & Materials:** Materials:

*Bacillus brevis* (NCIM 2533), Muller Hinton Broth, Silver nitrate, Silica gel. *Staphylococcus aureus* and *Salmonella typhi*.
Methods:
Biosynthesis of AgNPs:
The absolute concentration of 1 mM AgNO3 (0.017 g AgNO3/100 ml) was added in to 100 ml of cell filtrate and incubated for overnight in dark condition at 37°C. The synthesized Ag-BNPs was stored under ambient condition for further characterization and biomedical applications.

Characterization: UV-Visible spectroscopy, TLC, FTIR, AFM and SEM. Size and morphology of Ag-BNPs were determined with line profile using WSxM software, Nanotech Electronica. Determination of antimicrobial efficacy by Well diffusion & Disc diffusion method.

Statistical analysis were done in triplicate and then values were expressed as mean ± standard deviation using SPSS 11 version.

Results: The synthesised Ag-BNPs have the surface Plasmon resonance peak at 420 nm and in the size range of 41-68 nm with spherical shape. To confirm the presence of bioactive compounds, TLC and FTIR was carried out which determined the compounds responsible for bioreduction with B. brevis (NCIM 2533). SEM analysis of lyophilized Ag-BNPs are spherical in shape with an average size of 41 nm.

The antimicrobial activity is found to be maximum for Staphylococcus aureus and moderate for Salmonella typhi using disc and well diffusion methods. Our study corroborated with others that Ag-BNPs penetrate through bacterial cell membrane and disturb its functions.

Conclusion: AgNPs were synthesized by greener approach due to ecofriendly, cost effective and efficient alternative routes to conventional chemical and physical methods. Synthesis of Ag-BNPs and the extracellular proteins of bacterial extract are responsible for reduction of Ag+ ions. The particle size of the biosynthesized Ag-BNPs were 22-60 nm with spherical shape. The synthesized Ag-BNPs have higher antibacterial efficacy against multi drug resistant clinical isolates of Staphylococcus aureus and moderate efficacy towards Salmonella typhi. Therefore, it is suggested that Ag-BNPs from Bacillus brevis (NCIM 2533) show ideal antimicrobial agent against human pathogenic MSSA and MRSA clinical isolates.

19.007 Antibiotic susceptibility and β-lactamase production of Gram-negative bacteria from swimming pools in Slovenia

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Purpose: β-lactamases are enzymes which hydrolyze β-lactam antibiotics. The first β-lactamases were reported before the β-lactams were introduced into the clinical practice, mostly from environmental bacteria. Therefore, it is likely to expect that there is a pool of β-lactamase encoding genes in the environment. Gram-negative bacteria are ubiquitous in nature and are widely distributed in soil and water. Extended-spectrum β-lactamases (ESBLs) hydrolyze penicillins, cephalosporins of the 1st, 2nd, 3rd and 4th generation and monobactams. Carbapenemases hydrolyze carbapenems and belong to class A, B (metallo-β-lactamases or MBLs) and D (carbapenem-hydrolyzing oxacillinases). The aim of the study was to analyze antibiotic susceptibility and β-lactamase production of Gram-negative isolates collected from the swimming pools in Slovenia.

Methods & Materials: The collection included 10 Pseudomonas aeruginosa strains, 1 Pseudomonas putida, one Serratia marcescens, 1 Citrobacter farmeri, one Citrobacter brackii, one Citrobacter koseri, one Klebsiella oxytoca, one Escherichia coli, three Acinetobacter baumannii and one Cryseobacterium spp.

In total 21 Gram-negative strains were collected from various swimming pools in Slovenia. The antimicrobial susceptibility was determined by disk-diffusion and broth microdilution method according to CLSI. Production of ESBLs was detected by double-disk synergy test (DDST) and combined disk test with clavulanic acid. MBLs were detected by combined disk test with EDTA. Resistance genes including those encoding ESBLs (blaTEM, blaSHV, blaCTX-M), carbapenemases (blaKPC, blaIMP, blalux, blambl) and fluoroquinolone resistance (qnr A, B, and S) were determined by PCR.

Results: A. baumannii and P. aeruginosa strains showed resistance to amoxycillin alone and combined with clavulanate, and first and second generation of cephalosporins. DDST was positive in eight strains. The Enterobactericeae were resistant only to ampicillin and two to cefuroxime as well. CTX-M β-lactamases belonging to 8 and 25 group were identified in five Citrobacter spp, E. coli and S. marcescens strains. VIM-1 β-lactamase was identified in one Citrobacter brackii strain.

Conclusion: Unexpectedly high number of ESBL positive enterobacteriaceae was found in spite of the evident susceptibility to expanded-spectrum cephalosporins. We can assume that the expression of blaESBL genes anb blaMBL is weak in environmental bacteria due to the lack
of selection pressure. The study demonstrated spread of Gram-negative isolates carrying resistance genes into the environment.

19.008  What's happening in invasive pneumococcal resistance, a single center experience

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**Purpose:** *Streptococcus pneumoniae* is a major cause of invasive infections such as acute bacterial meningitis, pneumonia and sepsis. *S. pneumoniae* has high morbidity and mortality. An increased trend in antimicrobial resistance in invasive streptococcal infections is observed in Europe. The aim of this study is to evaluate the antimicrobial susceptibility of invasive pneumococci isolated at a 650-bed university hospital between January 2011 and March 2016.

**Methods & Materials:** 45 clinical isolates of *S. pneumoniae* from blood and cerebrospinal fluid were analyzed. Penicillin and ceftriaxone minimum inhibitory concentrations (MIC) were determined using standard E-test method using EUCAST breakpoints. Penicillin and ceftriaxone resistance for CSF isolates were defined as MIC >0.06 ug/mL and MIC >2 ug/mL respectively. For blood isolates penicillin and ceftriaxone resistance were defined as MIC >2 ug/mL (Table 1). Macrolide susceptibility was defined using disk diffusion method, zone diameter <19 mm were considered as resistant. *S. pneumoniae* American Type Culture Collection 49619 was used as weekly panel quality-control strains.

**Results:** Out of 45, 14 were CSF and 31 were blood isolates. Penicillin resistance was present in 57% of meningitis and 4% of blood isolates. No resistance was detected for ceftriaxone in both groups. Macrolide resistance was present in 50% of meningitis isolates. Penicillin resistance increased from 53.8% to 71% between 2011-2013 and 2014-2016 time periods.

<table>
<thead>
<tr>
<th></th>
<th>Susceptible</th>
<th>Resistant</th>
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</thead>
<tbody>
<tr>
<td>Penicillin G breakpoint</td>
<td>0.06</td>
<td>0.06 (2 for non-meningitis)</td>
</tr>
<tr>
<td>Meningitis isolates (n=14), n (%)</td>
<td>6 (43%)</td>
<td>8 (57%)</td>
</tr>
<tr>
<td>Non-meningitis isolates (n=31), n (%)</td>
<td>25 (80%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Ceftriaxone breakpoint</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Meningitis isolates, (n=14), n (%)</td>
<td>8 (57%)</td>
<td>0</td>
</tr>
<tr>
<td>Non-meningitis isolates (n=31), n (%)</td>
<td>26 (89%)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Conclusion:** Increasing penicillin and cephalosporin resistance in *S. pneumoniae* is a universal threat. Growing ceftriaxone/cefotaxime resistance has lead to Vancomycin recommendation in meningitis treatment. Penicillin resistance is 0-25% in Europe according to ECDC 2014 report. Several studies showed penicillin resistance is 20-40% in meningitis isolates in Turkey. Cephalosporin resistance was 1% of penicillin resistant isolates. An increasing trend in penicillin resistance is observed in current study. Emergence of resistance in this life threatening infection limits the therapeutic options for physicians.

19.009  Genomic characterization of Neisseria gonorrhoeae isolates with reduced susceptibility to cephalosporins in Guangdong, China

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**Purpose:** The emergence of antimicrobial resistance in *Neisseria gonorrhoeae* raises concerns over future treatment failures of gonorrhoea in China. Since 2011, an increase in the prevalence of *N. gonorrhoeae* with reduced susceptibility to cephalosporins was observed in Guangdong. This study was conducted to examine the resistance determinants for cephalosporins using next-generation sequencing.

**Methods & Materials:** Six *N. gonorrhoeae* with reduced susceptibility to cephalosporins (defined as MIC of ≥0.25 µg/ml for cefixime or MIC of ≥0.125 µg/ml for ceftriaxone) were included and next-generation sequencing was performed using Illumina MiSeq platform.
Purpose: Antibiotic use in the veterinary sector has been identified as a contributory factor in the global antimicrobial resistance crisis. In India, periurban ecosystems have emerged secondary to rapid urbanization. Owing to poor regulatory and quality support infrastructure, farmers in these areas practice industry style livestock rearing, indulging in harmful practices to boost productivity. A scoping review was undertaken to identify the market and policy drivers of antibiotic use in smallholder dairy farms of peri-urban India.

Methods & Materials: The Arksey and O'Malley framework was adopted for this review. This six-step process included: identifying the research question (market and policy drivers affecting antibiotic use in smallholder periurban dairy farms); identifying relevant studies (published and grey literature, using systematic search strategies); relevance screening; data charting; collating, summarizing and reporting the data; and expert consultation. Following expert consultation the data was iteratively interpreted to develop a conceptual framework.

Results: Data was extracted based on reported variables. These variables were then used to identify themes, which helped identify factors at three levels – market, farm, and systems – at which various factors operated to drive up antibiotic usage. These were further grouped into proximal and distal drivers based on inputs from the expert consultation. Proximal drivers included adoption of intensive production norms, a preference for rearing high-yield exotic breeds in an environment with poor hygiene and sanitation, with minimal infection control and biosecurity measures leading to high morbidity levels that necessitate higher antibiotic use in farms. Distal drivers included market-level factors like urbanization, population growth, globalized markets and changing food preferences. Systemic weaknesses, like the absence of antibiotic stewardship, monitoring frameworks, and surveillance and laboratory networks also drove higher antibiotic use. Lack of development of new drugs and incentives to reduce antibiotic use further contribute to the problem.

Conclusion: Farm level antibiotic use is driven by a complex interplay of market, farm and systems-level factors. These factors, either directly (proximal drivers) or indirectly (distal factors), increase antibiotic consumption, worsening risks of developing resistance in the community. A holistic approach needs to be adopted to combat overuse in order to effectively contain emergence of antibiotic resistance.

Veterinary antibiotic use in smallholder periurban dairy farms of India: A qualitative study

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Purpose: Although veterinary use of antibiotics has been identified as a major contributor to antibiotic resistance in community and hospital acquired infectious agents, there is limited evidence on identifying the drivers of antibiotic use at a local level. In this study, we endeavored to look at knowledge-attitude-practices, influences, and drivers in the key actors in the dairy farming enterprise in peri urban India.

Methods & Materials: The study was conducted in the periurban belts of Ludhiana, Guwahati and Bangalore. A stakeholder mapping was done to identify the key actors in the dairy farming enterprise. In-depth interviews were conducted based on thematic guides
developed through an intense review of literature and improved through pretesting. Data collection was continued till the attainment of saturation across key themes. Open coding of responses was done, followed by axial coding to establish relationships between categories. Selective coding to identify core themes was done, followed by etiological enquiry and generation of a conceptual model.

**Results:** The stakeholders identified were: dairy farmers, veterinarians, veterinary field assistant, traders, pharmacists, and civic officials or union representatives. Cost was a deterrent in seeking veterinarian care for sick animals and quacks were the major service providers at the farm level. Veterinarians were approached as a last ditch effort. Although civic officials emphasized the availability of laboratory services, farmers denied having ready access. Screening of animals was universally absent, and routine surveillance was also very weak. The risk of losing the razor thin margins to sick animals fueled antibiotic overuse. Direct to farmer marketing of drugs, easy availability of antibiotics over the counter (either without prescription or by reusing old prescriptions), and a plethora of informal prescribers providing antibiotics for animal health issues further drove antibiotic consumption in the farms.

**Conclusion:** The study revealed several important drivers that operate locally in the perirurban milieu in India, causing antibiotic overuse in farm animals. It is important to understand such local dynamics in order to develop sustainable policies to address the issue of irrational antibiotic consumption and overuse, precipitating antibiotic resistance.

19.012  **Pseudomonas aeruginosa** profile of resistance to quinolones and carbapenem parallelly to usage of quinolones and carbapenem

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**Purpose:** *Pseudomonas aeruginosa* (*P. aeruginosa*) present increasing high rate of resistance to carbapenem and fluoroquinolones (FQs) over years. This leads to a raise in the burden of its infection, the length of hospital-stay and the mortality. Our study aims specifically to show if a prior utilization of carbapenem or FQs influence the resistance of *P. aeruginosa* to imipenem (IMP) and to ciprofloxacin (CIP).

**Methods & Materials:** The study was conducted in Hotel-Dieu de France Hospital (HDF – Beirut). It’s an observational retrospective case-control study concerning all the patients hospitalized to our center between the date of January 24th and June 31st 2013 and having during their stay a positive culture for *P. aeruginosa*. They were classified into three groups on the following basis defining the cases: patients infected with *P. aeruginosa* resistant only to CIP, patients infected with *P. aeruginosa* resistant only to IMP, patients infected with *P. aeruginosa* resistant to both CIP and IMP. The controls are all patients in this study not belonging to the group in question. A univariate analysis and a multivariate analysis were done. The p-value adopted is 0.05.

**Results:** 135 patients were enrolled in the study with a mean age of 60.6 +/- 21.3 years.
18.5% of patients are infected with *P. aeruginosa* resistant only to IMP, 8.9% of patients are infected with *P. aeruginosa* resistant only to CIP, 28.9% of patients are infected with *P. aeruginosa* resistant to both CIP and IMP. 43.7% of patients are infected with *P. aeruginosa* susceptible to these antibiotics. Utilization of carbapenem in the 3 months prior to the positive culture is a risk factor for developing resistance to IMP *(p=0.006, OR=4.5, CI [1.5 – 13.1]) only and to both IMP and CIP *(p=0.001, OR=5.3, CI [2.0 – 13.9]).

Immunocompromised patients are more common to have germs resistant to both IMP and CIP *(p=0.013, OR=3.3, CI [1.2 – 8.5]).

**Conclusion:** A well guided antibiotherapy allows in some extent reducing the rate of morbimortalities and attenuating the burden of infections.

19.013  Risk factors for the acquisition of colistin and carbapenem-resistant Klebsiella pneumoniae in Medellin-Colombia, an endemic region for carbapenem resistance

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**Purpose:** Colistin is considered the last resort antibiotic for treating infections caused by carbapenem-resistant bacteria. However, the emergence of colistin resistance emphasizes the urgent need to understand its associated factors. The aim of this study is to identify risk factors for the acquisition of colistin and carbapenem-resistant *Klebsiella pneumoniae* (CRK) in an endemic region for carbapenem resistance.

**Methods & Materials:** A case-control study enrolling all inpatients infected by CRK between June-2012 and June-2014 in four tertiary-care centers in Medellin-Colombia was conducted. Cases were defined as patients infected by colistin-resistant CRK and controls as patients infected by colistin-susceptible CRK. Sociodemographics and medical history in the past year
from the beginning of infection were retrieved from clinical charts. Antibiotic susceptibility testing, multilocus-sequence-typing (MLST) and detection of mcr-1 a plasmid-mediated colistin resistance gene were performed. A bivariate and multivariate analysis using generalized estimating equations were implemented to account for the intra-class correlation within hospitals.

**Results:** A total 32 cases and 129 controls were enrolled. Most patients were males (63.4%) and all were adults (median=54 years, IQR 35–70). Frequent infections were urinary tract (28.7%), bloodstream (23.1%) and intra-abdominal infections (19.4%). MLST showed high heterogeneity among colistin-susceptible CRK, while colistin-resistant CRK belonged mostly to CG258 (84%). Detection of mcr-1 was negative in all resistant strains. Bivariate analysis showed patients with longer hospitalization were more likely to be infected by colistin-resistant CRK (23% risk increase by every 10 days). The strongest risk factors were infection by CG258 (OR=12.01, 95%CI 4.31-33.47) and previous colistin use in the past six months (OR=6.72, 95%CI 1.68–26.75). Factors associated with colistin-resistant CRK in the multivariate analysis were infection by CG258 (OR=17.57, 95%CI 4.93–62.62), previous use of colistin (OR=7.21, 95%CI 1.10–47.01), longer time at risk (coefficient 0.025, 95%CI 0.006–0.044) and mechanical ventilation (OR=3.43, 95%CI 1.11–10.66).

**Conclusion:** Infection by CG258 and previous use of colistin play an important role in colistin-resistant CRK acquisition. The emergence of resistance in this clone is worrisome due to its international spread and the paucity of therapeutics options for its treatment. Our findings suggest both infection-control practices and stewardship programs are essential to control infections by colistin-resistant CRK.

19.014 Antibiotic resistance in the Western Pacific Region: a systematic review

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**Purpose:** There have been increasing reports of bacteria carrying antibiotic-resistant genes discovered in the Western Pacific Region (WPR) in recent years. However, the impact on human health of these resistant pathogens has not been well documented due to the lack of region-wide and national surveillance programs on antimicrobial resistance (AMR) in the region. The objective of this review is to systematically review published evidence on the prevalence of AMR in selected bacterial pathogens in the region over the past 15 years.

**Methods & Materials:** A literature search for published data on AMR in eight selected pathogens (methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli, Enterococcus faecalis, Haemophilus influenzae, Klebsiella pneumoniae, Neisseria gonorrhoeae, Pseudomonas aeruginosa, and Streptococcus pneumoniae) was conducted on bibliographic database PubMed for five pre-selected locations in the WPR, namely mainland China, Japan, Australia, the Republic of Korea and Hong Kong.

**Results:** Our search identified 4,674 articles, 207 of which reported the prevalence of the eight selected pathogens in the five pre-selected locations. There were 43 studies on E. coli, 41 on MRSA, 25 on K. pneumoniae, 25 on N. gonorrhoeae, 24 on P. aeruginosa, 24 on S. pneumoniae, 15 on H. influenza, and 10 on E. faecalis. In the five selected locations, greatest levels of resistance were reported for E. coli (33.7% - 93.3% against ampicillin) and S. pneumoniae (4.1% - 97% against erythromycin). High prevalence of MRSA was recorded in the region over the past 15 years (up to 73% in Korea, 69% in Hong Kong, 64.8% in China, and 63.4% in Japan), with the exception of Australia (16% - 30.3%). Moderate to high levels of resistance against third-generation cephalosporins were reported in P. aeruginosa, and high levels of resistance against penicillin and fluoroquinolones were also reported in N. gonorrhoeae.

**Conclusion:** Generally, high prevalences of antibiotic resistance were identified in the 5 selected locations in the WPR, particularly in E. coli, N. gonorrhoeae, P. aeruginosa, and S. pneumoniae. However, longitudinal trends of antibiotic resistance in any of the selected pathogens were difficult to determine as most published data were generated from cross-sectional studies and few were from systematic surveillance through national or region-wide networks.

19.015 Survey on antimicrobial resistance of Helicobacter pylori to tetracycline and metronidazole by Etest and Real Time-PCR methods

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**Purpose:** *Helicobacter pylori* antibiotic resistance is the main factor affecting on current therapeutic regimens. Prevalence of bacterial resistance varies in different geographic areas,
Persistence of bacteria in sanitized hands and prevalent antibiotic resistotypes

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Purpose: The hand is an important vehicle in disease transmission both in the healthcare setting and in communities. Though guidelines exist for use in hospitals to reduce spread of communicable diseases, none has been formulated for use in public places. This study identified factors responsible for the persistence of microorganisms in the hand even after hand hygiene and determined whether the use of sanitizers will predispose to antibiotic resistance.

Persistence of bacteria in sanitized hands and prevalent antibiotic resistotypes

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Purpose: The hand is an important vehicle in disease transmission both in the healthcare setting and in communities. Though guidelines exist for use in hospitals to reduce spread of communicable diseases, none has been formulated for use in public places. This study identified factors responsible for the persistence of microorganisms in the hand even after hand hygiene and determined whether the use of sanitizers will predispose to antibiotic resistance.

and it has been correlated with the consumption of antibiotics in the general population. The aim of the present study was to investigate the prevalence of resistance rate in H. pylori against commonly used antibiotics in Iranian patients and the occurrence of 16S rRNA mutations associated with resistance or reduced susceptibility to tetracycline.

Methods & Materials: H. pylori isolates were obtained from gastric biopsies from patients in Tabriz, Iran. All positive cultures were tested for antimicrobial susceptibility by used of disk agar diffusion and Etest. A LightCycler assay developed to detect these mutations was applied to DNA extracted from culture. The 16S rRNA of these isolates were sequenced and resistance-associated mutations were identified.

Results: 104 H. pylori isolates were obtained from 322 patients. The prevalence’s of resistance of H. pylori isolates were; Tetracycline (10.6%), Metronidazole (77.9%), Clarithromycin (15.4%), Amoxicillin (22.1%), Ciprofloxacin (39.4%), Nitrofurantoin (4.8%), Erythromycin (24%), Rifampin (26.9%). 11 isolates showed reduced susceptibility to Tetracycline. Mutations in 16S rDNA were detected in 7 of the reduced susceptibility isolates, five single base mutation of A926G and two A928C. Also was found a novel mutation in 2 strains and exhibition of an A939C mutation.

Conclusion: The prevalence of H. pylori antibiotic resistance to metronidazole was high, while resistance to tetracycline was low in this area. There was an increasing trend in resistance rate of Clarithromycin, Metronidazole and ciprofloxacin. Therefore, surveillance of antibiotic susceptibility test for H. pylori is essential.

19.016 Patient level risk factors for Carbapenem-Resistant Enterobacteriaceae at a low prevalence tertiary care center in the United States

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Purpose: To characterize the clinical epidemiology of Carbapenem-Resistant Enterobacteriaceae (CRE) at Virginia Commonwealth University Health System (VCUHS).

Methods & Materials: A manual chart review was performed for all CRE-positive cultures identified at VCUHS between January 2014 and May 2016. The chart review assessed patient risk factors for CRE infection such as individual carbapenem utilization, recent hospital admission, and residence in a long term care facility. Subsequently, a point prevalence survey was performed on 2 high risk units in June 2016. As part of an ongoing quality improvement initiative, patients voluntarily agreed to a peri-rectal swab that was inoculated onto HardyChromCRE agar for incubation in ambient air, 35 °C, for 24 hrs.

Results: From January 2014 through May 2016, 39 out of 12,947 Enterobacteriaceae clinical cultures were positive with CRE (0.3%). Of these, 25 of 33, had a positive Modified Hodge Test (76%). Most specimens were from urine (N=23, 59%), followed by blood (N=4, 10%). Klebsiella spp. comprised 49% of organisms isolated; E. coli comprised 23%, Enterobacter spp. 24%, Citrobacter freundii complex and Serratia marcescens were both isolated from single specimens (3%). Patient level risk factors included recent admission(s) in 15/39 (38%) and skilled nursing or long term care residency in 13/39 (33%). Most frequent associated medical conditions were neurologic (26%), surgical complications (10%), transplantation (13%) and malignancy (15%). Of the inpatients (32/39), between 1 and 5 specimens were isolated from each of 18 different units without a pattern suggesting clustering. Eighteen of 33 patients with available pharmacy data were exposed to a carbapenem in the 30 days preceding their positive culture (55%) with an average duration of 8 days. The point prevalence survey tested 19 of 23 surgical ICU patients and 14 of 22 medical ICU patients. All of the surveillance swabs were negative for CRE.

Conclusion: These data suggest that the CRE at VCUHS are imported and/or individually developed at the patient level rather than transmitted within the hospital.

19.017 Persistence of bacteria in sanitized hands and prevalent antibiotic resistotypes

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Purpose: The hand is an important vehicle in disease transmission both in the healthcare setting and in communities. Though guidelines exist for use in hospitals to reduce spread of communicable diseases, none has been formulated for use in public places. This study identified factors responsible for the persistence of microorganisms in the hand even after hand hygiene and determined whether the use of sanitizers will predispose to antibiotic resistance.
Methods & Materials: Swab samples of washed and sanitized hands of 500 undergraduate students were collected and cultured following stipulated hand washing/sanitization guidelines. The roles of keeping long nails and toilet hygiene after defecation on the microbial load and types were determined. Bacteria isolated were tested by agar diffusion method against tigecycline, linezolid, trimethoprim-sulphamethoxazole, teicoplanin, tetracycline, vancomycin, fosfomycin, oxacillin, chloramphenicol, gentamicin, mupirocin and levofloxacin as applicable.

Results: Improper application of hand washing guidelines, keeping of long nails, and poor toilet hygiene were contributory to the persistence of microorganisms in hands. Forty bacterial strains were isolated from washed and sanitized hands (Staphylococcus aureus, coagulase negative staphylococci (CoNS), Enterococcus faecalis, Streptococcus pneumoniae, Bacillus spp, Escherichia coli, Klebsiella oxytoca, and Pseudomonas aeruginosa). Levofloxacin was the single most active inhibiting all the bacteria including P. aeruginosa. Multidrug resistance was observed against the other antibiotics and with only 2 of the 40 strains sharing the same resistotypes. Isolates from sanitized hands did not exhibit appreciable difference in resistance to those from washed hands. E. faecalis recorded 100% resistance to teicoplanin, tetracycline, fosfomycin and gentamicin.

Conclusion: The multiple factors associated with the persistence of microorganisms in the hand together with the diversity of antibiotic resistotypes amongst the isolates underscore the importance of the hand in the transmission of infectious diseases.

19.018 Bloodstream infections and antibiotic susceptibility of anaerobes isolated from orthopedic patients

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Purpose: Blood cultures for anaerobes are not routine practice in many hospitals in Nigeria, resulting in underreporting of the incidence of anaerobic septicemia in bloodstream infections. This also means absence of targeted treatment against anaerobes in septicemia requiring antibiotic intervention.

Methods & Materials: Forty five patients with orthopedic (hip joints, prosthetic joints and fractured jaw) wound sepsis that developed pyrexia was investigated for anaerobic bacterial etiology in two specialist hospitals in Lagos, Nigeria over a period of 6 months. Three of the patients with septic hip joint wounds were diabetes and infections in two of the fractured jaw patients have persisted for 4 months. Both needle aspirates from infection sites and venous blood samples were processed by culture for anaerobic bacteria. The anaerobes were tested against penicillin, amoxicillin, amoxicillin-clavulanic acid, cefoxitin, moxifloxacin, metronidazole and tigecycline.

Results: Anaerobes were isolated from twelve of the patients including the diabetic and those with chronic infections. Finegoldia magna was isolated from two patients with prosthetic joint infections (PJI) and a patient with hip joint infection. Bacteroides fragilis was isolated from two patients, one with PJI and the other hip joint infection. Prevotella intermedia and P. melaninogenica were isolated from two and one patient with fractured jaw sepsis respectively. Blood cultures were positive only for B. fragilis and P. intermedia. Finegoldia magna wound and blood isolates were sensitive to all the antibiotics. Prevotella species both wound and blood isolates were sensitive to the antibiotics except penicillin, and amoxicillin. One of the two B. fragilis from blood was resistant to the b-lactam antibiotics, and tigecycline. The other B. fragilis isolate from blood was sensitive to tigecycline in addition to metronidazole and moxifloxacin. All B. fragilis wound isolates were resistant to the b-lactam antibiotics except cefoxitin, but sensitive to metronidazole, moxifloxacin and tigecycline.

Conclusion: The difference in antibiotic susceptibility of blood and wound isolates highlights the need for full anaerobic investigation of septicemia originating from a local sepsis for which anaerobes are isolated. This study also emphasized the need for regular blood cultures for anaerobes in high risk patients.

19.019 Detection of extended spectrum beta lactamase producing strains among clinical isolates of Escherichia coli and Klebsiella pneumoniae in Alexandria using Chrom-ID ESBL agar and molecular techniques

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**Purpose:** The increased prevalence of *Enterobacteriaceae* producing ESBLs creates a great need for identifying these organisms for infection control and epidemiological surveillance. We aimed to compare the abilities of different phenotypic methods available in the routine laboratory to detect ESBL-producing strains, evaluate the ESBL detection performance of chromogenic media (ChromID<sup>TM</sup> ESBL) and to estimate the prevalence of the most prevalent β-lactamase genes in the tested isolates.

**Methods & Materials:** A total of 173 clinical isolates of *E. coli* (107) and *K. pneumoniae* (66) were collected. Phenotypic characterization of ESBLs by combined disc test, double disc synergy test (DDST), and the modified version of the DDST (MDDST) was performed simultaneously with antibiotic susceptibility testing. The combined disc test using both cefotaxime and ceftazidime alone and in combination with clavulanic acid was used. ChromID ESBL agar (bioMérieux) was evaluated for ESBL detection using isolates of *E. coli* and *K. pneumoniae*. ESBLs positive strains were tested for the presence of ESBL encoding genes, *bla*<sub>TEM</sub>-M, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub>, using PCR.

**Results:** An ESBL phenotype was confirmed in 100/173 (57.8%) isolates according to the CLSI guideline by combined disc test using both cefotaxime and ceftazidime alone and in combination with clavulanic acid. Using this technique, cefotaxime combined disc achieved the highest sensitivity of 98%. By using DDST at 30 mm, the sensitivity was 8%, while sensitivity achieved 44% when the distance kept at 20 mm apart. Cefepime yielded the highest performance among the β-lactams, with sensitivity 70% when tested with amoxicillin-clavulanate and 86% when tested with piperacillin-tazobactam. The highest sensitivity among phenotypic method (98%) was obtained with chromID ESBL. PCR of TEM, SHV and CTX-M genes revealed that 80% were positive for at least one of the studied genes. On the other hand, 20% had none of these three genes. None of the ESBL negative isolates were harboring any of these genes.

**Conclusion:** Both CCA and CCT combined discs appear to be optimal combination for ESBL detection since it significantly increased the sensitivity of the test. Agars with chromogenic substrates could be applicable as screening methods for ESBLs producing strains.

19.020  Antibiotic susceptibility testing on bacterial isolates from wound infections

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**Purpose:** The understanding of antimicrobial susceptibility profile of bacterial pathogens is a critical guide to empirical and pathogen specific chemotherapy. The aim of this study was to determine the antibiotic susceptibility profile of bacterial species isolated from wound infections.

**Methods & Materials:** The method employed in the analysis included antibiotic sensitivity testing using single antibiotic discs. Four different species of bacteria were isolated from fifty samples collected from patients at different wards of the University of Port Harcourt Teaching Hospital (UPTH) in Port Harcourt, Nigeria.

**Results:** The bacteria species isolated were *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. Results showed that *Klebsiella pneumoniae* and *Escherichia coli* showed high resistance to aztreonam and amoxicillin-clavulanic acid. The microbial isolates all showed multi-drug resistance. *Pseudomonas aeruginosa* showed 100% resistance to ceftazidime as well as other isolates and 100% resistance to cefepime, aztreonam, piperacillin-tazobactam, ticarcillin and was susceptible to a large extent to imipenem, also showing variable susceptibility pattern to gentamicin, and 50% susceptibility to ciprofloxacin and levofloxacin. *Staphylococcus aureus* showed 100% resistance to ceftazidime, and oxacillin, 76% resistance to erythromycin, 53% resistance to sulphamethoxazole-trimethoprim, and a slightly above average susceptibility pattern to ciprofloxacin, gentamicin and levofloxacin of 53%, 53% and 65% respectively. *Klebsiella pneumoniae* showed multiple drug resistance, with 100% resistance to ceftazidine, gentamicin, erythromycin, sulphamethoxazole-trimethoprim, aztreonam, amoxicillin-clavulanic acid, 90% resistance to polymyxin B and ciprofloxacin, 80% resistance to levofloxacin and a high susceptibility (80%) to imipenem. *Escherichia coli* also showed multiple drug resistance, with 100% resistance to ceftazidime, aztreonam, amoxicillin-clavulanic acid, 91% resistance to polymyxin B and erythromycin, 73% resistance to sulphamethoxazole-trimethoprim, 64% resistance to ciprofloxacin, levofloxacin and gentamicin and was only susceptible to imipenem (100%).

**Conclusion:** Our findings indicates imipenem to be the most effective antibiotic against all bacteria isolates, while all of the isolates showed high frequency of resistance to cefepime and ceftazidime. In view of the level of antibiotic resistance observed from this study, prudent use of antibiotics by physicians and patients is advised to help control the emerging trend of antibiotic resistance.
Purpose: The recommended antimicrobial agents for treatment or chemoprophylaxis of pertussis are erythromycin, azithromycin and clarithromycin. Trimethoprim-sulfamethoxazole (co-trimoxazole) can also be used. We tested the susceptibility to first-line and alternative antibiotics of 135 Bordetella pertussis (B. pertussis) strains recovered from patients with whooping cough through national pertussis surveillance in the Czech Republic (CR) in 1967 – 2015. Isolates of B. pertussis were divided according to the year of isolation into 3 periods: from the years 1967 to 1999 (42 strains), 2004 - 2010 (43 strains) and 2011 - 2015 (50 strains).

Methods & Materials: The minimal inhibitory concentrations (MICs) of erythromycin, clarithromycin, azithromycin, ciprofloxacin, and co-trimoxazole were tested by the reference agar dilution method on Bordet-Gengou agar with 15 % defibrinated sheep blood.

Results: Individual MICs of antibiotics were nearly identical in all three time periods. Each of the 135 study strains of B. pertussis was inhibited by two concentrations of erythromycin and azithromycin (0.06 and 0.12 mg/l) with the exception of one strain. All strains were inhibited by three concentrations of clarithromycin (0.03, 0.06, and 0.12 mg/l). All study strains were inhibited by a single concentration of ciprofloxacin (0.06 mg/l) and three concentrations of co-trimoxazole (0.12, 0.25 mg/l and 0.5 mg/l).

Conclusion: The MICs of antibiotics were in the narrow range of two to three low concentrations in all three time periods. The group of 135 Czech strains of B. pertussis appears to be homogeneous in terms of the MICs of the antibiotics tested, with two to three low concentrations being effective against all strains. To be inhibited, no strain required a higher concentration of erythromycin, clarithromycin, azithromycin, ciprofloxacin, or co-trimoxazole. No changes were observed in the MICs range of individual antibiotics during the period of 55 years.

Purpose: India was the largest consumer of antibiotics in 2010 in the world. Several studies have reported increasing bacterial resistance to antibiotics in India especially Carbapenem resistant Gram-negative Enterobacteriacea. The objective of our study was to investigate the trends in consumption of major antibiotic subgroups in India during January 2008 to December 2012 and compared them with European Surveillance of Antimicrobial Consumption Network (ESAC-Net) countries.

Methods & Materials: For our study we used state-specific data on total antibiotic sales (January 2008- December 2012) procured from the IMS Health Drug Sales Audit. We used ATC classification system and the defined daily dose (DDD) measurement unit (developed by the World Health Organization Collaborating Centre (WHOCC) for Drug Statistics Methodology) to standardize dosage trends. We assigned DDDs (2015 index) to all formulations based on the ATC/DDD index. To control for the size of the population we expressed our data in DDD per 1000 inhabitants’ daily (DID) and to compare antibiotic use of India with ESAC-Net countries through standardized matrices of DID, we rely on data ESAC-Net data, which was publicly available at the ATC third and fourth level. The antibiotic use was plotted and reported by year and antibiotic class. We used statistical software STATA 13.0 to perform our analytics.

Results: Our main findings are that in India per capita antibiotic consumption has increased from 13.1 DID in 2008 to 16.0 DID in 2012 - an increase of ~22%; antibiotic consumption rates in India are still low as compared to ESAC-Net countries (16.0 DID vs. 21.54 DID); use of newer class of antibiotics like carbapenems (J01DH) glycopeptides (J01XA), 3rd generation cephalosporins (J01DD) and Penicillins’s with beta-lactamase inhibitors has risen during 2008 to 2012; the antibiotic consumption pattern is seasonal in nature with peaks in July, August and September every year.

Conclusion: Our study has provided first reliable estimates of antibiotic use in India vis-à-vis ESAC-Net countries, through application of same methodology. Through this benchmarking exercise we have identified areas for quality improvement (for example, reduce use of newer
class of antibiotics). In addition, our study could provide a reference point to measure the impact of antimicrobial stewardship interventions.

19.023  Advancement of Dairying in Austria (ADDA): Mastitis in dairy cows – milk sample testing, antibiotic use and antimicrobial resistance
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Purpose: The ADDA project aims to provide practical solutions to farmers and veterinarians by combining expertise from academia, agriculture, commercial dairies, veterinary agencies and government authorities. This study assesses current antibiotic use (AMU) for the treatment of mastitis and in the dry period, the prevalence of antimicrobial resistance (AMR) in bacteria isolated from cows with acute mastitis, and the impact of possible management factors.

Methods & Materials: AMU data are currently being collated from 18 veterinary practices throughout Austria, with respect to 247 dairy farms. Milk samples from all cases of acute mastitis are being collected, cultured and the respective pathogens sensitivity tested according to EUCAST standards for antimicrobial classes relevant to veterinary medicine (clinical breakpoints). Farmers, veterinarians, commercial dairy employees/milk recorders have been asked to complete farm management surveys on each of these farms.

Results: To date (June 2016), 71.7% of farmers (177/247) have completed the questionnaire. Initial results show that 67.2% of these farms have loose housing systems, while 32.8% tether their dairy cows; 55.9% provide access to pasture. Prophylactic dry cow therapy is routinely implemented on 44.6% of farms, with 55.4% preferring to be selective in their use of antibiotic treatments in the dry period. Of all farmers, 14.7% regularly cultured milk samples from all animals prior to drying off, 69.5% carried out this precaution only in cows with udder problems and 15.8% never cultured prior to dry-off.

The resistance profiles of Staphylococcus aureus (n=56) isolates from mastitic milk samples have been analysed. Nine of these isolates were determined to be resistant to at least one antimicrobial class; with six being resistant to penicillin, one to tetracycline and two to both penicillin and tetracycline. Multiple resistance (≥3 classes) was not detected. The remaining isolates, including Streptococcus uberis, CNS, Streptococcus dysgalactiae and E.coli, are currently being assessed.

Conclusion: Initial results show that Austrian dairy farmers and veterinarians are proactive in collecting milk samples for culture and sensitivity testing. However, improvements in prudent AMU can still be made. Further analyses are planned to assess the relationship between AMU and AMR on Austrian dairy farms.

[COMET-K-Projekt ADDA]
[*CLF & AS contributed equally to the study]

19.024  Pathogens causing device-associated infections and Carbapenem resistance among gram-negative pathogens causing infections in ICU
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Purpose: 1-To identify the prevalent pathogens in ICU patients with device associated infections (DAIs)
2-To test the in- vitro susceptibility of gram negative pathogens to carbapenems.

Methods & Materials: A prospective surveillance study was conducted on 815 patients admitted to Intensive Care Units (ICUs) of New Emergency Hospital, one of Cairo University hospital, over one year starting from September 2014. Patients who were using one or more of the following devices were monitored: vascular catheters , urinary catheter , mechanical ventilation, In collaboration with the hospital Infection Control Team. The data was analyzed according to the specific case definitions provided by the Centers for Disease Control and Prevention (CDC,2014).

Clinical specimens from patients with suspected infections were collected by the physician in charge, submitted to the hospital microbiology laboratory to provide identification of pathogens by conventional method and antimicrobial susceptibility of all isolates was determined by the standard Kirby Bauer disc diffusion method (CLSI,2015).

Results: Out of 815 patients, 67 had DAIs. Device rate and device utilization (DU) ratio were calculated according to the NHSN definitions . Gram- negative pathogens represented 64.2%. Carbapenem- resistant isolates represented 58.1%. The most prevalent organisms were Acinetobacter baumannii followed by Pseudomonas aeruginosa then Klebsiella pneumoniae.
Isolation and preservation of the isolates: before the plates were discarded as negative for growth. Carbenopenem resistant gram-negative isolates in VAP cases were 87.5%. Central line associated blood stream infection represented 28.4%. Klebsiella was the predominant pathogen and carbenopenem resistance in gram negative pathogens was 54.5%.

Conclusion: Gram-negative pathogens were the commonest causes of DAI in ICUs. -Carbenopenem resistance among the gram negative pathogens was very high, representing a significant clinical and public health concern.

19.025 Poblational and biochemical-structural analyses on CTX-M β-lactamases harboring the D240G mutation reveal that E. coli populations with decreased susceptibility to ceftazidime preexist under a multifactorial resistance profile B. Ghiglione1, M. M. Rodríguez1, F. Brunetti1, M. Dropa2, L. Curto1, P. Power1, G. Gutkind1
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Purpose: CTX-M β-lactamases possess high catalytic efficiency towards cefotaxime (CTX) but spare ceftazidime (CAZ). Variants harboring the replacement of D240 by a glycine (D240G substitution) have been associated with a decreased susceptibility to CAZ. We analyzed the influence of D240G, and spontaneous porin deficient subpopulations in the five CTX-M sub families in relation to final MICs.

Methods & Materials: Parental blaCTX-M- genes and their corresponding D240G mutants from all 5 blaCTX-M clusters (2, 3, 8, 9, 25) were cloned in pK19 under regulation of the vector’s promoter and transformed in wild type and OmpF porin-deficient E. coli K12 strains. ESBL screening and susceptibility tests were performed according to CLSI recommendations. Selection of preexisting CAZ-resistant mutants with increasing concentrations of CAZ was performed. Steady-state kinetic parameters were determined for CTX and CAZ.

Results: Upon D240G substitution, CTX-M producing clones remained susceptible to CAZ, while those lacking OmpF were resistant. Although kcat/Km toward CAZ for all CTX-M variants carrying Gly was between 5-10 times higher than that for CTX-M variants carrying Asp, it remained between 200-725 times lower than for cefotaxime, due to a slight increase in kcat values, while Km values remain at the millimolar range. In vitro selection of CAZ-resistant E. coli strains yielded D240G CTX-M producers with MIC values of 32 and 64 µg/ml while those with no substitution was only 8 µg/ml.

Conclusion: D240G mutation leads to a remarkable increase in ceftazidime resistance in CTX-M producing clones only when expressed in an OmpF deficient background, suggesting the importance of this porin in the entrance of ceftazidime. Kinetic analysis showed that G240 variants have only weak activity toward ceftazidime. The presence of this mutation may favor the selection of porin deficient variants, and primary characterization as "susceptible" may mask the possibility for in vivo selection of this variants.

19.026 Antimicrobial resistance bacteria isolated from mastitic cows
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Purpose: In this study, 150 milk samples were obtained from cows in East Nilefarms. Samples positive for bacterial growth were identified using the Gram stain and various conventional biochemical tests. Out of 126 isolates 100 (79.4%) were found to be Gram positive, 23(18.2%) Gram negative bacteria and 3(2.4%) were yeast. Among the total isolates 87(53.2%) were Staphylococcus spp., 22(17.4%) Streptococcus spp., 14(11.1%) Enterobacter spp., 6(4.7%) Corynebacterium spp., 5(4%) Klebsiella spp., 4(3.2%) Bacillus spp., 2(1.9) E. coli, and 1 (0.7%) of each of Actinomycetes spp., pseudomonas spp., Proteus mirabilis in addition to 3(2.4%) Yeast. The samples showed no growth when cultured were 39 (26%).

Methods & Materials: Culture Cultural Procedures
Isolation of bacteria was attempted from the collected samples by using a sterile swab dipped into each of the test tube then streaked onto 5% sheep blood agar plates and incubated at 37°C for 24 hours. If no growth was observed, the incubation was continued for 48 hours before the plates were discarded as negative for growth.

Isolation and preservation of the isolates:
Well isolated representatives of the bacterial colonies were selected according to their morphology; sub cultured onto blood agar plates and incubated at 37 °C for 24 hours. Then stored at 4 °C for further analysis.

Identification of isolates:

Conventional methods:
The purified isolates were identified by using standard bacteriological methods as described by Barrow and Feltham (1993) and Quinn et al (1994).

Results: Of the 126 isolates 100 (79.4%) were gram positive, 23 (18.2%) gram negative bacteria and 3 (2.4%) were yeast. Among the total isolates 67 (53.2%) were Staphylococcus spp., 22 (17.4%) Streptococcus spp., 14 (11.1%) Enterobacter spp, 6 (4.7%) Corynebacterium spp, 5 (4%) Klebsiella spp, 4 (3.2%) Bacillus spp. 3 (2.4%) Yeast, 2 (1.9) E. coli, and 1 (0.7%) each of Actinomyces spp., pseudomonas spp. and Proteus mirabilis.

Conclusion: The organisms diagnosed were highly sensitive to Ciprofloxacin, Norfloxacin, Gentamycin and Streptomycin which are the most effective drugs to the tested isolates, while the resistance was more frequent to penicillin G, amoxicillin, lincomycin, cefalexin, and then Tetracycline.

19.027 Antibiotic resistance in Enterobacter cloacae with derepressed/partly derepressed/inducible AmpC and extended-spectrum beta-lactamase in Zenica-Doboj Canton, Bosnia and Herzegovina

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Purpose: To investigate the prevalence of derepressed, partly derepressed, inducible and ESBL producing AmpC-carrying Enterobacter cloacae isolates and treatment options for infections associated with those isolates in Zenica-Doboj Canton, Bosnia and Herzegovina.

Methods & Materials: Antibiotic susceptibility was determined by disc diffusion and broth microdilution according to CLSI guidelines. Double-disk synergy test (DDST) was performed in order to screen for ESBLs and combined disk test with phenylboronic acid to detect AmpC β-lactamases. PCR was used to detect blaESBL/blaTEM genes. Genetic relatedness of the strains was determined by pulsed-field-gel-electrophoresis (PFGE).

Results: Among 14 isolates with the ESBL positive E. cloacae producing isolates, four (28.6%), nine (64.3%) and one (7.1%) isolates were derepressed, partly derepressed and inducible AmpC producers, respectively. Eleven (out of 14) isolates were resistant to cefotaxime, ceftazidime, ceftriaxone, aminoglycosides and fluoroquinolones. All isolates were susceptible to imipenem and meropenem, 79% to cefepime. Five (out of 14; 35.7%) isolates (four derepressed and one inducible AmpC carrying E. cloacae) were negative in phenotypic test for ESBLs, but positive for broad spectrum TEM-1 β-lactamase. One (out of four derepressed) also produced CMY-2 β-lactamase. Four (out of nine) partly derepressed isolates were positive with the DDST, but did not yield PCR products with primers targeting TEM, SHV and CTX-M beta-lactamases. Four positive partly derepressed isolates carried a blaCTX-M-1 gene, two blaOXA-1 and one blaCTX-M-15, OXA-1, and one blaCTX-M-28, OXA-1. PFGE showed no genetic relatedness.

Conclusion: AmpC enzymes which encoded by chromosomal or plasmid genes are evolving to hydrolyze broad-spectrum cephalosporins more efficiently. Microbiology laboratories must be able to detect and recognize AmpC-carrying isolates in a timely manner, especially those that are falsely susceptible in vitro to drugs that may be considered for therapy of infected patients.

19.028 High occurrence of multiple genes in ESBL-producing inpatient and outpatient isolates in Zenica-Doboj Canton, Bosnia and Herzegovina

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Purpose: To investigate the prevalence and antimicrobial resistance of Gram-negative isolates producing more than two types of beta-lactamases, obtained from in- and outpatient infections in Zenica-Doboj Canton, Bosnia and Herzegovina.
Methods & Materials: Antibiotic susceptibility was determined by disc diffusion and broth microdilution methods according to CLSI guidelines. Double-disk synergy test was performed to screen for ESBLs and pAmpC beta-lactamases. PCR was used to detect blaESBL, blaAmpC, bla carb genes. Genetic relatedness of the strains was determined by PFGE.

Results: Among 88 inpatient (n=126; 10.0%) and 62 outpatient (n=184; 6.4%) beta-lactamase producing isolates, 50.0/29.0% K. pneumoniae, 12.5/30.6% E. coli, 11.4/4.8% A. baumannii, 8.0/14.5% K. oxytoca, 8.0/4.8% E. cloacae, 5.7/8.1% Proteus spp., and less than 3.5% of other isolates, respectively, were found. Sixty-eight (out of 88; 77.3%) inpatient and 28 (out of 62; 45.2%) outpatient isolates possessed more than two genes. Among inpatient isolates, Klebsiella spp. and E. coli was the most prevalent strain producing more than two types of genes, in 90.0% and 70% cases, respectively. The combination of blaTEM+blaSHV+blaCTX-M+blaOXA-1 were noticed in 34 (out of 68; 50.0%) inpatient isolates; blaCTX-M+blaSHV+blaOXA-1 in seven (10.3%); blaCTX-M+blaOXA-1 in six (8.8%). In outpatient isolates combination of blaTEM+blaSHV were noticed in seven (out of 28; 25.0%) and blaTEM+blaSHV+blaCTX-M+blaOXA-1 in six (21.4%) cases. The most prevalent combination of blaTEM+blaSHV+blaCTX-M+blaOXA-1 was noticed in 28 (out of 38; 73.7%) inpatient Klebsiella pneumoniae. Prevalence of antimicrobial resistance were higher to all cephaplospirons, amynoclicosides and piperacillin in Klebsiella spp., and E. coli inpatient isolates producing more than two types of beta-lactamases comparing to isolates producing only one type of beta-lactamase. PFGE showed no genetic relatedness between the isolates.

Conclusion: High prevalence of Gram-negative isolates producing more than two types of beta-lactamases, especially in inpatient isolates were found. Continuous surveillance, and implementation of infection control and prevention measures will help in limitation of further spread of these isolates.

19.029 Molecular epidemiology and antimicrobial susceptibility of AmpC- and/or extended-spectrum (ESBL) β-lactamase- producing Proteus spp. clinical isolates in Zenica-Doboj Canton, Bosnia and Herzegovina

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Purpose: To investigate prevalence, antimicrobial susceptibility, molecular characteristics, and genetic relationship of AmpC- and/or extended spectrum beta lactamase (ESBL)-producing Proteus spp. clinical isolates in Zenica-Doboj Canton, Bosnia and Herzegovina.

Methods & Materials: Antibiotic susceptibility was determined by disc diffusion and broth microdilution methods according to CLSI guidelines. Double-disk synergy test was performed in order to screen for ESBLs, and combined disk test with phenylboronic acid to detect AmpC β -lactamases. PCR was used to detect blaESBL/blacarb genes. Genetic relatedness of the strains was determined by pulsed-field-gel-electrophoresis (PFGE).

Results: Eleven ESBL-producing isolates were included in the study (six inpatients and five outpatients). Susceptibility rate to amoxicillin-clavulanic acid, imipenum and meropenem was 100%. Resistance rate to cefturoxime was 100%, gentamicine 90.9%, piperacillin/tazobactam 81.8%, cefotaxim, ceftriaxone and cefazidim 72.7%, cefoxitine and ciprofloxacine 63.6% and to cepemip 45.5%. In five (out of 11) isolates it was detected multi-drug resistance (MDR) to cephaplospirons, cefamycines, amynoclicosides and fluoroquinolones. Besides TEM-1 which was detected in all isolates, CTX-M+OXA-1 β-lactamases were detected in seven (out of 11; 63.6%) isolates (five blaCTX-M-1 and two blaCTX-M-15 genes), and CMY-2 β-lactamase in two isolates. PFGE showed no genetic relatedness.

Conclusion: Because of high prevalence of MDR strains in epidemiologically unrelated patients with AmpC- and/or ESBL producing Proteus spp. infection, further surveillance is needed. Molecular characterization and strain typing, or at least phenotypic test for AmpC/ESBL production of is important for appropriate therapy and the detection of the sources and modes of spread, which is a main step in order to design targeted infection control strategies.

19.030 Evaluation of tetracycline resistance genes during avian manure composting process.

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Purpose: Antimicrobial resistances (AMRs) are an emerging threat for animal and public health. Among the different mechanisms of AMRs, plasmid-mediated antimicrobial resistance genes (PM-ARGs) are of major concern due to their capability of mobilization and transfer between bacteria. Since at least an 80% of bacteria are not cultivable, PM-ARGs should be studied directly in the microbiome, without previous culture. AMRs are mainly studied in every step “from the farm to fork”, but less known is how can they be spread into the environment through animal waste. Animal manure is commonly used as agricultural fertilizer and may carry on PM-ARGs which could reincorporate to food chain by crops. An interesting alternative is composting the animal manure, which reduces the presence of pathogenic bacteria and improves its quality as fertilizer, offering an added value to the product. In this work, we tested plasmid-mediated tetracycline resistance genes (tet) during four different small-scale avian manure composting processes.

Methods & Materials: Four different avian manure composting processes (avian manure+straw; avian manure+straw+fresh eggs; avian manure+straw+egg ashes; avian manure+straw+fresh eggs+egg ashes) at small scale were evaluated during eight weeks. Seven tet genes were quantified by real time PCR (tet(A), tet(B), tet(C), tet(K), tet(M), tet(Q) and tet(S)). Besides, the 16SARN gene was amplified for sample validation and to calculate the relative concentration of every gene (expressed as log of percentage of bacteria which carries a determinate gene). All genes, with the exception of tet(C), were detected.

Results: No apparent differences were observed among the types of composting. For every gene and every type of compost, there is an increase of gene quantity during the first week of composting. Also, there is a dramatic decrease of gene quantity during weeks 5-7, depending the gene. After this decrease, tet(M) and tet(S) genes were still under detection limit up to the final week; however, the rest of genes increase during the last weeks, to at least to initial levels.

Conclusion: Composting during 8 weeks doesn’t seem to reduce the quantity of tet genes. Increase the number of weeks of composting as well as analyze other PM-ARGs will be evaluated. This work is funded by RTA2014-00012- C03-02.

19.031 Detection of plasmid-mediated colistin resistance (mcr-1) in E. coli isolated from pig caecum in Austria

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Purpose: Colistin is regarded as a last line defence against infections caused by multidrug-resistant Gram-negative bacteria. Plasmid-encoded colistin resistance mediated by the mobile colistin resistance-1 (MCR-1) protein has been identified in food animal, human, food, and environmental isolates on every continent. We report on the first colistin-resistant E. coli strain documented in Austria.

Methods & Materials: A total of 257 pig caeca were collected at slaughterhouses in the framework of an EU monitoring program targeting antimicrobial resistance between January and December 2015 in Austria. Samples were screened for the presence of ESBL/AmpC producing E. coli using non-selective enrichment and McConkey agar supplemented with 1 mg/L cefotaxime. Antimicrobial susceptibility was determined by Sensititre microbroth dilution using EUVSEC and EUVSEC2 plates. The colistin-resistant isolate was sequenced using the Illumina MiSeq platform. Raw reads were de novo assembled based on Velvet algorithms using the pipeline available from the Center for Genomic Epidemiology (CGE). The assembled genome was analyzed using pipelines MLST, PlasmidFinder, pMLST, and ResFinder available from CGE.

Results: In November 2015, an E. coli isolate harboring the mcr-1 gene was isolated from pig caecum sampled in a slaughterhouse in Austria. The isolate belonged to MLST sequence type 101 (ST101) and showed a MIC of 8 mg/L for colistin. Besides mcr-1, the isolate contained seven additional resistance genes (blaCTX-M-1, aadA5, dfrA17, strA, strB, sul2, and tet(B)) conferring resistance to extended-spectrum beta-lactam antibiotics as well as aminoglycosides, tetracyclines, trimethoprim, and sulfamethoxazole. Plasmid replicons detected were IncFIB(AP001918), IncI, IncFIC(FII), pO111, IncX4, and ColRNAI. The Inc groups identified were IncI[ST-3] and IncF[F46:A:-B24]. Analysis of contigs showed that mcr-1, insertion sequence ISApl1, and replicon IncX4 were located on the same contig indicating that mcr-1 might be situated on plasmid IncX4.
Conclusion: The detection of the \textit{mcr-1} gene in an \textit{E. coli} strain isolated from a pig shows its presence in livestock in Austria. In Austria, the implementation of a selective screening program in existing monitoring programs will be necessary to ascertain the true prevalence of plasmid-mediated colistin resistance in \textit{Enterobacteriaceae} in livestock and food.

19.032 Antimicrobial resistance of \textit{Pseudomonas aeruginosa} strains isolated from surgical wards
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Purpose: \textit{Pseudomonas aeruginosa} is one of the main opportunistic pathogen agents that cause increased morbidity and mortality in the hospital environment, mainly in surgical and intensive care units.

The purpose of this study was to analyze the resistance profiles of \textit{P. aeruginosa} strains isolated from patients hospitalized in surgical wards in Regional Institute of Gastroenterology and Hepatology, Cluj Napoca, Romania.

Methods & Materials: We conducted a retrospective study during January–December 2014. Isolation of \textit{P. aeruginosa} strains was performed on selective media for Gram-negative bacteria. Identification of strains was done both by conventional methods and by automated methods using Vitek\textsuperscript{®} 2 Compact. Antibiotic susceptibility testing was done using the Vitek\textsuperscript{®} 2 Compact, but also by Kirby-Bauer method. Interpretation susceptibility testing was performed according to the CLSI standard.

Results: They were analyzed 259 isolates from 244 patients hospitalized in surgical wards, 50.8% male, respectively 49.2% women. These strains were isolated from different pathological products: lower tract respiratory (75), pus (65), peritoneal fluid (26), urine (18), central venous catheter insertion (17), blood (13), faeces (9), bile (8) and other secretions (13). Patients were aged between 19 and 98 years, with a median value of 60.5 years. Most patients were immunosuppressed by malignancy, pancreatitis, gastric and duodenal ulcers, peritonitis, gallstones, and others. Clinical outcome was infaust, registering death in 49.6% of patients.

\textit{P. aeruginosa} strains showed the following levels of antibiotic resistance, respectively, 86.1% to ticarcillin, 62.1% to piperacillin, while resistance to the penicillins combined with beta-lactamase inhibitors, ticarcillin with clavulanic acid and piperacillin with tazobactam was 75.1%, respectively 51.8%. Resistance to antipseudomonal cephalosporins was 61.8% to ceftazidime and 48.8% to cefepime, while the level of resistance to carbapenems was 68.7% to imipenem and 69.8% to meropenem. Resistance to aminoglycosides was 76.6% to gentamicin, 65.4% to tobramycin and 75.6% to amikacin. 66.4% of strains showed cross-resistance to fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin), while resistance to colistin was only 0.6%.

Conclusion: The studied strains showed high levels of resistance to all classes of antibiotics, colistin remains one of the only therapeutic options. Judicious administration of antibiotics in combination with nosocomial infection control measures need to be introduced in hospitals to prevent the circulation of these multidrug resistant strains.

19.033 Species of non-fermentative Gram–negative bacilli other than \textit{Pseudomonas aeruginosa} isolated from surgical wards in Regional Institute of Gastroenterology and Hepatology Cluj Napoca, Romania
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\(^1\)University of Medicine and Pharmacy, Cluj- Napoca, Romania, \(^2\)Regional Institute of Gastroenterology and Hepatology, Cluj- Napoca, Romania

Purpose: Nonfermentative Gram – negative bacilli represent an important cause of morbidity and mortality in hospital environments with intrinsic resistance to multiple classes of antibiotics and their ability to gain new resistance factors.

In this study we aimed to identify the etiology of nonfermentative Gram - negative bacilli in 2014 from surgical wards of Regional Institute of Gastroenterology and Hepatology “O. Fodor” Cluj - Napoca and to analyze their resistance profile.

Methods & Materials: We analyzed 280 clinical specimens from hospitalized patients between January - December 2014. Their identification was achieved with automatic system Vitek\textsuperscript{®} 2 Compact (bioMérieux, Marcy - l’ Étoile, France). Antibiotic susceptibility testing was performed both with automatic system VITEK\textsuperscript{®} 2 Compact and with disc diffusion method. Antibiotic susceptibility for \textit{Stenotrophomonas maltophilia} was done by disc diffusion method with Levofloxacin 5 μg, Minocycline 30 μg and Sulphamethoxazole-Trimethoprim 1.25 / 23.75 μg. Interpretation sensitivity testing was done according to current CLSI.
Results: 308 nonrepetitive strains of nonfermentative Gram-negative bacilli were isolated. The involved species are presented in the table that follows.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achromobacter denitrificans</td>
<td>1</td>
<td>0.3%</td>
</tr>
<tr>
<td>Achromobacter xylosoxidans</td>
<td>1</td>
<td>0.3%</td>
</tr>
<tr>
<td>Burkholderia pseudomallei</td>
<td>1</td>
<td>0.3%</td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>1</td>
<td>0.3%</td>
</tr>
<tr>
<td>Elizabethkingia meningoseptica</td>
<td>8</td>
<td>2.6%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>259</td>
<td>84.1%</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>1</td>
<td>0.3%</td>
</tr>
<tr>
<td>Rhizobium radiobacter</td>
<td>1</td>
<td>0.3%</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>33</td>
<td>10.7%</td>
</tr>
<tr>
<td>Sphingomonas paucimobilis</td>
<td>2</td>
<td>0.7%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>308</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

P. aeruginosa was the main pathogen isolated (84.1%), followed by S. maltophilia (10.7%) and E. meningoseptica (2.6%). The antimicrobial resistance profile for S. maltophilia was: 3.9% to levofloxacin, 3.3% to minocycline, and 32.1% to sulphonamethoxazol-trimethoprim (SXT). E. meningoseptica was resistant to all tested antibiotics, except ciprofloxacin, minocycline and trimethoprim. B. cepacia was resistant to aminoglycosides. P. putida, R. radiobacter and S. paucimobilis were sensitive to all antibiotics. Achromobacter species were resistant to aminoglycosides, fluoroquinolones, colistin and SXT.

Conclusion: These species require automated methods for identification, their diversity is a challenge for microbiological diagnosis. Although these species are rarely isolated, they are important due to the intrinsic resistance that limits therapeutic options.

19.034 **In Vitro** efficacy of essential oils alone and in combination with fluconazole against azole-resistant strains of Cryptococcus neoformans

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Purpose: The present study was aimed to screen certain plant essential oils and active compounds for antifungal activity and their *in vitro* interaction with fluconazole against azole-resistant strains of *C. neoformans*.

Methods & Materials: The present study was aimed to screen certain plant essential oils and active compounds for antifungal activity and their *in vitro* interaction with fluconazole against azole-resistant strains of *C. neoformans*.

Results: The two test strains viz. *C. neoformans* 01 (clinical strain) and *C. neoformans* MTCC4424 (standard strain) exhibited common resistance to azoles including fluconazole, ketoconazole and itraconazole out of six drugs tested. Among the 21 essential oils or active compounds tested, ten showed promising antifungal activity. Eugenol and cinnamaldehyde showed most promising antifungal activity and killing potency against *C. neoformans* 01. Eugenol showed strongest synergy with fluconazole against *C. neoformans* 01 by reducing the MICs of fluconazole and its own up to 16-fold.

Conclusion: This study highlights the potential antifungal activity from oils of *C. verum, C. citratus, C. martini, C. coticum* and *S. aromaticum* against the azole-resistant strains of *C. neoformans*. Moreover the compounds like eugenol and cinnamaldehyde were found to be highly active against the *C. neoformans* and also potentiated the antifungal activity of fluconazole.

19.035 Quinolone resistance determinants of *Salmonella* Enteritidis isolated from patients in Thailand
Purpose: *Salmonella* Enteritidis has emerged as global concern regarding quinolone resistance and invasive potential. Although quinolone-resistant *S*. Enteritidis has been observed with high frequency in Thailand, information on the mechanism of resistance acquisition is limited. This study aimed to investigate the quinolone resistance determinants in clinical nalidixic acid (NAL) resistant *S*. Enteritidis isolates collected nationwide in Thailand.

Methods & Materials: To elucidate the quinolone resistance mechanism, a total of 190 clinical isolates of NAL-resistant *S*. Enteritidis were collected throughout Thailand, and the quinolone resistance-determining region (QRDR) mutational status and the plasmid-mediated quinolone resistance (PMQR) determinants were investigated in the context of resistance levels to NAL, norfloxacin (NOR) and ciprofloxacin (CIP). In addition, the relationship between these resistance determinants and multi-locus variable number of tandem repeat analysis (MLVA) was to elucidate the dissemination of specific clones.

Results: The most commonly affected codon in *gyrA* was 87, followed by 83. Double codon mutation in *gyrA* was found in an isolate with high-level resistance to NAL, NOR and CIP. A new mutation causing serine to isoleucine substitution at codon 83 was identified in eight isolates. Eighteen *qnrS1*-carrying isolates showing non-typical quinolone resistance. One isolate carrying both the *qnrS1* gene and a *gyrA* mutation showed a high level of quinolone resistance. Genotyping by MLVA suggested the presence of a possible clonal expansion of NAL-resistant *S*. Enteritidis nationwide.

Conclusion: Our data suggested that NAL-resistant isolates with single quinolone resistance determinant may potentially become fluoroquinolone resistant by acquiring secondary determinants.

Purpose: To describe the incidence and treatment response for Colistin only sensitive *Acinetobacter baumanii* infection involving prosthetic joints

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Purpose: Uncontrolled usage of antibacterial agents can cause widespread antibiotic resistance, which then becomes more and more difficult to treat and to eradicate. Nowadays,
antibiotic resistance is a huge problem for developing (as well developed) countries. With rapid dissemination of multi-drug resistant bacterial pathogens, the interest in alternative remedies to antibiotics, including bacteriophage treatment, is gaining new ground. In contrast to antibiotics, bacteriophages have high specificity, and are self-propagating, and self-limiting organisms. The G. Eliava Institute is a world-known institution that houses the largest collection of bacteriophage in the world and has work ongoing in various research areas.

Phages from the institute were used to examine their activity against antibiotic-resistant bacterial strains isolated from human samples.

**Methods & Materials:** Bacterial strains were isolated from patients from different countries (Bulgaria, France, Germany, UK, Ireland, Israel, Switzerland, Turkey, UK, China, Singapore, Vietnam, and the US). Isolated pathogens included methicillin-resistant *Staphylococcus aureus* (MRSA), Beta-lactamase producing *Klebsiella* spp. and *Escherichia coli*, *Acinetobacter baumannii*, and *Enterobacter* spp. Phage susceptibility of the strains was examined by spot test. Commercially available phage preparations (Pyophage, IntestiPhage, and staphylococcal bacteriophage) and laboratory phages were used for susceptibility testing.

**Results:** The best results were demonstrated for *S. aureus* strains with 96-98% of isolates being susceptible to the staphylococcal phage preparation. Commercial phages revealed high activity against *Acinetobacter* strains (~94%), *Enterococcus* spp. (~84%), *E. coli* (~87%), and *Klebsiella* spp. (~90%) clinical isolates. The clinical strains, resistant to commercial phages were tested against laboratory phages. The laboratory phages reveal 97-100% lytic activity against the isolates.

**Conclusion:** The growing problem of antibiotic-resistance in combination with the environmental burden caused by the unrestricted use of antibiotics, provides sufficient motivation for developing alternative solutions to bacterial infections. Bacteriophage treatment of various bacterial infections, including those that are caused by antibiotic-resistant bacteria, is considered a reliable and effective treatment approach. Selection and preparation of specific phages, active against multidrug resistant pathogens is an effective tool to eradicate and treat infections, caused by those pathogens. Use of phages can also decrease the distribution and spread of antibiotic-resistance in medical settings and various environments.

**19.038** Exotoxin-profiling and typing of clinical Panton-Valentine Leukocidin positive MSSA versus MRSA
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**Purpose:** *Staphylococcus aureus* (SA) is one of the most important human pathogens worldwide. SA is causing infections ranging from mild to severe, especially Panton-Valentine leukocidin (PVL) came into focus for the severity of clinical symptoms. Within the last 20 years community acquired PVL positive MRSA strains spread globally and proved to be a public health burden. PVL seems to be highly associated with CA-MRSA but its role in pathogenesis is still controversial and in MSSA neglected.

**Methods & Materials:** We investigated 79 PVL positive SA (18 MSSA and 61 MRSA) from Austria for SCCmec type, spa-type and 30 exotoxin-genes (sea-see, tst-I, seg-ser, seu, eta, etb, ahl, bhl, ghlabc, ghivar, dhl, lukDE).

**Results:** The most common agr-type was agr III (51%) followed by agr I (24%). The MRSA strains carried mainly SCCmec IV or V (93%). Analysis of spa-types revealed a diverse genetic background for MSSA and a narrower one for MRSA. The exotoxin-genes seb, seg, sei, sem, sen, seu, ahl, bhl, and ghlabc were detected significantly more often (p<0.05) in MSSA compared to MRSA. 50% of MSSA carried 11 or more (min. 5 - max. 15; average 10) exotoxin-genes whereas 50% of MRSA carried only 6 or more (min. 1 - max. 12; average 7) exotoxin-genes.

**Conclusion:** The PVL positive MRSA carried significantly less toxin-genes than PVL positive MSSA isolates. This finding supports the theory that the methicillin resistance together with to many toxin-genes would be too much of a cost of fitness or because of the benefit of methicillin resistance less toxin-genes are more sufficient.

**19.039** Prevalence and characterization of carbapenem-resistant gram-negative bacteria isolated from Tamil Nadu, India
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**Purpose:** Carbapenemases have increasingly been reported among gram-negative bacteria worldwide. Plasmid bound carbapenemases are more common hence, resistance can easily
spread. Carbapenem-resistant gram-negative bacteria are reported to cause 30-50% of mortality globally. We set out to determine the carbapenem resistance level and to establish the mechanism of carbapenem resistance among gram-negative clinical isolates obtained from two centres in Tamil Nadu.

**Methods & Materials:** This was a prevalence study with a total of 182 gram-negative clinical isolates previously collected from blood, urine, pus, wound swab, bronchial waste, bile fluid, cerebrospinal fluid culture and Vaginal swabs. All the isolates were identified at species level by 16S rRNA analysis and subjected to phenotypic carbapenemase screening using agar dilution MIC, Modified Hodge and E-test. In addition, PCR assay was performed to test the presence of carbapenemase-encoding genes and prevalence of class1-3 integrons.

**Results:** The study included 182 gram-negative clinical isolates that belong to 9 genera. Phenotypic tests found that 46.2% (84/182) of the isolates were carbapenemase producer and genotypic prevalence was only 30.8% (56/182). The most prevalent gene was *blaNDM*-1 (27, 14.8%), followed by *blaIMP* (11, 6%), *blaVIM* (8, 4.4%), *blaKPC* (5, 2.7%) and *blaOXA*-48 (5, 2.7%). None of the meropenem susceptible isolates by phenotypic analysis carried tested carbapenem-resistant genes. Among 56 isolates encoding carbapenemase genes, *E. coli* species had the highest number 37.5%, followed by *K. pneumoniae* (30.4%). In addition, prevalence of integrons was 76.9%, among class 1 was 44.5% (81/182), class 2 was 26.9% (49/182) and class 3 was 5.5% (10/182). To a small extent, 26.8% (15/56) of carbapenem encoded genes had either class 1 or class 2 integrons and most of these carbapenemase genes were in bacteria isolated from urine samples.

**Conclusion:** Our result confirm the high prevalence of carbapenemases and dissemination of carbapenemase-encoding genes among gram-negative bacteria in Tamil Nadu, India. This study showed the emergence of carbapenem encoded genes in isolates harbouring integrons. This could lead us to a serious threat of an outbreak of MDR development, which makes the treatment of infections more complicated in the future.

19.040 Prevalence and antimicrobial-resistance features of *Staphylococcus* spp. from pets.

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**Purpose:** Staphylococci have been developing, in the last decades, high resistance to beta-lactam antimicrobials, first to penicillin, then to methicillin. In recent years, the number of methicillin-resistant staphylococci due to the presence of *mecA* gene contained in the SCCmec, isolated from pets, has been raising. The *mecA* gene confers resistance to the semi-synthetic penicillins and increases the risk of developing multidrug-resistance. The aim of this study was to characterize the antimicrobial-resistance traits of potentially zoonotic *Staphylococcus* spp. strains isolated from dogs and cats with infection.

**Methods & Materials:** One hundred ninety-nine samples from 160 dogs and 39 cats referred to the Veterinary Teaching Hospital of Padua University were cultured using bacteriological standard techniques. The identification of staphylococci at the species level was assessed by biochemical determination and by multiplex-PCR assays for *Staphylococcus Intermedius Group* and *S. aureus*. Antimicrobial susceptibility to the main antimicrobial classes was determined by disk diffusion method based on CLSI guidelines. The presence of *mecA* genes, conferring resistance to beta-lactam antimicrobials, was detected by PCR and was correlated to phenotypic multidrug-resistance profile.

**Results:** Forty-six *Staphylococcus* spp., 36 from dogs and 10 from cats, were isolated. 76% were *S. pseudintermedius* while the remaining 24%, were represented by other species including *S. aureus*, *S. epidermidis* and *S. hyicus*. The prevalence of *mecA* gene was 35%. Almost all *mecA*-positive strains were also multidrug-resistant, but the diffusion of multidrug-resistant strains was higher, with a prevalence of 47.8%.

The prevalence rates of *mecA* gene and of multidrug-resistance were similar between *S. pseudintermedius* and the other *Staphylococcus* species.

**Conclusion:** *S. pseudintermedius* was the most frequently detected species. Indeed this species often occurs as saprophyte on mucocutaneous surfaces and as opportunistic pathogen of pets. In addition to this species, other zoonotic *Staphylococcus* spp. were isolated with similar resistance features.

Our results confirm that multidrug-resistance is associated with the presence of *mecA* gene, although it is also a common feature of potentially pathogen *mecA*-negative strains that should be monitored.

The presence of some strains resistant to almost all drugs tested raises concern because they could spread in veterinary hospitals and to humans, seriously compromising patient outcomes.
Fecal carriage of extended spectrum beta lactamase-producing *E. coli* and *K. pneumoniae* among street children in Mwanza, Tanzania

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**Purpose:** Increasing prevalence of healthcare-associated infections due to Extended Spectrum Beta Lactamase (ESBL)-producing strains in a tertiary hospital in Mwanza necessitated the investigation of the surrounding community to assess the clonal spread of genotypes and the prevalence.

**Methods & Materials:** Between April and July 2015, 108 street children were enrolled in a study. Demographic and other relevant data were collected. ESBL screening and confirmation were done using MacConkey Agar supplemented with 2µg/ml of cefotaxime and VITEK® 2 system respectively. Molecular characterization of the isolates was done by multilocus sequence typing. Data were analyzed using STATA-13 software.

**Results:** The mean age of the enrolled children was 14.2 ± 3.7 years. Among 108 children, 34 (31.5%, 95% CI; 22.7-40.3) carried ESBL-producing *E. coli* and *K. pneumoniae*. Two children were colonized by both species resulting in a total number of isolates of 36. Of 36 ESBL isolates 30 (83.3%) were *E. coli* and 6 were *K. pneumoniae*, p<0.001. Out of 36 isolates 16 (44%), 25 (69%) and 35 (97%) were found to be non-susceptible to gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole, respectively. Notably, 4 of the 6 *K. pneumoniae* isolated were resistant to tigecycline. Eighteen different *E. coli* sequence types were observed of which ST131 (5/30), ST10 (3/30), ST448 (3/30) and ST617 (3/30) were the most prevalent. ESBL isolation rate was significantly high in children reported to use local herbs (48.9% vs. 21.5%, p=0.003).

**Conclusion:** A high carriage rate of ESBL-producing Enterobacteriaceae was observed in street children which warrants close monitoring and surveillance. Detection of STs 131, 10, 38 and 648 which were previously detected in *E. coli* isolates from patients, companion and domestic farm animals in the same region calls for “One Health” approach to combat Antimicrobial resistance burden.

**19.042**

**Antimicrobial resistant pattern of methicillin-resistant *Staphylococcus aureus* isolated from stray dogs’ nasal swabs to fifteen antimicrobials in Myanmar**


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**Purpose:** The objective was to determine the antimicrobial resistant patterns, MDR, PDR and XDR of MRSA isolates from healthy stray dogs’ nasal swab in Yezin, from public health point of view.

**Methods & Materials:** A total of 65 stray dogs’ nasal swab were collected. Isolation and identification of MRSA, was performed according to Baba et al. (2010). Antimicrobial sensitivity test was carried out by using standardized disk agar-disc diffusion method to 15 antimicrobials, ampicillin (10µg); cephalexin (30µg); cefazolin (30µg); ciprofloxacin(5µg); erythromycin (15µg); gentamicin (10µg); vancomycin (30µg); linezolid (30µg); norfloxacin (10µg); rifampin (5µg); imipenem (10µg); clindamycin(2µg); tetracycline (30µg); trimethoprim (5µg) and teicoplanin (30µg). Antimicrobial resistance pattern of 31 MRSA isolates were determined based on (NCCLS, 2002) and (CLSI, 2012) methods. Using *Staphylococcus aureus* ATCC 25923 strain as quality control. The zone diameter breakpoints were observed according to NCCLS (2002) and CLSI (2012). Antimicrobial resistance levels were determined according EFSA (2015) report. International standard definitions for acquired resistance for S.aureus; antimicrobial categories and agents used to define MDR, XDR and PDR was described as Magiorakos et al. (2012).

**Results:** A total of 31/65 (47.7%) MRSA isolates was identified and resistance between 1 to 13 no. of antimicrobials. The % of MRSA isolates resistance to the number of antimicrobials were 1/15(6.7%); 2/15(13.3%); 3/15(20.0%); 4/15(26.7%); 7/15(46.7%); 8/15(53.3%); 9/15(60.0%); 10/15(66.7%); 11/15(73.3%) and 13/15(86.7%). All MRSA isolates were identified as: extremely high resistant to ampicillin (90.3%) and trimethoprim (80.6%), very high for rifampin (54.8%), high level to clindamycin (48.4%), vancomycin (45.2%); tetracycline (38.7%); linezolid (38.7%); clindamycin (38.7%); cefazolin (38.7%); cefoxitin (35.5%) and erythromycin (32.3%) Moderate level resistance to norfloxacin (19.4%) and ciprofloxacin (19.4%), and least resistance to gentamicin (3.2%) was observed. MDR
isolates 23/31 (74.2%) and not MDR isolates 8/31 (25.8%) while none XDR and PDR isolates was observed.

**Conclusion:** This study indicates that these stray dogs may serve as reservoirs of MRSA, imply that MRSA was present in stray dogs’ population and also represent as a potential public health risks.

19.043 The antibiotic resistance pattern of water polluting cocci around the Danube river mouth
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**Purpose:** Fecal pollution of waters in the Danube Delta was considered to be one of the most important risk factors for resident population, tourists and livestock. We hypothesized that the pollution with fecal cocci/staphylococci will be lower due to the dilution process, but their antibiotic resistance/infectious risk will be higher due to intense tourism at the Danube river-Black sea junction. The research aimed to monitor the bacterial load and antibiotic resistance pattern at the Ciotica channel.

**Methods & Materials:** A number of 42 paired samples of water and sediment were collected in June 2015 from the Ciotica channel (N lat 44.8210° and E long 29.4983°), connecting the Danube river with the Black sea. Bacteriological techniques used TCBS Cholera Medium, MSA manitol salt agar (Oxoid), m Enterococcus Agar (Sigma Aldrich). The antibiotic sensitivity patterns (Kirby Bauer) to marbofloxacine, oxytetracycline, eritromycine, amikacine, ampicilline, enrofloxacin, ciprofloxacine, streptomycine were adapted to CLSI standards. MAR (multiple antibiotic resistance) index for each bacterial genus and strain as well as mean values were calculated and analyzed by Statistica program.

**Results:** The results suggested a heavy bacterial pollution of both water and sediment. A total of 47 strains (21.44.68%; sediment 26, 55.32%) were isolated, namely each Enterococcus spp. and Staphylococcus spp., equally distributed in the water and sediment (47.62% and 52.38%, respectively), while E. faecalis (5 strains) was prevalent in the sediment (80%). There were no significant differences between the sensitivity to antibiotics of enteroccci from water (w) and sediment (s), but although the latter were less resistant to ampicilline (42.86% versus 100%), their MAR index was higher (0.2±0.072 versus 0.156±0.05) indicating the strains originated from high risk sources. The staphylococci were least sensitive to ampicilline (w 66.67%, s 20%), but resistance to eritromycine, enrofloxacin, ciprofloxacine and streptomycine was present, ranging from 16.4 to 40%. The MAR index was the highest for the strains in the sediment (0.33±0.072).

**Conclusion:** The results of the study partially supported our hypothesis, indicating a large numbers of enterococcal and staphylococcal isolates, with an increased antibiotic resistance posing a high health risk in the ecosystem. Supported by PNII-61-2012

19.044 Resistance of nosocomial pathogens in burns unit (Yaroslavl, Russia)
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**Purpose:** In patients in burn units (BU) with infectious complications are often problems with the appointment of antimicrobial agents, including in relation with a higher level of resistance of pathogens. The aim of our study was to evaluate the resistance of the main problematic pathogens in the BU.

**Methods & Materials:** Pathogen isolates were collected from patients hospitalized in BU of Yaroslavl Emergency Clinical Hospital in 2015 year. Susceptibility (S) and resistance (R) of Ps.aeruginosae, Acinetobacter baum., E.coli, Klebsiella pneum. and S.aureus was interpreted to principal antimicrobial drugs according to current EUCAST standard by disk-diffusion.

**Results:** In 2015 was isolated from patients in the BU: S. aureus - 26, Acinetobacter baum. - 9, Ps. aeruginosae - 7, Klebsiella pneum. - 6 and E. coli - 3. The localization of pathogenic material were burn wounds. Of the 26 strains of S.aureus only 7 were MRSA. These strains were susceptible to aminoglycosides, rifampicin and fusidin. R of Ps. aeruginosae was extremely high for all drugs, including carbapenems (R to imipenem and to meropenem - 28.5% and 42.8%, respectively). High activity (R <25%) maintained: ceftazidine, aztreonam, gentamicin (S for all - 100%), amikacin, tobramycin (S both - 14.3%). Acinetobacter baum. showed a higher level of resistance. Activity retained: cefoperazone / sulbactam (R - 0%),
ampicillin / sulbactam (R - 11%), imipenem (R - 22.2%), while the level R to other drugs (cefazidime, meropenem, aztreonam, piperacillin / sulbactam, ciprofloxacin, amikacin, tigecycline) was much higher - 89, 100, 100, 88, 83, 75 and 38% respectively. All strains of *E. coli* were producers beta-lactamases (ESBL (+), AmpC (+)). Pathogens were resistant to carbapenems. At the same time, all strains of *E. coli* were susceptible to cefoperazone / sulbactam and piperacillin / tazobactam.

**Conclusion:** Among pathogens from patients in BU there is no clear predominance of Gram (-) or Gram (+) flora. The highest levels of resistance have been reported in Gram (-) pathogens. The spread of strains resistant to carbapenems is a serious problem for practical public health.

19.045 Prevalence of ESBL-producing *Escherichia coli* from different canine populations in Italy

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**Purpose:** The increased diffusion of antimicrobial-resistant bacteria, especially extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in humans, animals, and their surrounding environments is a severe problem of Public Health. Cephalosporins represent one of the few therapeutic options to treat many bacterial infections, so they are defined as critically important drugs for their broad activity. Since the spread of these bacteria implies different transmission routes and reservoirs including pets, the aim of this study was to provide data on the prevalence and characterization of ESBL-producing *E. coli* from dogs of different origins.

**Methods & Materials:** Faecal samples of 511 dogs (237 from households, 193 from kennels and 81 from public gardens) were plated on MacConkey agar supplemented with cefotaxime and Brilliance-ESBL Agar (Oxoid). Presumptive ESBL-producing *E. coli* were confirmed by combination disk diffusion tests. Susceptibility to the non-beta-lactams was determined by the disk diffusion method according to the CLSI guidelines.

**Results:** Of the 511 samples, 31 (6%) carried an ESBL-producing *E. coli*. Most of the ESBL-producers (22 strains; 71%) were isolated from kennels, followed by 7 (22.6%) from faeces from public gardens and 2 (6.4%) from household dogs. Most of these isolates were also classified as multidrug-resistant, showing resistance to at least three antimicrobial classes. All the ESBL-producing isolates indeed showed resistance to at least one of the quinolones tested and a range of resistance to carbapenems from 22% in samples from kennels to 50% in samples from households animals and public gardens.

**Conclusion:** The presence of commensal ESBL-producing *Escherichia coli* in canine faeces confirms the role of pets as potential homely and environmental sources of resistant bacteria to critically important drugs for humans. The high number of pets, their close relationship with humans and the increased attention to their health poses risks of transmission of antimicrobial-resistant bacteria among dogs and humans. Indeed, the spread of resistance due to the use in pets of drugs for treatment of infectious diseases, including compounds of primary importance in the treatment of human infections, should not be underestimated. Therefore, this study highlights the need of monitoring antimicrobial-resistance also in companion animals to have a complete picture of the dynamics of this phenomenon.

19.046 Prevalence and antimicrobial-resistance characterization of vancomycin resistant enterococci (VRE) strains in healthy household dogs in Italy

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**Purpose:** Enterococci are one of the leading causes of nosocomial infections for humans and the emergence of vancomycin-resistant enterococci (VRE) and high-level aminoglycoside-resistant enterococci (HLRA) caused public health concern because these drugs are often one of the few therapeutic alternatives for treatment of multidrug-resistant (MDR) enterococcal infections. The widespread effect of MDR enterococci is partially driven by the selective pressure produced by the use and overuse of drugs in both human and veterinary medical practice. In recent decades, the analyses of their virulence and antimicrobial-resistance traits in hospital-acquired infections have been extensively reported in humans. However, less attention has been given to the role of pets as reservoir of MDR enterococci, despite their relationship with humans. The aim of this study was to evaluate the prevalence and the antimicrobial-resistance traits of VRE from household dogs.

**Methods & Materials:** Faecal samples of 237 healthy household dogs were tested to detect *Enterococcus* spp. using the classical bacteriological procedure. The species identification was confirmed by PCR assays as previously described. Antimicrobial-resistance to
vancomycin was assessed by plating single colonies on Brilliance VRE Agar (Oxoid). The antimicrobial susceptibility to high-level aminoglycosides and to other compounds (together with the confirmation of vancomycin-resistance), was evaluated by the disk diffusion method based on recommendations of the CLSI.

**Results:** Enterococci were detected in 72% (n=170) 170 of dogs and, in accordance with other studies, the most prevalent *Enterococcus* species were *Enterococcus faecalis* (n=118; 69.4%) and *Enterococcus faecium* (n=50; 29.4%). The 49% of the isolates (n=84) were VRE and 2.4% (n=2) and 21.5% (n=18) of VRE strains showed HLR to gentamycin and streptomycin respectively. Interestingly, most of these isolates were also classified as multidrug-resistant.

**Conclusion:** These results demonstrate that dogs are commonly colonized by antimicrobial-resistant enterococci and highlight the role of pets as reservoir of last-line drug resistances. Moreover, the close contact with humans confirms the possible transmission of antimicrobial-resistant bacteria from dogs to humans. Therefore, the study stresses the need of surveilling this phenomenon also in companion animals.

**Purpose:** The mammalian defensins are considered as the best substitute of antibiotics for their broad-spectrum activity, especially the advantages over the conventional antibiotics against multi-drug resistant bacteria. In our previous study, a recombinant neutrophil peptide-1 (NP-1), a rabbit deprived defensin, was cloned and characterized from a transgenic chlorella ellipsoidea. The goal of present study is to evaluate its antimicrobial activity against drug-resistant bacteria.

**Methods & Materials:** In this study, two clinical strains of multi-drug resistant bacteria were applied, one strain is methicillin-resistant Staphylococcus aureus (MRSA) and the other strain is multi-drug resistant Pseudomonas aeruginosa (MDR-PA). Moreover, MICs and MBCs of NP-1 were detected via broth microdilution method, and colony counting was performed to determine bactericidal dynamics. Skin infection model established with MRSA in rat was established to evaluate the efficacy of NP-1 for anti-MRSA-induced skin infection. Scanning electron microscopic and arginine competition assays were conduct for the mechanism study.

**Results:** The results of broth microdilution method showed that the MIC of mNP-1 against clinically isolated MRSA was 16 µg/ml, and for clinically isolated MDR-PA, the MIC of mNP-1 was 8 µg/ml. Bactericidal kinetics curves indicated rapid and continues bactericidal capability of mNP-1. Topical application of mNP-1 displayed obvious effect on skin-infection caused by MRSA in immunosuppression SD rats. Furthermore, certain concentrations of arginine could reverse the antibacterial effect of mNP-1, which demonstrated the key role of positive charge carried by mNP-1 in combating bacteria. Moreover, bacterial morphological observation via scanning electron microscope indicated different action modes of mNP-1 against MRSA and MDR-PA.

**Conclusion:** Taken together, our data demonstrate that NP-1 is a potential antibacterial agent for treatment of drug-resistant bacteria infection, at least for external application. To our knowledge, this is the first systematic study about the bactericidal activities of chlorella expressed mNP-1 against multi-drug-resistant bacteria.

**Detection of carbapenemase production by multidrug-resistant acinetobacter baumannii isolates from selected wards in dr george mukhari academic hospital**

**Purpose:** Carbapenem-resistant *Acinetobacter baumannii* has emerged as an important cause of nosocomial outbreaks worldwide. Detection of carbapenemase production by modified Hodge test using Mueller Hinton agar has been reported to be less sensitive. The objectives of this study were two-fold: i. to compare the performance of MacConkey agar (MA) and Mueller Hinton agar (MHA) for the detection of carbapenemases; ii. to determine the type of carbapenemases that are produced by *A. baumannii* isolates from Dr George Mukhari Academic Hospital (GDM).

**Methods & Materials:** Twenty four stored carbapenem-resistant and 2 carbapenem-susceptible *A. baumannii* isolates were identified using Vitek 2 automated system (bioMerieux, France). The modified Hodge test (MHT) was done according to CLSI guidelines on MHA (DMP, South Africa) and MA (DMP, South Africa) using *Escherichia coli* ATCC 25922 and meropenem disks. All the plates were incubated at 37°C overnight. Detection of
carbapenem-resistance genes (OXA-23, OXA-40, OXA-58, SIM, VIM-1, VIM-2 AND IMP-like) was done according to published molecular methods.

**Results:** Of the 24 carbapenem-resistant isolates which were screened, 87.5% (21) and 62.5% (15) were found to be carbapenemase positive by MHT on MA and MHA, respectively. The cloverleaf pattern on MHA appears to be absent for some of the isolates but it is accentuated on MA. Both MHA and MA showed 50% false positive result for the 2 susceptible isolates. Of the 24 resistant isolates which were screened for bla genes, 87.5% (21) and 25% (6) were found to have OXA-23 and OXA-40, respectively.

**Conclusion:** MA may be used in place of MHA for the screening of carbapenem-resistant A. baumannii and thus will impact on infection control practices. OXA-23 bla gene was the most prevalent carbapenemase type at DGM Hospital. Though the numbers were small, the study confirms that phenotypic methods lack specificity and sensitivity, hence their results have to be confirmed by molecular methods.

19.049 Antimicrobial resistance of opportunistic microflora in adults with acute infectious diarrhea and approaches to improve its susceptibility to basic antimicrobials

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**Purpose:** To evaluate the status of opportunistic intestinal microflora (OIM) in acute intestinal infections (AII) in adults and effectiveness of aminodimethylaniline sodium (АДФNa) as part of its combined therapy.

**Methods & Materials:** Investigation of OIM in rectal swabs in the dynamics of disease was performed in 2 groups of patients. Each of the groups included 30 patients of both genders aged 18 to 60 years with time from the onset of symptoms not more than 48 hours, fever less than 38.5°C less and without signs of hematocolitis. 1st group received common standard therapy. АДФNa was administered to patients of the 2nd group in addition to standard therapy. ADFNa is Russian immunomodulator that can influence cellular and humoral immunity, in particular, increase the functional activity of macrophages, the synthesis of interferons and lysozyme and regulate the balance of pro- and anti-inflammatory cytokines. Qualitative and quantitative composition of OIM, as well as its sensitivity to various antibacterial drugs were estimated.

**Results:** Microflora released from all patients were only OIM that in 68-90% had resistance to the main classes of antimicrobials: penicillins (93.3±4.5% in both groups), macrolides 90±5.4% (in both groups) fluorhinolones (83.3±6.8% in 1st group and 86.6±6.2% - in 2nd group), cephalosporins (93.3±4.5% in 1st group and 83.3±6.8% in 2nd group), aminoglycosides (73.3±8.1% and 80±7.3%, accordingly) despite the fact that patients were not treated with antibiotics. Administration of АДФNa in addition to the standard treatment regimen led to reduce the duration of clinical manifestations of disease, recovery of normal quantity of OIM and more than two times reduced antimicrobial resistance to penicillins and cephalosporins I, II and III generation.

**Conclusion:** The results of the study allow to recommend АДФNa in the combined therapy of the AII. The administration of АДФNa also can be reasonable during antibiotic therapy with penicillins or cephalosporins for patients with any other infectious diseases.

19.050 Establishing phenotypic susceptibility testing of influenza virus to oseltamivir as a laboratory diagnostic method

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**Purpose:** To establish phenotypic susceptibility testing of influenza virus to Oseltamivir as a laboratory diagnostic method.

**Methods & Materials:** Reference panels of neuraminidase inhibitor (Oseltamivir) - sensitive and -resistant viruses were cultured in MDCK cells. Culture supernatant was stored at -80°C in single-use aliquots of 0.2 mL. Susceptibility to Oseltamivir was determined by NA-Star (Applied Biosystems), a chemiluminescent enzyme inhibition assay. The assay was performed at final drug concentrations ranging from 0 to 1000 nM. Chemiluminescent signals were collected using Tecan Infinite M200 PRO reader and the IC50 was calculated by using Magellan Software V7.2. The acceptable IC50 range for each reference strain was determined through 10 repetitive testing. Reference strains must be included with each run of assay and must fall within the established range in order for an assay to be valid. A total of five
A(H1N1)pdm09 virus isolates which were previously examined for the neuraminidase H275Y substitution by RT-PCR/RFLP method developed by CDC, USA and confirmed by sequencing were tested using the established assay. The criteria set by WHO to define the antiviral susceptibility of viruses based on the fold change of their IC_{50} value compared to reference IC_{50} values in reporting surveillance data were used. Influenza A viruses that have <10-fold, 10-100-fold or >100-fold increase in IC_{50} are interpreted as influenza A viruses showing normal, reduced or highly reduced inhibition, respectively.

Results: The acceptable ranges of IC_{50} for Oseltamivir–sensitive and –resistant reference strains were 0.18 – 0.31 nM and 60.27 – 101.11 nM, respectively. Three isolates that had shown no H275Y substitution had <10-fold increase in IC_{50}. Of the other two isolates that consisted of mixed populations of wild and oseltamivir-resistant genotypes, one had 10-20-fold increase in IC_{50} while the other had >100-fold increase. The results obtained by the phenotypic susceptibility testing were consistent with the genotypic susceptibility testing.

Conclusion: Phenotypic susceptibility testing of influenza virus to oseltamivir as a potential laboratory diagnostic method was established in the study. Furthermore, the established assay, when used to analyse previously genotyped isolates, yielded consistent results and shows promise for diagnostic use in the laboratory.

19.051 Kinetics of Candida tropicalis with antifungal medicinal Plant with reference to synergism

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Purpose: Fungal infections are most commonly caused by Candida species. Candida tropicalis has been popular since last decades as a pathogen for Candidiasis, because of its leading prevalence throughout the world and in South East Asia. This unicellular yeast and dimorphic fungi able for the invasion in host cells & responsible for the potential cause of multiple infections from simple mucocutaneous to life threatening diseases, specifically in immunocompromised patients. Antifungal susceptibility of Candida is limited. Species of genus Candida generate broad resistance against available antifungal drugs. This resistance creates complications in the treatment of candidiasis. So now a day’s researcher all over the world believing on natural herbal products for the treatment of Candidiasis because they have antibacterial and antifungal activity and also has minimum side effects.

Methods & Materials: In this concerned study we have observed growth kinetics of resistant strains of C.tropicalis with reference to one of the available antifungal and find out their percent inhibition. Ethanolic extract of fruit of Oak Manna tree was prepared with the initial concentration of 10 mg/ml. After six hours of exposure % inhibition was calculated and compared with fluconazole. Antifungal activity with fluconazole has been observed with some modification with the method provided by NCCLS Micro dilution M27-P guide line.

Results: Average percent inhibition of different strains of Candida tropicalis by used medicinal plant was found to be 26.16% at initial concentration and with fluconazole MIC 90 was observed at 15.36µg/ml and MIC 100% was at 122µg/ml. The result showed that oak manna fruit has some therapeutic efficacy in comparison with the fluconazole.

Conclusion: Natural antifungals could be the therapeutic agent for the treatment of Candidiasis. These have most affectivity against bacteria and fungi with no side effects.

19.052 Monitoring of antibiotic therapy in Acute Respiratory Infections (ARIs) complicated with community-acquired pneumonia in children

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Purpose: To increase the efficiency of antibacterial therapy of ARIs through the use of antibacterial card that allows to carry out the monitoring of antibiotics used in the prehospital phase

Methods & Materials: A retrospective analysis of case histories of patients with a clinical diagnosis of ARIs complicated with community-acquired pneumonia in children. Age of children ranged from one month to five years. Patients were treated in viral diseases branch of municipal children's infectious diseases hospital in Astana. Patient’s data of antibacterial records were analyzed in outpatient stage in order to monitor antibiotic therapy. We developed an antibiotic map which includes the data on antibiotics obtained before entering the hospital for the last 3 months, indicating the dose and duration of outpatient treatment, as well as antibiotics that the patient received in the hospital, the dose and duration. This card was put into practice in the hospital from 2009. This allows us to track the quantitative and
qualitative characterization of antibacterial therapy in hospital. Statistical data calculation was performed using Excel 2010 software and SPSS Statistics 20.0. **Results:** We analyzed 395 cases patients in the viral disease branch with a clinical diagnosis of ARIs complicated with community-acquired pneumonia. The average age of studied patients was 20.48 ± 13.95 months. The antibacterial card was revealed that 57 (14.4%) patients out of 395 got antibiotics before hospitalization. At the time of admission, the period from the beginning of illness averaged 3.2±2.1 days. Among the antibiotics the most commonly used were semi-synthetic aminopenicillin (40.3%), inhibitor protected semi-synthetic aminopenicillin (17.5%), macrolides (15.6%) 2-generation cephalosporins (12.3%), 3rd-generation cephalosporins (8.7%). During the appointment of antibacterial therapy, physician will base on the obtained data in order to avoid irrational use of antibiotics. **Conclusion:** The use of antibacterial cards, with the aim of monitoring used antibiotics in the hospital, allowed to improve the quality of the causal treatment, to reduce significantly the time of the patient's stay in hospital and to reduce drug polypharmacy.

19.053 Bacterial load occurrence and antibiogram of salmonella species from cattle carcasses and the processing environment in abuja abattoirs nigeria

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**Purpose:** To: Assess the level of bacterial contamination on cattle carcasses and the slaughter environment Isolate Salmonella species and determine the resistance profiles of the isolates Confirm the presence of invA gene in the Salmonella isolates

**Methods & Materials:** A cross sectional study was employed in which a total of 422 samples comprising carcass swabs, rectal swabs, floor swabs, hide swabs, knife swabs, hand swabs and water were investigated at Kubwa, Karu and Gwagwalada abattoirs in Abuja, Nigeria. Bacterial load was enumerated while using the spread plate technique while Salmonella was detected via culture. Suspect Salmonella isolates were identified using conventional biochemical methods and Microbact™12E kit. Biochemically positive isolates were confirmed using Salmonella polyvalent A-S antisera and further screened for the presence of invA virulence gene using PCR.

**Results:** Mean and SD of TVCs in the entire study was 5.09±0.57 cfu/cm², 7.50±0.61 cfu/swab, 7.43±0.35 cfu/cm², 7.26±0.45 cfu/cm², 5.22±0.61 cfu/cm², 5.04±0.51 cfu/cm² and 4.47±0.59 cfu/ml from carcass swabs, rectal swabs, floor swabs, hide swabs, knife swabs, hand swabs and water respectively. The overall mean log carcass TVC was higher than the 5.0 cfu/cm² limit of the EC. A Welch ANOVA and a Kruskal-Wallis H test showed that there was no statistically significant difference (p> 0.05) in the TVCs from carcasses, rectum, hides, knives and hands across the abattoirs. Salmonella was isolated from 11 (2.6%) of the total 422 samples of which 1 (1.6%) carcass swab, 1 (1.6%) rectal swab, 4 (6.3%) floor swabs, 3 (4.8%) hide swabs, 1 (1.6%) hand swab and 1 (1.6%) water were positive. 100% of the Salmonella isolates were found to harbour the invA gene and exhibited multidrug resistance most commonly to ampicillin, erythromycin and amoxicillin/clavulanic acid.

**Conclusion:** Cattle carcasses were generally contaminated beyond the acceptable limit with even higher contamination recorded from most of the processing materials. Multi-drug resistant Salmonella were isolated from carcass, rectum, floor, hide, hands and water. Beef from abattoirs in Abuja, Nigeria, therefore have the potential to transmit multi-drug resistant Salmonella species to consumers.

19.054 Pefloxacin as a surrogate marker to determine susceptibility to quinolones drugs in Salmonella enterica Serovar Typhi: Structural and molecular analysis

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**Purpose:** Quinolones remain the mainstay for enteric fever treatment since past four decades after the emergence of MDR strains of S. Typhi. CLSI recommends the pefloxacin susceptibility as a surrogate marker for ciprofloxacin, ofloxacin and levofloxacin susceptibility for treatment of enteric fever susceptibility. We analyzed the sensitivity and specificity of pefloxacin as a surrogate marker for all quinolones by using susceptibility analysis with current CLSI guidelines, detection of molecular mechanisms of resistance and drug target interactions by molecular docking.

**Methods & Materials:** Antibiotic susceptibility to ciprofloxacin, ofloxacin and levofloxacin were determined in 321 isolates of S. Typhi as per CLSI guidelines. The correlational analysis was done for pefloxacin zone of inhibition and MIC of ciprofloxacin, ofloxacin and
levofloxacin. Temporal analysis was done by Chi-Square test for trend using SPSS ver 17.0. DNA sequencing was done for quinolone resistance determining region (QRDR) of gyrA, gyrB, parC and parE genes of target enzymes and mutations were compared to pefloxacin susceptibility. Drug target interaction was studies by molecular docking. Homology model of DNA gyrase-DNA complex was determined using templates 1AB4 and 2XCT in prime modeling software. Molecular docking of ciprofloxacin, ofloxacin, levofloxacin and pefloxacin drug molecules was done. Binding mode and docking score were determined for each drug.

**Results:** Pefloxacin susceptibility and resistance showed a predictive value of 0.99 and 1 respectively. Most common mutations were Ser83 and Asp87 of gyrA gene. No mutation was found in QRDR of pefloxacin susceptible strains. Docking confirmation was same for all quinolones studied. The binding was stabilized by stacking interaction of quinolone ring and aromatic base pairs of DNA and carboxylate group interacts with the ser83 residue of GyrA subunit. Docking score for ciprofloxacin, ofloxacin, levofloxacin and pefloxacin were -11.666, -11.349, -11.348 and -8.269 respectively.

**Conclusion:** The mode of binding and conformation of pefloxacin is same as ciprofloxacin, levofloxacin and ofloxacin but binding affinity is less than all these three antibiotics as confirmed by docking score. It is concordant with the CLSI criteria that an isolate susceptible with pefloxacin is automatically susceptible to all fluoroquinolones, hence it is a good surrogate marker for fluoroquinolones susceptibility.

19.055 Effect of sub-lethal concentration of vancomycin on biofilm formation by *Staphylococcus* species  
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**Purpose:** Staphylococcus is responsible for many community- and hospital-acquired infection in different sites of human body. Drug resistant Staphylococci such as Methicillin resistant Staphylococcus (MRSA) and vancomycin resistant Staphylococcus aureus (VISA) has become common concern. The drug resistance increases 10 to 1000 folds when there is production of biofilm. This study was aimed to assess the level of biofilm production without and on exposure to sub-lethal concentration of vancomycin against the clinical isolates of Staphylococcus.

**Methods & Materials:** A total 103 pure growth of staphylococci isolated at Department of Microbiology, Tribhuvan University Teaching Hospital (TUTH), Kathmandu, was included in the study. Of them 25 were MSSA, 25 MRSA, 23 MS-CONS and 30 MR-CONS. All isolates were subjected for determination of MIC of vancomycin by following the standard method describe by CLSI and for biofilm detection by method describe by Christensen et al and modified by Stepanovic et al, 2007.

**Results:** Among 103 isolates of Staphylococcus species, many (97.1%) were found to have MIC of vancomycin within susceptible range, while few (2.9%) were found to have MIC of intermediate range. But, still many isolates shows the MIC value of upper limit of sensitive region of vancomycin, i.e. 24.0% of Staphylococcus aureus and 45.2% of CONS. This shows the increasing resistant pattern of Staphylococci towards vancomycin. Among Staphylococci isolates, 63.1% were producing different degree of biofilm with CONS sharing the larger percentage. Sub-lethal concentration of vancomycin has significantly (p<0.05) induced biofilm formation in both MRSA and MSSA; however, inducing/enhancing effect seems to be higher in MRSA isolates. On the other hand, in cases of CONS, sub-lethal concentration of vancomycin could not show significant induced effect (p>0.05)

**Conclusion:** This study has concluded that the MIC value of clinical isolates of Staphylococcus is increasing suggesting MIC creep of vancomycin and the sub-lethal dose of Vancomycin was found to induce biofilm production, thus increasing the biofilm forming capacity of staphylococci species. These phenomenon can further reduce the treatment success rate of vancomycin. Therefore, result may suggest the need the MIC determination prior to therapy and proper dosing of vancomycin should be done.

19.056 Risk assessment of the exposure of people to methicillin-resistant *Staphylococcus aureus* (MRSA) from dogs  
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**Purpose:** The purpose of this study was to assess the risk of exposure of people to MRSA from dogs, via direct and indirect pathways, with special emphasis on companion animal veterinarians. This scenario was used as an example of cross-species spread of antimicrobial resistance genes.
Methods & Materials: We conducted a risk assessment to quantify the probability of exposure of people, specifically veterinarians to MRSA. Risk pathways included both direct and indirect (e.g. environment) transmission. A 24h-time period was considered to estimate the probability of potential exposure. Input data was collected through a literature review. When information was not available, expert opinion was considered. Palisade decision tools (@Risk 7.0.1 Pro edition) were used to construct the risk simulation model. The outcome was the probability of exposure within the defined time window. Repeated exposure and exposure load were not considered. Sensitivity analysis was performed to quantify the relative significance of each of the model input parameters.

Results: The risk of exposure to MRSA from dogs appears to be low, for individuals with no occupational exposure (2.4x10^-8) and one order of magnitude higher for companion animal veterinarians. Most of the available literature focused on reporting cases where similar isolates of MRSA were found in dogs and humans, but there are important data gaps for parameters including exposure load. It is therefore currently not possible to reliably quantify the significance of the different transmission pathways or even to rank them.

Conclusion: No significant exposure to MRSA and therefore no indication of related adverse health effects from dog ownership or occupational exposure of small animal veterinarians were identified. However, we only considered a 24h-time period of potential exposure. In a scenario of prolonged colonization of an animal the repeated daily contact will result in cumulative exposure. Also, exposure does not necessarily mean risk. More work is needed to understand the health consequences of exposure to resistance genes, particularly MRSA.

19.057 Population-based surveillance of antibiotic dispensing in Alberta, Canada

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Purpose: Overuse or misuse of antibiotics can lead to antibiotic resistance, leading to an inability to effectively treat bacterial infections. Our purpose was to establish public health surveillance related to the of antibiotics in using a population-based data from the Alberta Pharmaceutical Information Network (PIN).

Methods & Materials: The province of Alberta (population = 4 202 617), Canada maintains a publicly funded, universally available health care system. As part of managing the system, the Ministry of Health maintains a number of population-based administrative health databases that are all linkable.

Data were extracted from the Alberta Pharmaceutical Information Network (PIN) database for 2015. Over 97% of community pharmacies in Alberta contribute data to PIN on prescriptions dispensed. The data include a personal health number (unique identifier), birth date, gender, pharmacy location, drug identification number (DIN), Anatomical Therapeutic Chemical classification (ATC) code, dispense date, quantity, dosage, and other administrative information.

All events under the ATC rubric J01 were extracted. Using the full ATC code, the data were organized into 22 groupings. Population data were extracted from the Alberta Health Care Insurance Plan Central Stakeholder Registry which contains a record for all residents of the province. Age-specific dispensing rates, by antibiotic class, were computed.

Results: In 2015, 2.49 million prescriptions were filled in community pharmacies. Overall, 30.2% of the population (n=1 270 504) filled one or more prescriptions for an antibiotic. The most commonly prescribed antibiotics included beta-lactam antibacterials (penicillin), macrolides, and quinolones, with 16%, 9.2%, and 5.2% of the population filling a prescription, respectively. 34.2% of children under 5 filled at least one prescription. Among these, 26.5% received a beta-lactam and 9.2% a macroglide. Among those over 70 years of age, 15.2% received a beta-lactam and 13.6% a quinolone. Use of fluoroquinolones increased with age.

Conclusion: Antibiotic use is common with 30.2% of the population in Alberta filling at least one prescription. The use of antibiotics varied by class and patient age. Further review of the high use of fluoroquinolones in older populations is required to determine the appropriateness of use and opportunities to improve prescribing behaviours.

19.058 Uropathogens and antimicrobial susceptibility patterns in urinary tract infections diagnosed in the primary care setting in Singapore

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**Purpose:** Urinary tract infection (UTI) is a frequent condition encountered in primary care. Treatment is usually empirical without urine culture or susceptibility testing because the causative uropathogens and their antimicrobial susceptibility profiles are considered to be predictable. However, there is increasing evidence of antimicrobial resistance in community-acquired uropathogens. This cross-sectional study aims to describe the distribution of uropathogens and their antibiotic susceptibilities amongst patients who were diagnosed with UTI in primary care clinics in Singapore.

**Methods & Materials:** Patients with UTI diagnoses (by their primary care physicians) were recruited from two public sector primary care clinics under the SingHealth Polyclinics in Singapore. Upon enrolment, data on diagnosis, symptoms, risk factors for antimicrobial resistance and antibiotics prescribed for this UTI episode were collected. Each patient provided a clean-catch mid-stream sample for urine culture at the routine diagnostic laboratory of Changi General Hospital in Singapore. A positive urine culture was defined as isolation of ≥10³ colony forming units (cfu)/ml of primary urinary pathogens (*Escherichia coli, Klebsiella spp. or Staphylococcus saprophyticus*) in pure culture, or isolation of ≥10⁵ cfu/ml of these organisms as predominant growth. The significance cut-off for culture of other potential urinary pathogens were ≥10³ cfu/ml. Susceptibility to antibiotics commonly used for UTI was assessed using disc diffusion methods using standard cut-offs. Descriptive statistics on the prevalence of different uropathogens and their antibiotic susceptibilities are presented, with Pearson’s chi-square test used to assess differences between subgroups.

**Results:** Of 380 urine samples collected, 177 (46.2%) yielded positive cultures. There were no significant associations between the urine cultures result and ethnicity or age group. *E. coli* (n=135, 76.3%) and *Klebsiella* spp (n=14, 7.9%) were the two most common pathogens isolated, for all gender and age groups. The *E. coli* strains demonstrated high nonsusceptibility to Amoxicillin (46.7%), Cephalexin (60.7%), Ciprofloxacin (30.4%), Trimethoprim(29.6%) and Trimethoprim-sulphamethoxazole (28.9%), while the *Klebsiella* spp strains showed 42.9% non-susceptibility to Nitrofurantoin. Susceptibility to Augmentin and Fosfomycin remain high (at least 90%) for both *E. Coli* and *Klebsiella* spp.

**Conclusion:** While oral Amoxicillin, Cephalexin, Ciprofloxacin, Trimethoprim-sulphamethoxazole and Nitrofurantoin are commonly used for UTI treatment, increasing community-acquired resistance to these antibiotics in Singapore may complicate the efficacy of empirical treatment.

19.059 Canine leishmaniosis: Serological comparison of a commercial rapid test with a quantitative enzyme-linked immunosorbent assay test for detection of anti-Leishmania infantum antibodies

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**Purpose:** The aim of this study was to evaluate a serological immunochromatographic test for the detection of anti*-Leishmania infantum* canine antibodies.

**Methods & Materials:** The rapid test was compared to a reference quantitative serological technique: ELISA in-house (Enzyme-Linked Immunosorbent Assay). This quantitative ELISA in-house was used to define the sera as positive or negative. One hundred canine serum samples were evaluated, 41 were considered negative and 59 were considered seropositive with different levels of anti-*Leishmania* antibodies: low levels (n=7), medium levels (n=28) and high levels (n= 24). Based on these results, the FASTest® LEISH (Diagnostik Megacor, Austria) was evaluated: sensitivity, specificity, positive predictive value, negative predictive value, Kappa index and accuracy were calculated.

**Results:** The sensitivity and specificity were 0.97 and 0.98, respectively. In an endemic area to *Leishmania infantum* infection with a low seroprevalence (10%), the positive predictive value was 0.82 and the negative predictive value was 1.00. By contrast, for a high seroprevalence area (25%), the positive predictive value was 0.93 and the negative predictive value was 0.99. For the Kappa index, FASTest® LEISH obtained 0.95 and the accuracy for this qualitative test was 0.97.

**Conclusion:** The study showed that FASTest® LEISH is a reliable diagnostic test that complies with all requirements for a sensitive (relative sensitivity 97%) and specific (relative specificity 98%) rapid test. Compared to other commercial tests the FASTest® LEISH is a commercial rapid test with high sensitivity and specificity for early detection infected dog with a reasonable cost-benefit balance.
Scenario-based assessment of decontamination methodology for biosafety and biosecurity applications

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Purpose: In laboratory biosafety and in public biosecurity, there is the requirement to call upon effective methods to decontaminate occupied spaces following a biological release. Not all methods will be suitable for a specific environment or against a specific bioagent. Consequently it is important to use realistic scenarios in performance evaluation.

Methods & Materials: A range of decontamination methods, including fumigants, spray delivery of disinfectants and physical methods, have been tested using either non-hazardous surrogate biological agents or, under contained conditions, high hazard infectious agents. A controlled environment test chamber was used for initial proof-of-principle evaluations, followed by realistic scenarios to mimic laboratories, offices, domestic rooms, railway carriages and outdoor environments. Decontamination of personal protective suits worn by emergency service workers was also evaluated. Biological test agents were immobilised onto test coupons of material surfaces typical in those environments, or delivered into enclosed spaces as bioaerosols. Log kill achieved by decontamination methods was measured against unexposed controls.

Results: Results achieved are a combination of effectiveness and practicality. In laboratory biosafety, room fumigation with complete kill is achievable but some chemical agents are less effective than others against some infectious agents, and can be compromised in some circumstances such as large volumes of spilled biological material. In the wider environment, disinfectant fogging devices can be effective when combined with a systematic clean-up strategy to return the affected area to normal use.

Conclusion: The cost and health and safety implications of dealing with an accidental or deliberate biological release are significant. No single decontamination method is universally effective. Therefore it is important to develop a portfolio of methods from which the one most suited to an environment can be selected. Validation of methods using representative scenarios is vital in achieving this.

Imported melioidosis cases increasing in Portugal

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Purpose: Melioidosis, which is caused by the bacterium Burkholderia pseudomallei (B. Pseudomallei), is a potentially fatal tropical infection, little known outside its main endemic zone of Southeast Asia and northern Australia. Sporadic cases have occurred in other locations, such as in South America, particularly north-eastern Brazil. and evidence suggests that the epidemiology of this disease is changing. B. pseudomallei share some characteristics of other biological agents with potential to be used as weapons in bioterrorism events. However, the principal threat from melioidosis is a result of naturally occurring events.

Methods & Materials: Practical problems with definitive laboratory diagnosis, antibiotic treatment and the current lack of a vaccine, underline the need for prevention through exposure avoidance and other health measures. It's likely that the increasing population burden of the tropical zone and extraction of resources from the humid tropics will increase the prevalence of melioidosis. Climate change-driven extreme weather events will increase the prevalence of infection and gradually extend its main endemic zone. Its clinical features are so many, varied and non-specific that there is a risk that an infected patient may go undiagnosed. A further difficulty in reaching an accurate diagnosis of confirmed melioidosis is that it requires an unusual level of diagnostic laboratory expertise. This is lacking even in many developed countries where the infection is not endemic, and in remote parts of countries where the disease is known.

Results: The Portuguese National Institute of Health through the Emergency Response and Biopreparedness Unit (UREB) is the national reference laboratory for diagnosis of highly pathogenic microorganisms, namely bacteria, virus and toxins, which are considered bioterrorism agents. This Unit has skilled professionals, know-how, BSL-3 facilities, capacity to work 24h/7d and trained human resources to increase lab capacity in emergency situations.
In the last five years, UREB identified two imported cases of *B. pseudomallei* in Portugal, one from Thailand and one from Brazil. Both cases came as suspicious for laboratory diagnosis, having no relationship with deliberate events.

**Conclusion:** Considering that the signs of melioidosis are nonspecific (resembling tuberculosis), these cases reinforces the need for an appropriate screening for the disease etiology in people travelling from endemic areas.

19.062 Improving Biosecurity in Pakistan: Report from an IATA Guidelines Training for Transportation of Biological Agents

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**Purpose:** Every day thousands of hazardous samples are transported from one destination to another through aircraft which if uncontrolled could pose severe threat to the passenger, crew or the recipient of the shipment. The International Air Transport Association (IATA) Dangerous Goods Regulations (DGR) is the most reliable source to help prepare the samples for transportation. It ensures safety and health of shipper, carrier, public and environment. Periodic training and certification in a laboratory setting is mandatory for personnel shipping and receiving dangerous goods. Unfortunately in Pakistan this is one of the least addressed issues and individuals working in a laboratory are generally unaware of existing guidelines, despite remote locations of reference level laboratories and an emergent need to ship infectious patient samples to these laboratories.

**Methods & Materials:** In an effort to create awareness of IATA regulations for transportation of dangerous goods, two workshops were organized in Karachi, Pakistan sponsored by Fogarty Global Infectious Disease Research Training Program, National Institute of Health. This was an unprecedented activity as very few IATA training events were held in the past with no published reports. To assess baseline knowledge and improvement in knowledge among participants, we developed a standard questionnaire derived from the basic elements of IATA DGR and administered as pre- and post-test.

**Results:** Two full-day workshops were conducted in Karachi in April, 2016. Each workshop was attended by 20 technically qualified participants of which 57% were females and 43% were males associated with medical, biological and veterinary laboratories from Karachi. Pre-test questionnaire revealed extremely poor knowledge concerning the IATA guidelines as only 30% of participants were able to answer >80% of the questions correctly, while post-test questionnaire showed an overwhelming response as 78% of participants answered > 80% questions correctly. Over 90% of participants expressed that the training was relevant and useful.

**Conclusion:** Given the lack of adequate baseline knowledge of IATA regulations for infectious substances, we conclude that IATA trainings in Pakistan are an unmet gap in laboratory training of professionals. We recommend that a government-led, nationally-funded regulatory body be established to ensure regular training of the laboratory personnel in Pakistan.

19.063 An outbreak of scrub typhus in nepal following the 2015 Gorkha earthquake

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**Purpose:** To describe the epidemiology and clinical features of 23 cases of scrub typhus seen in a tertiary infectious diseases hospital following the massive April 2015 earthquake in Nepal.

**Methods & Materials:** In addition to routine clinical examinations and laboratory tests, all patients with undifferentiated fever admitted to the Sukraraj Tropical and Infectious Disease Hospital (STIDH) in Kathmandu, Nepal between August to October 2015 had serum tested by ELISA for IgM antibodies against *Orientia tsutsugamushi*.

**Results:** Scrub typhus cases surged in Nepal in late 2015 following the Gorkha earthquake. The vast majority of 23 cases diagnosed at STIDH were individuals rendered homeless in the most devastated districts, and living in temporary shelters with a high reported incidence of mouse infestation. Fever and anorexia occurred in 100%, and chills or rigors, myalgia or arthralgia, headache, or nausea occurred in >75%. Physical findings of eschar formation, red eye, or lymphadenopathy were noted in <1/3 of these patients. The majority of patients had significant thrombocytopenia and transaminitis. Although all patients with undifferentiated fever had been empirically started on ceftriaxone, azithromycin and doxycycline were the most effective treatments once the diagnosis of scrub typhus was made. All of the patients treated at STIDH recovered without disease complications or adverse drug effects.
Conclusion: Major causes of undifferentiated fever in Nepal include malaria, dengue, enteric fever, brucellosis, chikungunya, leptospirosis, and scrub typhus. We observed a singular spike in cases of scrub typhus following the 2015 Gorkha earthquake, which we attribute to large-scale human habitat destruction and population dislocation, leading to increased contact with rodents infested with the trombiculid mite vectors of O. tsutsugamushi. Only a minority of patients present with characteristic physical findings, so the diagnosis of scrub typhus can only be established by specific IgM assay. With appropriate diagnosis and treatment, clinical outcomes from scrub typhus can be good, even in resource-limited environments.

19.064 Naegleria fowleri an old organism become new emergence in Karachi, Pakistan
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Purpose: Primary Ameobic Meningoencephalitis (PAM) become an emergence infection in Karachi Pakistan due to steep rise temperature upto 40 degrees Celsius or higher. So conventional epidemiologic investigation is required to overcome Naegleria fowleri the causative agent of PAM cases. Naegleria fowleri a thermophilic amoeba found in environment, cause Primary Ameobic Meningoencephalitis (PAM) in human. Naegleria fowleri invades via contaminated water through nose in brain. There are 133 PAM infections have been recorded from 1962 to 2014 worldwide. The fatality rate is over 98%. In Pakistan around 80 to 100 deaths occur in Karachi alone since 2008, 12 people died in 2012. Approx 14 Naegleria fowleri related deaths were reported in 2014. The total number of deaths due to the “brain-eating amoeba”, Naegleria fowleri leapt to 11 on june 2015. To date.

Methods & Materials: A short study was conducted during summer in the year 2014 - 2015 to isolate Naegleria fowleri. Thirty water samples were collected from different sites of Karachi including swimming pools and stagnant water. Motile amoeba was identified by wet mount microscopy. For growth of Naegleria fowleri sample was added on NNA seeded with heat killed suspension of E.coli. The plates were incubated at 45°C selected for thermophilic amoeba. Further the flagellated forms of amoeba were confirmed by enflagelation test. Qualitative chlorination test of water samples also done.

Results: The motile amoeba (20%) was identified by wet mount microscopy. Among 30 samples 18 (60%) were positive for plaque formation when grown on NNA agar plate. Positive enflagelation test indicate the presence of thermophilic Naegleria fowleri.

Conclusion: As the number of PAM cases were increasing in Karachi due to thermophilic amoeba, the swimming pools must be chlorinated with exact quantity of chlorine in order to protect public from deadly infection.

19.065 Trends in re-emergence of FMD in bovines in varying Geo-climatic conditions in Pakistan
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Purpose: Foot and mouth disease outbreaks and its endemic nature in livestock had always remained a major problem in Pakistan. The present study was aimed to estimate the association between re-emergence of FMD and Geo-ecological variations in selected rural villages of Khyber Pakhtunkhwa province.

Methods & Materials: In a cross sectional survey total of 2511 sera samples were collected from cattle and buffaloes respectively over a period of 1 year “between” July 2013- June 2014 from three different zones based on variation in geo-ecological conditions. Using 3ABC ELISA serum samples were tested identifying antibodies against FMD serotypes.

Results: From a total of 2511 sera, 9.83% (n=247) tested FMD positive. The highly prevailing serotype recorded was Asia-1. Overall true prevalence calculated was 7.42%. The highest prevalence was observed in sub humid zone (13.69%), followed by humid (8.95%) and semi arid zone (3.26%) (P<0.05). Seasonal pattern of FMD was also identified, showing significantly (p<0.05) higher prevalence in monsoon followed by summer and post monsoon.

Conclusion: It was concluded that several factors i.e. farming system, unchecked livestock movements (during religious festivals and trading of livestock from Punjab to KPK) and change in meteorological factors play a vital role in the prevalence of FMD. Based on this pertinent prevention measures are required to reduce future outbreaks considering local climatic conditions in different geographical conditions.
Purpose: Human impacts on the global environment suggest that we have created a new epoch in Earth history, the "Anthropocene", although its starting date is widely debated. We review the Anthropocene debate in order to explore its utility from an integrated One Health perspective for understanding emerging infectious diseases and to enhance effective communication with the public.

Methods & Materials: We examine the major definitions of "the Anthropocene" since the term was first used in 2000. The date initially proposed for the onset of the Anthropocene is the late 1700s with the start of the Industrial Revolution. Some historical scientists have placed the onset of the Anthropocene at the Pleistocene-Holocene boundary ca 11,000-9,000 years ago, when humans worldwide began to develop agriculture and domesticate plants and animals. The Anthropocene Working Group of the Geological Society of London, however, recently recommended a starting date of 1945 CE (the first atomic bomb detonation, coincident with the beginning of the Great Acceleration era). Using these three proposed dates, we examine how the Anthropocene may correlate with major changes in the emergence and spread of emerging diseases over time.

Results: For the events and processes that define these three periods (from the late 1700s, from 11,000-9,000 BP, and from 1945 CE), we assess their relevance from an integrated One Health perspective for cross-species spillover (e.g. deforestation and livestock farming), human-to-human transmission (e.g., population growth and urbanization), "pan-epidemic" spread (e.g., globalization, plane travel), and control (e.g., sanitation and vaccination) of emerging diseases. We find that competing definitions of the Anthropocene encapsulate different but equally important factors significant in human-pathogen interactions and historical trends in disease emergence.

Conclusion: The Anthropocene is a useful concept for the international infectious diseases and integrated One Health community that could facilitate global efforts to educate, prepare, and involve the public in understanding emerging infectious diseases past, present, and future.

Purpose: To evaluate the association between environmental features and changes in the incidence of VL across time in the neighbourhoods of Teresina, Brazil.

Methods & Materials: This is a mixed ecologic study in which the units of analysis are the 102 neighbourhoods of Teresina in the years of 1996 to 2009. Outcome was defined as quintiles of the distribution of empirical Bayes smoothed visceral leishmaniasis (VL) incidence rates for each year and neighborhood. Primary predictors were environmental variables derived from remote sensing imagery (Landsat TM) from the years of 1995, 1997, 2000, 2003, 2005 and 2007. Environmental variables include changes in landcover/landuse classes across years and the normalized vegetation index (NDVI). Since there are repeated measures of the incidence of VL by neighborhoods we employed multilevel ordinal logistic regression models to determine the association between environmental variables and the incidence of VL across years. Associations between variables were expressed as odds ratios and respective 95% confidence intervals (95%CI).

Results: From 1995 to 2007, around 15% of the city’s area covered by vegetation was substituted by residential and commercial structures, with some neighbourhoods experiencing this type of change in more than 50% of their area. The chance of a neighbourhood be at a higher quintile of VL incidence increased by 19% (OR=1.19, 95%CI: 1.06-1.34) for each increase in 0.10 in the mean value of the NDVI and by 24% (OR=1.24, 95%CI: 1.04-1.48) for every 10% of the area covered by vegetation in 1995 that changed to residential and commercial structures by 2007. Even after considering these two variables, about 17% of the variability of the ordinal responses across years could be attributed to variation among neighbourhoods.

Conclusion: Along the years of the study, the city of Teresina experienced significant environmental changes with a substantial increase in urban features. Both the levels of vegetation cover, as measured by the NDVI, and urbanisation, measured by the replacement of vegetation by residential and commercial structures, were important factors in defining
neighbourhoods at higher risk of VL. These variables might be used to predict high-risk areas for guiding interventions against the disease.

19.068 Epidemiology of crimean-congo hemorrhagic fever (CCHF) in Pakistan, 2012-2015

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Purpose: In Pakistan, since the first fatal case in 1976, sporadic cases of CCHF have continued to occur. This study provides descriptive epidemiology of the suspected cases referred to National Institute of Health Islamabad for diagnostic purposes.

Methods & Materials: Descriptive analysis of CCHF cases referred to NIH from 2012-15 was conducted. Samples were confirmed through ELISA and RT-PCR techniques. Standard WHO case definition for suspected and confirmed cases were used. Data from all suspected cases were collected on standard case reporting form and analyzed by using SPSS.

Results: A total of 929 CCHF suspected cases were referred to NIH. Out of which 254 were laboratory confirmed. Among confirmed cases 204 (80%) were male. 45 deaths were reported with 18% CFR. 213 (84%) cases were reported between the months of April to October. Balochistan remained most affected province with 136 cases (53.5%) followed by Punjab, KPK and Sindh 41 (16%), 33 (13%) and 28 (11%) cases respectively. CFR of Balochistan, Punjab, KPK & Sindh was 19 (14%), 7 (17%), 8 (24%) and 10 (36%) respectively. The median age was 32 years, ranging from 8 to 80 years. Most affected age group was 21-40 years (n=118, 46.5%) followed by 41-60 years of age group (n=70, 28%). High risk occupations included animal handler 89 (35%), butcher 47 (18%), tannery worker 36 (14%), Housewives 35 (14%), labor 28 (11%), students 11 (4%) and drivers 8 (3%). Animal and animal tissue contact history was reported by 122 (48%) cases, contact with positive CCHF case was reported by 17 (7%) and travel history to endemic areas was reported by 27 (10%). Clinical presentation of the cases include fever 254 (100%), body aches 229 (90%), skin spots 94 (37%), nose bleeding 94 (37%), blood in mouth 36 (14%), blood in stool 30 (12%) and gum bleeding 25 (10%). Ribavirin was taken by 76% of cases.

Conclusion: Animal/meat handlers and healthcare providers were found to be more at risk of developing CCHF. Prevention and controls measures targeting “at risk” occupational groups need to be in place. Surveillance system for the vector-borne diseases should be strengthened for early detection and timely response.

19.069 Avian influenza risk from live bird markets to local poultry and the impact on local poultry farmers livelihood

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Purpose: Over a billion people living in abject poverty in the developing world keep local poultry (LP) which was identified as a tool for poverty alleviation. This paper assessed qualitatively, risk of HPAI transmission from live bird markets (LBM) to local poultry and impact on farmers’ livelihood in Nigeria.

Methods & Materials: OIE risk assessment framework was used with categorical scale used in assessing probabilities. Data to assess likelihood of occurrence of each pathway steps were obtained from literature.

Results: Identified release and exposure pathways were live birds, fowl sellers, manure, scavengers, consumers and transporters. Most release pathways were associated with high risk of occurrence were live birds, fowl sellers, manure, consumers and transporters. The release risk estimates for scavengers was medium. Most risk estimates were associated with medium level of uncertainty, due to few documented evidence except for live birds and fowl sellers estimated with low uncertainty. The risk of infection of local poultry through exposure to HPAI from LBMs was assessed as medium via scavengers and consumers; high via live birds and transporters; very high via manure and fowl sellers. The overall risk estimate for the transmission of HPAI from LBMs to local poultry is medium to high with medium level of uncertainty. Pathways associated with highest risk of transmission are live birds, manure, fowl sellers and transporters (high risk), followed by scavengers and consumers (medium risk). Purchasing of local poultry breeders from LBMs increases chances of local poultry exposure to HPAI.

Conclusion: High level of uncertainty for most pathways indicates limited or non available data. Since most poor and landless people own local poultry, HPAI outbreak in local poultry will result in income losses thereby worsening their poverty. This would adversely affect efforts to reduce poverty and food insecurity in Nigeria while posing a threat to farmers’
health. Local poultry are at high risk of being infected with HPAI virus from LBMAs with adverse impact on their livelihood and health.

19.070 Strengthening One Health epidemiological surveillance in Armenia with EIDSS

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Purpose: A number of zoonotic diseases is registered in Armenia: Brucellosis (200-400 human cases annually, 60% higher than the EU average), Salmonellosis, Echinococcosis, Anthrax, etc. The sources of infection are mainly dairy products or professional activities. The epi-surveillance of these diseases needs strengthening and further integration of human epidemiological and veterinary services within the One-Health and risk management concepts.

Methods & Materials: The infectious diseases epi-surveillance system that includes 170 human and 86 veterinary cases was analyzed. There are 25,000 cases including 500 zoonosis registered annually. All epi-surveillance data is collected and transferred on paper. Assisted by the DTRA, the Ministries of Health and Agriculture started deployment of an open source Electronic Integrated Disease Surveillance System (EIDSS) to integrate epidemiological, veterinary and vector surveillance with laboratory component and joint data analysis capabilities.

Results: Active EIDSS deployment began in 2015 at the national and regional ("marz") level first priority sites: 124 workstations deployed at 71 branches; 296 professionals trained; EIDSS configured to the surveillance priorities. The full EIDSS implementation is planned to include the district level by 2016-2017 (additional 48% of workstations and 45% of employees).

EIDSS provides capabilities for epidemiologists and veterinarians to collect Urgent Notifications at the district level, and then send them in an electronic form to the regional level government institutions for response actions and joint analysis. Laboratory research is monitored in joint database at the Ministries’ Central Facility in Yerevan. It is planned that notifications from the primary medical institutions will also be received electronically through integration with IHISA (Integrated Health Information System of Armenia) Electronic Medical Record system upon its implementation in 2016-2017. The Veterinary Service also plans to implement the EIDSS-based new procedures of notification on current situation in the real-time mode.

Conclusion: EIDSS implementation strengthens the epidemiological surveillance providing the tools and best practices for human, veterinary, and vector data joint analysis, rapid data collection and transmission, and simplifying risk management. Potential integration and data exchange using EIDSS will support regional surveillance and planning of disease control actions. Within the One-Health integrated approach, EIDSS will become an important element for all reportable diseases surveillance, including zoonosis.

19.071 Coagulase positive staphylococcal colonization of HIV-infected and healthy humans and their household pets

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Purpose: Persons with HIV infections appear to be at increased risk for colonization and infection with coagulase positive staphylococci (CPS) especially caused by Staphylococcus aureus. Animals may be contaminated, colonized or infected with CPS and also may act as a potential reservoir of the pathogen for humans. The objective of the study was to test the possibility of colonization and interspecies transmission of CPS in HIV-infected and healthy humans groups and their household pets.

Methods & Materials: The study was conducted in the years 2015-2016. There were collected samples from HIV-infected and healthy humans. Swabs from each person were taken from: nostrils, skin behind the ear, flexion of the elbow and pharynx. Swabs from animals were taken from nares, conjunctival sacs, skin and anus. Staphylococcal isolates were identified as species of CPS using coagulase tube test and BLAST analysis from the 16S RNA PCR product. Specific PCR primers of the meca genes were used to determine methicillin-resistant strains.

Results: There were isolated only three CPS species: S. aureus, S. pseudintermedius and S. schleiferi subsp. coagulans from humans (n=39) as well as from animals (n=23). 47.8% of HIV-infected people were colonized with one or more CPS strain while in healthy people group there were 25% colonized humans. There were no statistically significant difference
between groups. We observed that the same staphylococcal species were isolated from the owners and their pets. The most frequently isolated species of staphylococci was S. aureus in humans as well as in pets. Much higher percentage of healthy people (18.75%) were colonized with methicillin-resistant Staphylococcus aureus (MRSA) than in the group of HIV-infected people (8.7%). Methicillin-resistant staphylococci were isolated from pets kept in households where humans were confirmed as carriers of MRSA.

Conclusion: HIV-infection was not a significant risk for colonization with S. aureus or other CPS species. The recognition of similar CPS species in humans and animals has shown their role as potential reservoir or vectors for human CPS infection in the community.

19.072  Timeliness for visceral leishmaniasis detection in Albania
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Methods & Materials: A cross-sectional study analysing Tirana university hospital centre cases was conducted between April to November 2015.

Results: All cases that underwent treatment in Tiran university hospital were confirmed by microscopic identification of bone marrow aspiration and serologic diagnosis in some cases. Only 34.85% of the cases had serologic diagnosis where 10.1% in negative microscopy cases, 11.2% in positive microscopy cases and 13.5% had only serology. 93% of serologic diagnosis has been performed in private laboratories even when IPH has offered the diagnosis. The mean time from diseases onset to hospital admission was 22.6 ± 36.1 days ranging from one day to one year. Most of the cases have been admitted to the hospital within first 6-30 days of disease onset and in 28.5% of them within 10 days. The timing is age related and is increased in older adults so there is positive significant correlation of admission time and patient age (rho =0.2, 95%CI=0.1-0.3, p<0.01). The mean time from admission to laboratory diagnosis and diagnosis confirmation is 1.9 ± 4.1 days. The shortest time is within a day but also in very few cases there were delays within 25 days from the admission. The treatment has started after the diagnosis was confirmed. 80% of cases with a delay in clinical diagnosis were from different districts outside the capital.

Conclusion: The recent analysis shows the differences of diagnostic capacities in different areas in Albania and the lack of implementation of diagnostic algorithm protocols. The late detection time contributed to late response and late treatment and an increased morbidity and mortality in the country.

19.073  Addressing bovine tuberculosis in smallholder periurban dairy farms of India: A qualitative study
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Purpose: The emergence of periurban fringes, with lax regulatory, quality-control, and infrastructural frameworks, has led to increasing vulnerabilities at the human-animal-environment interface, especially in intensive, industry-style smallholder units, functioning on razor thin profit margins. This study was undertaken to understand the health system and farm level factors that influenced the transmission of bovine tuberculosis (bTB).

Methods & Materials: The study was conducted in the periurban belts of Ludhiana, Guwahati and Bangalore. A stakeholder mapping was done to identify the key actors in the dairy farming enterprise. In-depth interviews were conducted based on thematic guides developed through an intense review of literature and improved through pretesting. Data collection was continued till the attainment of saturation across key themes. Open coding of responses preceded axial coding to establish relationships between categories. Selective coding to identify core themes was followed by etiological enquiry and generation of a conceptual model.

Results: Veterinarians were consulted as a final effort after home-remedies and quacks had failed. Damage control measures, like selling or abandoning animals, in a setting with limited screening and surveillance, adds to the risk of disease transmission. Although civic authorities believe in the adequacy of a functioning laboratory network, end users are aggrieved at the lack of available, accessible and affordable services. Despite the presence of extension services, knowledge and awareness of the disease is limited in the community, promoting risky behavior. The absence of cogent policies and predominance of cultural
beliefs in dealing with bovine tuberculosis are significant barriers. Overall, despite glaring vulnerabilities, bTB is not considered to be a major problem by all stakeholders.

Conclusion: Across the board, the stakeholders did not recognize bTB as a major concern. However, given the lack of epidemiological data on the disease, and inadequate laboratory network to support surveillance, the real situation remains unknown. With multiple factors contributing to the vulnerabilities, both at the individual (farm) and the systems level, it is possible that the problem is underestimated. The current study helps to identify glaring lacunae which need to be addressed through systems and policy research, and interventions to build community awareness.

19.074 First serological evidence of West Nile virus in horses and dogs from Corsica Island, France

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Purpose: West Nile virus (WNV) is widely distributed over the world, including Europe, Africa, Asia and spread over the last two decades to North and South America. In the South of France, sporadic cases are frequently described and two epidemics occurred in Camargue in 2010 and 2015. Corsica Island is a French island in the Mediterranean Sea close to the South-East French coast, Sardinia and the West Italian coast. To date, no circulation of West Nile Virus (WNV) has been described. The aim of the present study was to identify a possible WNV circulation in Corsica in horses and dogs as sentinel animals for the virus surveillance.

Methods & Materials: In 2014, 386 blood samples were collected from 219 sheep, 96 horses and 71 dogs, in 12 localities in Corsica, in the oriental coast of Corsica. Each sample was systematically tested for WNV immunoglobulin G (IgG) using an in-house enzyme-linked immunosorbent assay (ELISA) with inactivated WNV as antigen. Due to antigenic cross-reactivity among flaviviruses, all positive samples were confirmed as true positive by serum neutralization test.

Results: All the sheep sera were negative for the detection of WNV antibodies by ELISA. While, 9 horses over 96 (9.4%) and 6 dogs over 71 (8.4%) presented WNV antibodies. All the positive samples from horses and dogs were confirmed by seroneutralization. Globally, 50% of the WNV positive dogs and 44.4% of the WNV positive horses were native from Corsica, and consequently have been infected in this island.

Conclusion: Migratory birds could have brought the virus to the island during a stopover in Corsica. Although no symptomatic case has been reported to date, this study highlights the necessity to improve WNV surveillance in animals and humans, as well as in blood donors in Corsica.

19.075 A survey on Trypanosoma cruzi infection of dogs in French Guiana

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Purpose: Clinical cases of Chagas disease, an infection caused by the parasite Trypanosoma cruzi, have been recently described in humans and dogs in French Guiana, a French overseas department located in South America. Elsewhere in endemic countries for this disease, cases of asymptomatic infections are described. In this study we carried on a prevalence survey of the infection in dogs in Cayenne and Kourou, the main cities of French Guiana.

Methods & Materials: In 2014 and 2016, blood samples were taken from 153 dogs of Cayenne and Kourou. All the dogs were apparently healthy at the time of sampling. Sex and age of the dogs were recorded as well as the location where they lived. A rapid immunochromatographic test (Chagas Stat-Pak®Assay, Chembio, USA) was used on the sera of the dogs to detect anti-T. cruzi antibody. A T. cruzi kDNA real-time PCR was also performed on the blood samples of the dogs (EDTA anticlotting); the cutoff for positive samples was set at 35 Ct.

Results: Eight dogs (5.2%) were positive in serology and three (1.9%) in qPCR. One dog was positive for both tests. The rate of infection (positivity for one of the two tests) is 6.5% (10/153). There is no significant difference (X2 test) between Cayenne (5/100) and Kourou (5/53), between males (3/60) and females (7/93) and between 2014 (2/55) and 2016 (8/98).
Conclusion: Canine surveillance is a useful tool for public health risk assessment of Chagas disease. Positive dogs, even asymptomatic, should be treated, as they can serve as a reservoir for the parasite.

19.076 Wild birds as carriers of antimicrobial-resistant and ESBL-producing Enterobacteriaceae

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Purpose: Extended-spectrum β-lactamases (ESBLs) has been studied widely in Escherichia coli and Salmonella spp. from humans and livestock in Europe. In contrast, information on this phenomenon in wild animals is poor, although the close interaction between wildlife, other animals and human-influenced habitats potentially allows bacterial exchange. Moreover, migratory behaviour of wild birds make these animals potential carriers of antimicrobial-resistant bacteria from human-influenced environments over long distances. Thus, the aim of this work was to provide data on the prevalence and characterization of ESBL-producing E. coli and Salmonella spp. from wild birds in Northern-Italy.

Methods & Materials: One hundred-three cloacal samples from wild birds (belonging to the Accipitridae, Anatidae, Strigidae, Apodidae, Corvidae, Falco Linneaeus and 1 Trichoglossus haematodus. The Salmonella spp. isolate was not ESBL-producing. A considerable number of strains, including 3 of the 4 ESBL-producing strains, were also classified as multidrug-resistant, showing resistance especially to aminoglycosides, sulfonamides and tetracycline. All the isolates were susceptible to the quinolones tested and the unique Salmonella spp. strain was susceptible to all the compounds tested.

Conclusion: The presence of antimicrobial-resistant and ESBL-producing Escherichia coli in wild birds highlight the potential roles of these animals as source of resistant bacteria to critically important drugs. The migratory behaviour of some of these animals, together with the close contact with urban and peri-urban areas, suggests that wild birds can contribute to the global dissemination of resistance among different ecosystems.

19.077 Epidemiology of hydatid disease in Markazi province of Iran (2011-2015)

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Purpose: Hydatid disease or Echinococcosis is a zoonotic disease caused by the larva of Echinococcus species. The disease has a worldwide distribution. This study was carried out with the aim of describing the epidemiology of Cystic Echinococcosis in central province of Iran.

Methods & Materials: This is a descriptive cross-sectional study performed in patients with hydatid cysts. Information about age, gender, number of cysts, organ involvement, morbidity and mortality were collected from medical records of hydatid patients in the Arak University of Medical Sciences from 2011 to 2015.

Results: A total of 84 patients were included in the study period. Mean age of patients was 23.6±5.1 years. We observed that the rural populations are particularly at risk (65.5%). Females were the dominant sex affected by the disease (55.9 %). Of the total study patients, 72 (85.7%) had single organ involvement and 12 (14.3%) had more than one organ involved. Liver was involved in 44 patients (52.4%) either solitarily or in association with other organs. Lung involvement was seen in 43 patients (51.2%) either solitarily or in association with other organs and 9 patients had involvement of brain, kidney, heart, spleen and uterus. One of the patients died due to complications of the disease.

Conclusion: Prevalence rate of hydatidosis in Iran was reported to be 0.6-2 in 100000 populations. In our study the prevalence rate of hydatidosis in central province was 1.15 in 100000 populations. Hydatid of the liver and lungs were more common and the highest rate of infection and complications were in patients of 20-30 years age.
Epidemiology of anthrax in Markazi province of Iran (2011-2015)

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Purpose: Anthrax is a zoonosis produced by Bacillus anthracis, and as a human infection is endemic in several areas in the world. More than 95% of the reported naturally acquired infections are cutaneous, and approximately 5% of them can progress to meningoencephalitis.

Methods & Materials: In a descriptive cross-sectional study we review the clinical and epidemiological characteristics of the patients with definitive diagnosis of cutaneous anthrax evaluated from 2011 to 2015 in Markazi Province of Iran.

Results: Fourteen patients were included. Nine (64.3%) patients were men and mean age of patients was 25±4.2 years. The most common of the patients were farmer (64.3%). The source of infection was Contact with livestock and animal products in 13 (92.9%) patients. All the patients had ulcerative lesions, with a central necrosis. Four patients (28.6%) had several lesions and the lesions mainly located in the upper limbs (85.7%). One patients (7.1%) developed septicemia, and all patients recovered.

Conclusion: Prevalence rate of anthrax in Iran was reported to be 0.06-2.25 in 100000 populations. In our study the prevalence rate of anthrax in central province was 0.2 in 100000 populations. By considering its clinical and epidemiological characteristics, cutaneous anthrax must be included in the differential diagnosis of skin ulcers.

Seroprevalence and risk factors for equine arboviral infections (Eastern Equine Encephalomyelitis, Western Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis and West Nile Fever) in Costa Rica

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Purpose: To gain a better understanding of the epidemiological situation of arboviral infections in horses in Costa Rica, a national IgG seroprevalence study was performed in 2013. In Costa Rica, equine arboviral infections are caused by Alphaviruses such as Eastern equine encephalomyelitis virus (EEV), Western equine encephalomyelitis virus (WEV) and Venezuelan equine encephalomyelitis virus (VEV), as well as a Flavivirus: West Nile Virus (WNV).

This study was carried out in response to a number of lethal disease outbreaks in horses, which were primarily caused by VEV, but also WNV and EEV. Arboviruses, which are transmitted to horses and humans through blood-sucking mosquitoes, may cause pyrexia, neurological disease and even death in both animals and humans.

Methods & Materials: The representative sample size with respect to the national horse population was calculated to be 196, assuming a total population of 140,000, using a precision of 7%, a confidence level of 95% and an expected seroprevalence of 50%. In the absence of horse distribution data, a convenience sampling approach was required, collecting serum from 3 horses per canton.

Samples were evaluated for IgG at a single dilution of 1:100. Alphaviruses were assessed by indirect ELISA, antibodies to WNV were determined using a capture monoclonal, recombinant protein. Risk factors such as mosquito density, housing system and location for these arbovirus infections were analysed.

Results: A total of 241 horse serum samples from 80 cantons were collected and analyzed. The highest seroprevalence was determined for EEV (61%), followed by WNV (58%), VEV (with 43%) and WEV (16%). Detected risk factors were primarily related to the location (province), but also management (housing system).

Conclusion: The present study was the first nationwide seroprevalence study of equine arboviruses in Costa Rica. The high seroprevalence of IgG to these viruses demonstrates that horses are highly frequently exposed to these pathogens. However, we have not yet been able to distinguish individual responses from cross-reactions to these or other viruses of the genus, which might be resolved by serum neutralisation test. Nevertheless, this is an important finding which may have consequences for both veterinary and human public health authorities in the country.
Aortitis due to non-typhoidal *Salmonella* in a young immunocompetent man

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**Purpose:** Non-typhoidal *Salmonella* (NTS), which is a gram-negative anaerobic bacteria, tend to produce self-limiting gastroenteritis. It affects 1,200,000 people annually with 450 deaths in the United States. Invasive forms of NTS are infrequently observed in immunosuppressed patients, such as those infected with HIV. Herein, we describe a very rare case of *Salmonella* bacteremia leading to abdominal aortitis in a young immunocompetent man.

**Methods & Materials:** A 54Y Man, with a history of coronary artery disease, hypertension and prostate hypertrophy, presented with two weeks of fevers, chills and weakness associated with myalgias and arthralgias. Of note, the patient worked closely with animals such as horses and was recently exposed to diarrheal stools of a sick horse. On examination, he was febrile and tachycardiac. White blood count (6,500/uL) was normal but liver enzyme tests were mildly elevated (AP154, AST 82, ALT 60). Chest radiograph was unremarkable. Intravenous broad-spectrum antibiotics were initiated. Urinalysis and urine culture was negative but blood cultures revealed *Group D Salmonella*, prompting further imaging of the abdomen. CT scan of the abdomen showed mild inflammation of the infrarenal abdominal aorta. Intravenous ceftriaxone was prescribed for six weeks and a follow up CT scan. HIV serology was negative.

**Results:** NTS typically results in a self-limiting diarrheal illness. A small proportion of hosts go on to develop bacteremia and extra intestinal focal infections. The majority of these hosts have immunodeficiencies or predisposing risk factors such as HIV or malignancy. Cardiovascular salmonellosis is a very rare extra-intestinal manifestation, which can present as endocarditis, myopericarditis or endovascular infection. Fever (100%), heart murmurs (57%), and heart failure (71.4%) are commonly associated with NTS endovascular infections, but preceding gastrointestinal symptoms are uncommon. *Salmonella* aortitis is a rare disorder associated with a high mortality. In a study conducted by Chen et al., risk factors associated with endovascular complications in those with *Salmonella* bacteremia included male sex, history of coronary artery disease, hypertension and serogroup C1 infections.

**Conclusion:** Among patients with multiple risk factors and invasive NTS, it is imperative to maintain a high degree of suspicion for cardiovascular salmonellosis as early diagnosis and treatment is the key to reducing mortality.

Prevalence and predictors of type 2 diabetes among newly diagnosed and sputum positive tuberculosis patients in Western Cameroon

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**Purpose:** There is a growing awareness on a global scale on the possible relationship between tuberculosis [TB] and diabetes mellitus [DM]. Cameroon is a country with a high burden of TB and an increasing incidence of DM. We determined the prevalence of type 2 diabetes and associated risk factors among newly diagnosed sputum positive pulmonary TB patients.

**Methods & Materials:** This study was carried out in two referral TB management clinics in Cameroon. Trained clinicians carried out clinical screening of 222 newly diagnosed pulmonary TB patients between November 2014 and July, 2015. Laboratory analyses included screening of DM based on pre-therapy fasting blood glucose test using the One Touch Ultra glucometer and the oral glucose tolerance test conducted according to the W.H.O guidelines. A questionnaire captured known risk factors for diabetes among all participants. Risk factors for diabetes were analysed in a binary logistic model in which not being diabetic was considered the reference condition. The results presented as odds ratios were used to assess association between occurrence of diabetes and potential risk factors. A p-value < 0.05 was considered statistically significant.

**Results:** Of the 222 patients who accepted to participate in the study, 9.4% [21/222] were diabetic, 15.32% [34/222] had impaired glucose tolerance whereas 32.43% [72/222] had a family history of diabetes. Among the 18 diabetics, 17 were determined to be type 2 diabetics while one had type 1 diabetes. Three of them [16.67%] were known DM patients and had a poor glycaemic control [defined as RBS level > 200 mg/dl] despite being on glucose lowering therapy. We noted a threefold increase in the risk of diabetes among unmarried TB patients.
and a 32% increase in the risk of diabetes for every unit increase in the body mass index. Patients who were infected with HIV had a four-fold risk of being diabetic in our analysis.

**Conclusion:** The prevalence of DM among newly diagnosed sputum positive pulmonary TB patients in West and North West Cameroon was found to be 9.4%. The principal risk factors associated with the DM among newly diagnosed TB patients were increased BMI, being unmarried and being HIV infected.

19.082 Detection of *human rhinovirus* in cerebrospinal fluid of children with viral encephalitis

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**Purpose:** To investigate the relationship between the viral encephalitis in children and *human rhinovirus*(HRV). The application value of GenomeLab Gene Expression Profiler(GeXP) analyzer in detection of HRV in cerebrospinal fluid (CSF)of children with viral encephalitis.

**Methods & Materials:** We collected 140 CSF specimens from children with convulsions and fever, who were admitted to the pediatric intensive care unit (PICU) of Second Affiliated Hospital of Shantou University Medical College between January 2012 and December 2012. The CSF specimens were tested for common respiratory viruses including HRV by using GeXP genetic analysis system. Viruses including *Adenovirus*, *Respiratory syncytial virus*, *human Rhinoviruses*, *WU Polyomavirus*, *human Bocavirus*, *Influenza virus A, B, C type*, *Parainfluenza virus type 1,2,3,4*, *human Metapneumovirus*, *human Coronavirus*-NL63/229E and HKU1/OC43. The HRV positive specimens were typed by nested RT-PCR, and the positive PCR products were sequenced. The resultant DNA sequences were aligned with the reference HRV sequence in GenBank.

**Results:** Totally 6 HRV positive specimens were detected by GeXP genetic analysis system. Among them 4 specimens were HRV-C genotypes showing 95.8%~98.3% identity with reference sequences. The others were one HRV-A and one HRV-B genotype which show 96.0% and 99.0% identity with reference sequences respectively. The clinical manifestations of HRV central nervous system infection could febrile convulsion convulsions with mild gastroenteritis (CwG).

**Conclusion:** HRV was one of the pathogen of viral encephalitis in children probable. All types of HRV can cause central nervous system infection, among which HRV-C accounts for the majority. GeXP genetic analysis system could be used as a rapid and high throughput detection tool for viral encephalitis etiology research.

19.083 Pilot survey on brown rats as possible reservoirs of Q Fever and Leptospirosis in cattle

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**Purpose:** Q fever is a widespread zoonosis caused by *Coxiella burnetii* (*C. burnetii*). Previous studies suggested a possible epidemiological role of synanthropic rodents in the spread and maintenance of the infection among ruminants. On the other hand, genotyping studies are showing species-specific associations of *Coxiella* strains to animal species, in particular to bovine. As no data are available on direct transmission of *Coxiella* strains from rats to ruminants, the first goal of this study was to investigate the presence of *C. burnetii* in brown rats captured in dairy farms' surroundings. The characterization of rat's strains may confirm or exclude their epidemiological role in cattle infection. Brown rats are also well known reservoirs of Leptospirosis, mainly for the serovar icterohaemorrhagiae, but their role in bovine Leptospirosis is little investigated.

**Methods & Materials:** Since January 2016, 48 brown rats were conferred to the laboratory for necropsy analysis. Real-time PCR for *Coxiella burnetii* (targeting the IS1111 insertion) and *Leptospira* spp (targeting the genetic fragment rrs, 16S) were respectively performed on spleens and kidneys taken from 48 brown rats; the sampling is still ongoing. Coxiiela positive samples were submitted to MLVA and SNP analysis to attempt genotyping of bacterial DNAs and compare them to the strains already present in international available data bases, including the ones circulating in Italian ruminants farms. Leptospiira positive samples were
submitted to MLST and MLVA analysis to identify genospecies and, possibly, their respective serogroups and serovars.

**Results:** So far, diagnostic molecular investigations for *Coxiella burnetii* and *Leptospira* spp. have been respectively performed on spleens and kidneys taken from 48 brown rats. Four out of fifty spleens resulted positive for *Coxiella*, three of them unfortunately with Ct greater than 32. The fourth positive sample had a Ct value of 22 and it has been already partially typed with MLVA method. Ten out of 48 kidneys tested positive to *Leptospira* spp. and MLST and MLVA analyses are currently underway.

**Conclusion:** Brown rats are confirmed to be susceptible to *Coxiella burnetii* and *Leptospira* spp. infection, but the genotyping results will clarify their epidemiological role as reservoirs for cattle population.

19.084    Studying the role of diallyl sulfide in ameliorating ethanol induced adipose tissue injury

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**Purpose:** Alcohol consumption has been in existence in the world for many centuries and it is the major cause of death and injury worldwide. Alcoholic liver disease (ALD) is caused due to excess and chronic alcohol intake. Studies across the globe have identified several pathways leading to ALD. Adipose tissue which has been considered as an energystorage organ is also found to play a major role in ALD progression by secreting hormones and cytokines known as adipokines or adipocytokines. Ethanol affects the metabolic and innate immune activities of adipose tissue contributing to alcohol-induced injury of the tissues. The main objective of the present work is to understand the action of DAS in regulating ethanol induced adipose tissue injury.

**Methods & Materials:** To understand the effect of ethanol and DAS on adipose tissue function differentiated 3T3L1 cell line, isolated human primary adipocytes, and male Wistar rats were used in the study. Effect of DAS in ameliorating ethanol induced oxidative stress, ER stress, up-regulation in pro-inflammatory cytokines production, down-regulation in anti-inflammatory cytokines expression, and changes in the adipose mass and adipocyte morphology were studied. Further the role of DAS in macrophage switching was also studied.

**Results:** The cells exhibited decreased viability with increased dose and time of exposure of DAS. DAS was found to effectively reduce the ethanol induced oxidative and ER stress in adipocytes and adipose tissue. Further DAS restored the adipocyte morphology and adipose tissue mass that was altered by ethanol consumption in male Wistar rats. A down regulation in the production of pro-inflammatory cytokines and an up-regulation in the expression of anti-inflammatory cytokines were observed in the alcohol exposed cells and rats treated with DAS when compared to only ethanol treated cells and rodents. DAS treatment also caused macrophage switching from M1 or Classical phenotype to M2 or Alternative phenotype. Taken together, the results suggest that DAS normalized ethanol induced tissue injury in the adipose tissue.

**Conclusion:** The present study helps in understanding the role of DAS in reducing the injury caused to adipose tissue on ethanol consumption. The study results infer that DAS can be used in drug formulations to treat ethanol induced tissue injury.

19.085    Potential zoonotic pathogens in bat species in South Africa

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**Purpose:** Bats host a suite of pathogens and with several of these, the route of transmission to humans and other animals is unknown. Factors such as the availability of food resources, age, reproductive status, movement behaviour and population density have all been factors which effects the risk of infections within host populations. In addition to opportunistic short term surveillance, this study included longitudinal surveillance and the possibility of seasonal variation of pathogen prevalence. It also focus on pathogen presence in excretions to determine possible route and the risk of transmission.

**Methods & Materials:** In 2004 we initiated surveillance in bats with the initial objective to identify rabies–related lyssaviruses but this has since expanded to include several other pathogens. We used nucleic acid detection techniques to detect partial pathogen genomes (RT-PCR or PCR) and virus neutralization assays to test for presence of antibodies.
Furthermore, excretions (saliva, feaces and urine) from insectivorous bats were evaluated to determine the pathogen composition by using next generation sequencing techniques.

**Results:** We reported ten new isolations of the rabies related viruses, Lagos bat (LBV) and Duvenhage virus. We indicated that frugivorous bats (*Epomophorus wahlbergii* and *Rousettus aegyptiacus*) maintain a high level (30–67%) of seropositivity against LBV and *Nycteris thebaica* and *Miniopterus natalensis* tested seropositive for Duvenhage virus, implicating these species as possible reservoir hosts. We reported the first detection of *corona*, paramyxo and filoviruses in South African bats as well as the first reports of *Bartonella* and *Rickettsia*. Longitudinal studies, specifically focused on cave dwelling fruit bats and measuring the presence of LBV antibodies over time, indicated cyclic fluctuation of antibodies with marked increases shortly after the parturition period. Metagenomic studies on excrections of insectivorous bats identified possible zoonotic pathogens such as *Leptospira*, *Rickettsia*, *Bartonella*, *Coxiella*, *Adeno-*, *Herpes-*, *Picorna-*, and *Coronaviridae*.

**Conclusion:** Serological evidence obtained can be used to infer periods of increased virus circulation. These caves are used for traditional activities and information can be used to identify high risk periods for entry. We identified several pathogens that are being shed into the environment, potentially infecting other species. The collective data can be used to inform prevention and control strategies.

19.086 Tick-borne zoonotic bacteria in fallow deer (*Dama dama*) in Euganean Hills Regional Park of Italy

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**Purpose:** In the last decades an increasing incidence of tick-borne zoonoses (TBZ) has been reported in Europe. This trend regards in particular pathogens transmitted by *Ixodes ricinus*, which is the main vector of several viral, bacterial, and protozoan zoonotic infections. This tick species feeds on a broad range of mammals, birds and reptiles and frequently bites human. The aim of the study was to investigate the prevalence of zoonotic bacteria (*Borrelia burgdorferi* sensu lato, *Rickettsia* spp., *Anaplasma* spp. and *Ehrlichia* spp.) transmitted by ticks in a population of fallow deer (*Dama dama*) in the tourist area of Euganean Hills Regional Park, Veneto Region, Italy.

**Methods & Materials:** The study was conducted over a 13 months period from March 2014 to March 2015. The presence of *B. burgdorferi* s.l., *Rickettsia* spp., *Anaplasma* spp. and *Ehrlichia* spp. was evaluated by PCR and real time PCR assays on 61 blood samples of fallow deer culled during the population control campaigns by the park operators. PCR assays were targeted to *groEL* (*B. burgdorferi* s.l.), *gltA* (*Rickettsia* spp.) and 16S rRNA (*Anaplasma* spp. and *Ehrlichia* spp.) genes. Amplicons were purified and directly sequenced. The nucleotide sequences were analyzed using the basic local alignment search tool (BLAST).

**Results:** Eighteen samples (29.5%) were positive for *A. phagocytophilum* and one (1.6%) for *Ehrlichia* spp. BLAST analyses showed sequence identity ranging from 97% to 100% with those already present in the database. None of fallow deer tested carried *B. burgdorferi* and *Rickettsia* spp.

**Conclusion:** The results demonstrate the presence of zoonotic tick-borne pathogens in the studied area. Although the most dangerous pathogen, *B. burgdorferi*, was not detected, our results underline the risk of human recreational or professional exposure to infections during the outdoor activity or manipulation of hunted animals. Furthermore, the presence of deer populations in peri-urban areas promotes the diffusion of ticks and increases the TBZ of humans and domestic animals.

19.087 PREDICT-2: Bridging the gap between wildlife and human disease in Lao PDR

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**Purpose:** PREDICT-2, the second phase of the highly successful project of the Emerging Pandemic Threats program funded by the United States Agency for International Development and implemented by a consortium led by the University of California at Davis and including EcoHealth Alliance, Metabiota, the Wildlife Conservation Society and Smithsonian Institution, represents a major advancement in the surveillance of zoonotic
disease in regions with high risk of viral sharing between animals and humans. Building on an existing framework of animal disease surveillance, PREDICT-2 is introducing human disease and behaviour monitoring to better understand the factors involved in zoonotic disease spread. The purpose of this work is to present the case of human surveillance in Lao PDR as a representation of the larger PREDICT-2 program.

Methods & Materials: PREDICT-2 is partnering with local hospitals and clinics in Lao PDR to identify individuals who exhibit symptoms of disease of unknown origin. Patients with syndromes ranging across fever of unknown origin, fever with hemorrhage, fever with rash, fever with diarrhea, and encephalitis will be invited to participate in the PREDICT-2 program. Enrolled patients will provide biological samples and complete detailed behavioural questionnaires related to their health, travel history, and exposure to animals. Biological samples will be tested by specifically-designed consensus PCR protocols for filo-, paramyxo-, corona-, flavi-, and influenza viruses, and up to 20 other viral families depending on patient symptoms. All PCR-positive samples will undergo full-genome sequencing for viral confirmation.

Results: With partnerships in multiple locations within Lao PDR, PREDICT-2 aims to enroll over 1,500 individuals during its 5-year program. With detailed questionnaire data from these individuals, paired with PCR screening for several viral families of zoonotic potential, we aim to determine which human behaviours, practices, and beliefs are most associated with disease transmission events, and thereby produce region-specific recommendations on strategies to limit the spread of disease from animals to humans.

Conclusion: The PREDICT-2 program aims to improve the health and prosperity of people in regions at risk of zoonotic disease events by conducting concurrent surveillance of disease in both animals and humans on a global scale.

19.088 Japanese Encephalitis outbreak among children in Mayurbhanj, Odisha-India, 2015
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Purpose: Japanese Encephalitis (JE) is one of the leading causes of viral encephalitis in Asia. Annually 50,000 cases are reported in India with 30% deaths and 75% disability, but burden may be underestimated because many suspected outbreaks are not confirmed. During September-November, 2015 a viral encephalitis epidemic among children was reported from Mayurbhanj district, Odisha, India. We investigated to identify the etiology and understand epidemiological characteristics of the outbreak.

Methods & Materials: A case was defined as illness among children <15 years, presenting with fever, vomiting, altered sensorium and convulsions in Mayurbhanj during September-November 2015. We reviewed medical records to find cases. We collected serum and cerebrospinal fluid (CSF) samples from case-patients to test for JE IgM ELISA at the Regional Medical Research Centre (RMRC) laboratory. Environmental and entomological surveys were conducted and 80 mosquitos were processed in 8 batches for reverse transcriptase polymerase chain reaction (RTPCR) at RMRC.

Results: We identified 20 cases (67% female; median age 3 years [range: 1.5-4.6 years]) including 2 deaths. Among 20 serum and 4 CSF samples, 12 (60%) serum samples and all CSF specimens were JE IgM positive. Entomological survey showed the prevalence of Culex vishnui (larvae and adult), a known vector for JE. Rice field with stagnant water were found close to the affected households. Two batches of mosquito samples containing Culex vishnui and Culex gelidus species positive for JE based on RTPCR.

Conclusion: Our investigation confirmed the JE outbreak based on clinical specimens and substantiated by presence of JE virus in the vector mosquitoes. An integrated vector control approach with continuous monitoring and high JE vaccine coverage for eligible children is recommended to prevent epidemics.

19.089 Experience in treatment of residual cystic echinococcosis in children
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Purpose: Cystic Echinococcosis (CE) remains a relevant interdisciplinary health problem, due to a significant number of residual and recurrent forms and absence of consensus on effectiveness of anthelmintic drugs in treatment of recurrent and residual CE
Methods & Materials: We reported 2 cases of residual CE in clinic of the Rostov scientific research institute of microbiology and parasitology.

Results: The diagnosis of CE in first was diagnosed in a 8-year old girl in 2000. In 2003 the patient was operated due to liver CE. During an ultrasound examination of the abdominal cavity in 2004 2 new cysts (20 and 25 mm) in IV, V liver segments were recorded. Treatment was conducted with mebendazole in a daily dosage of 400 mg for 28 days with intervals of 14 days on the background therapy. Child received 5 courses of therapy. In 2004 abdominal CT scan detected large (80 mm) residual hepatic cyst, located below the diaphragm in the VII segment of the liver. Parasitic cysts had a thick calcificated shell (more than 7 mm), filled with heterogeneous content, and two cysts in the IV, V liver segments up to 20 mm with thick calcificated shells. The patient observed in the clinic during 11 years. Data of CT scan of the abdominal cavity in 2015 showed no increase in the cysts. In November 2010, a 7-year old child received operative treatment with multiple CE (liver and right lung). The cyst of the right lung (70 mm) was removed surgically. For prevention of recurrence CE child was observed and received conservative therapy in the clinic of parasitic diseases. Patient received 3 courses of therapy with albendazole. Treatment conducted by a dose of 15 mg / kg daily for 28 days with an interval of 14 days. The child observed in the clinic until now. X-ray examination in March 2016 did not show any signs of relapse of lung echinococcosis. Ultrasonic research of the abdominal cavity indicated a reduction of the size of the residual liver cysts.

Conclusion: These cases show the effectiveness of albendazole and mebendazole for the treatment of residual liver CE.

19.090 Eco-epidemiological analysis of rickettsia infection in rural areas from Colombia: A multilevel approach

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Purpose: Rickettsiosis is a re-emergent disease that is not the subject of epidemiological surveillance in Colombia. Rickettsia cases are generally undiagnosed and several lethal outbreaks have been reported in Colombia during the last decade. The aim of this study is to analyze eco-epidemiological aspects of rickettsia infection in two of the towns with previous reported outbreaks.

Methods & Materials: A cross-sectional study was conducted enrolling 597 people living in 246 houses from nine villages in two towns in Colombia from November 2015 to January 2016. Sociodemographic and housing conditions information (exposures) was collected by interview. Blood samples were collected to assess rickettsia infection in humans, horses and dogs (IFA, cut-off=1/128). In addition, ticks from humans and animals were evaluated for Rickettsia infection by qPCR targeting gltA and OmpB genes. All participants signed an informed consent. Data were analyzed by a multilevel logistic regression model using two levels (house and village) and seropositivity as the main outcome.

Results: Overall prevalence of infection by Rickettsia was 25.6% (95%CI 22.2-29.3). Four zones with higher proportion of infection in both people and animals were identified. Spotted Fever Group Ricketttsia was detected in Amblyomma ticks from humans and animals. The multivariate model showed the association between working outdoors and infection was confounded by age (years) and sex (male) (ORcrude= 2.25, 95%CI 1.47-3.46; ORadj=1.06, 95%CI 0.61-1.85). Sex and age is a risk marker for rickettsia infection in this zone (ORmale=1.88, 95%CI 1.15-3.07) and OR15years vs 10years=1.59, 95%CI 1.19-2.10). Not farming into the forest is a protection factor (OR=0.49, 95%CI 0.29-0.82) and the proportion of seropositive animals is strongly associated with human infection (ORproportion52.9% vs proportion8.7%=3.58, IC95:1.69-3.73). The model revealed variability among houses but not among villages (RandomEffect_houses=-0.65 ± Std. Err=0.25, 95%CI 0.30-1.39 and RandomEffect_villages=-0.11±StdErr=-0.29, 95%CI 0.00-21.46).

Conclusion: Studies on rickettsia infection in areas where previous outbreaks occurred are useful as an early approach for surveillance of re-emergence diseases in Colombia. Domestic animals could have an important role as sentinels for detecting zones with Rickettsia circulation. Finally, prevention measures should be taken to avoid invasion of primary and secondary forests where increased transmission of emergent and re-emergent pathogens such as Rickettsia can occur.

19.091 A study of bacterial contamination in feces of macaques in Lopburi Province, Thailand

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Purpose: According to overlapping of living space and food, macaque monkeys, play a vital role in human-monkey conflict in some part of Thailand, especially Lopburi Province, where the monkey is symbol of Province. Pig-tailed macaques live in Erawan non-hunting area, Chongsrarika Subdistrict destroy crops of local people nearby, while long-tailed macaques live in the property of people in the city of Lopburi as their own habitat. Community based resource management have been done by cooperation between key local authorities and community people for enhancing better quality of life of both human and monkeys. Zoonotic diseases that also may affect human who exposed which those monkey have to be investigated to minimize the risk of any infection

Methods & Materials: In this study, pathogens spread through exposure to the monkey feces have been examined. Samples were periodically collected by fresh fecal dropped on the ground was analyzed for bacterial contamination by using conventional bacterial identification methods.

Results: E. coli, Staphylococcus spp, Salmonella spp, was found in fecal swab samples.

Conclusion: Practicing good personal hygiene should be encouraged for people who have a chance to contact with these monkeys, especially local people and tourists. Additionally, other possible zoonotic diseases of non-human primate should be further investigated.

19.092 Leptospirosis in the Caribbean: A One Health approach


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Purpose: Leptospirosis is a global re-emerging zoonotic disease, caused by pathogenic spirochete bacteria called leptospires. It is common in the tropical and sub-tropical areas such as the Caribbean where there is significant rainfall. The purpose of this study was to describe the risk factors for the transmission of Leptospirosis from animals to humans and develop One Health guidelines to reduce the risk of transmission in the Caribbean

Methods & Materials: The CaribVET VPH Working Group conducted a subregional workshop to describe and analyse the prevalence and distribution of Leptospirosis in the Caribbean, describe the transmission and develop recommendations to reduce the risk of transmission from animals to people.

Results: The risk factors for Leptospirosis transmission from animals to people were categorised and described. Human risk factors included occupational and recreational exposure and sex. Occupations such as agricultural, sewage, garbage, abattoir and veterinarians were at high risk. Recreational exposure included walking barefoot through flood waters, hiking in tropical forests, and hunting wildlife. The incidence was twice as high in men and teenage boys as in females.

Domestic animal risk factors

Leptospirosis can be transmitted from domestic and wild animals and rodents, through exposure to animal urine. The causative organisms have been found in both wild and domestic animals, including rodents, insectivores, dogs, cattle, pigs and horses. In the Caribbean, the mongoose may also be a reservoir.

Environmental risk factors

Outbreaks of Leptospirosis in humans and animals are frequently found in flood conditions, usually associated with high rainfall and humidity. Recent Leptospirosis outbreaks in Dominica and Guyana occurred under persistent flood conditions. Climate change is believed to contribute to rising ambient temperature and humidity. Inadequate drainage can also contribute to flooding, particularly in lowlying areas. Increases in the rodent or mongoose populations increase the probability that ground water is contaminated with Leptospira infected urine, and can lead to outbreaks in animals and humans who are exposed to the contaminated water.

Conclusion: As risk factors include human, agricultural and environmental exposure, an effective prevention program should address all three sectors, which is a One Health approach.
Specific recommendations were formulated for each risk factor in order to prevent transmission.

19.093 Brucellosis in Cameroon: Seroprevalence and risk factors in beef-type cattle
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**Purpose:** Bovine brucellosis is a well-known zoonosis which also has profound negative impacts on cattle productivity and on international trade. In this study we aimed at investigating seroprevalence and possible risk factors associated to seropositivity of bovine brucellosis in two agro-pastoral ecologic zones of Cameroon: the Western Highland Plateau Savannah (WHPS) and the Guinea Highland Savannah (GHS) as they encompass the most productive regions in the country in terms of cattle production.

**Methods & Materials:** The subdivision and village herds were considered as clusters for a two-stage cluster sampling procedure. To enable us to sample animals by probability proportional to size, cattle population of each village (cluster) was noted against the corresponding village. Each beef-type cattle in Cameroon was assigned to a single cluster (village). Using C-survey 2.0 a final list of villages to be visited was established. Blood samples were collected and screened using the Rose Bengal Plate Test. A field questionnaire was designed and distributed to herd managers during sampling to collect information on intrinsic risk factors (age, sex and breed) and extrinsic risk factors (ecological zone, herd size, herd management system, third trimester abortion, interaction with wildlife, and interaction with sheep and goats during grazing) that can affect Brucella sero-status of the target population. Data generated from the field questionnaire were tested for significance against seropositivity using ANOVA and Chi-Square in R-Software®.

**Results:** The results of this work confirm that bovine brucellosis is endemic in Cameroon. With an uneven but wide distribution the study revealed a 4.61% and 16% seroprevalence at the animal-level and herd-level respectively. There was a preponderance of seropositivity in the GHS (87.5%) over the WHPS (12.5%). The extrinsic risk factors investigated were all positively correlated with seropositivity. It was interesting to note that cattle interactivity with sheep and goats (small ruminants), wildlife, herd size, administrative division, history of third trimester abortion, ecological zone, and herd management systems had significant effects on seropositivity.

**Conclusion:** To the best of our knowledge, these findings provide the first factual insight into the seroprevalence of bovine brucellosis and some of its associated risk factors in the most livestock-productive agro-pastoral ecozones of Cameroon.

19.094 Molecular screening for *Coxiella burnetii* in seropositive ruminant herds in Portugal
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**Purpose:** Domestic ruminants are recognized as major sources of infection for *Coxiella burnetii*, the Q fever agent. In Portugal, recent surveys showed an individual seroprevalence of up to 15% in ruminants. This study aimed to evaluate the shedding in milk and vaginal mucus of *C. burnetii* in ruminant females from seropositive non-vaccinated herds and to estimate their bacterial load.

**Methods & Materials:** A cross-sectional study was conducted between February and July 2012. In twenty four herds, 142 vaginal swabs and 92 milk samples were collected from females older than 6 months (n=142). The screening was performed by PCR, amplifying the IS1111. Bacterial load was estimated by qPCR in positive samples.

**Results:** *C. burnetii* shedding was confirmed in 20.8% of the herds and in 9.2% of females. The proportion of shedding was higher in cattle herds (66.6%) than in mixed (25.0%) and goat herds (22.2%). It was not found in sheep herds. Individually, excretion was higher in goats (15.0%), than in cows (10.9%) and ewes (3.6%). The quantification results categorized 7.7% of samples as strong positive (≥3 x 107 bacteria/ml); the bacterial load ranged from 460 to 600000 bacteria/ml. A significant association was found between seropositive individuals and bacteria shedding (p<0.05); a lower risk of excretion (p<0.005) was observed in animals older than 36 months.

**Conclusion:** To our knowledge, this is the first study confirming the shedding of *C. burnetii* in apparently healthy ruminants in Portugal. Overall, the study highlights that the identification of
shredders is central in the assessment for the risk of human infection and in Q fever control or surveillance schemes.

19.095 This is the abstract title for my abstract - Chronic opisthorchiasis: Current situation in the Republic of Kazakhstan

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**Purpose:** Aim: to investigate prevalence of human opisthorchiasis in natural foci of Kazakhstan for 2009 -2014.

**Objectives:** 1) to study prevalence of opisthorchiasis in Kazakhstani population; 2) to perform time series analysis, considering ecological zones and administrative division of Kazakhstan.

**Methods & Materials:** Consolidated reports 1, 2 of Ministry of Healthcare of Kazakhstan and data provided by Agency for population statistics of Kazakhstan. Statistical methods: 1) analysis of intensive values; 2) analysis of time series (average annual growth / decline rate (AAG/DR,%); 3) 95% confidence interval (CI) 1.96 × SE (CI)

**Results:** From 2009 to 2014, average annual incidence rate was 12.24 ± 0.86⁰/₀₀₀₀ (95% CI = 10.56-13.92⁰/₀₀₀₀). Trends of adjusted incidence rates of helminthiasis increased in Akmola (AAGR = + 19.18%), Kostanay (AAGR = + 16.9%), Aktobe (AAGR = + 15.7%) regions. In other regions morbidity trends declined, with average annual decline of adjusted incidence rates ranging from AADR = -1.84% in West Kazakhstan to AADR = -24.5% in North Kazakhstan.

Significant heterogeneity in distribution of incidence in various regions was observed, caused by specific lifecycle of *Opisthorchis*, prevalence of intermediate hosts of helminthiasis in reservoirs, large country area, including 9 water basins.

Current investigation revealed Ural, Caspian, Irtysh, Ishim, Tobol, Turgay basins (from 18.2⁰/₀₀₀₀ and 8.08⁰/₀₀₀₀) to be regions with high and medium levels of morbidity of opisthorchiasis. They are located in north-western and north-eastern parts of Kazakhstan and are co-integral parts of the two world’s natural foci of opisthorchiasis - Ob-Irtysh and Volga-Kama

Nura-Sarysu water basin and Almaty were determined to be regions with low indices (up to 0.08⁰/₀₀₀₀). However, majority of cases in Almaty were imported, with no tendency of morbidity reduction due to increase in population migration, trade and economic processes. Imported cases and infection of local population in Ishim water basin area were identified in Astana (23.25⁰/₀₀₀₀).

**Conclusion:** Uniqueness of natural environment and specificity of hydrological regime ensure stable functioning of opisthorchiasis foci. Despite the dynamic decline in morbidity during 2009 to 2014 in total population, a steady level of newly diagnosed patients with opisthorchiasis in northern and central regions of Kazakhstan remains.

19.096 First description of african horse sickness virus serotype 7 in healthy horses in northern Senegal

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**Purpose:** The purpose of the study was to survey African Horse Sickness (AHS) virus prevalence in healthy horses and identify the serotypes circulating in Senegal. This study was conducted one month prior to the notification of the first AHS clinical case in northern Senegal and before mass vaccination against eight serotyes took place.

**Methods & Materials:** Between 12-19 August 2007, 414 horses were randomly sampled in three zones in the Senegal River delta: St.-Louis, Ross Bethio and Richard Toll. The blood samples collected from 138 horses in each zone were centrifuged, stored at -20°C, and later tested by competitive ELISA. A random sample of 71 positive horse sera was analyzed by Plaque Reduction Neutralization Test (PRNT) on Vero cells against African Horse Sickness Virus (AHSV) serotypes. Cytopathogenic effects were read four days later. Statistical data analysis was done using R software.

**Results:** The overall true seroprevalence rate by ELISA test was 89.5% in 414 horse sera. In the subset of 71 infected horses, antibodies against AHSV 2 were detected in 67.5%, against AHSV 7 in 92.96% and against AHSV 9 in 100% of horses. AHSV 7 antibodies were found in 80.95 % of the positive horses from Ross-Bethio, 95.45% from St.-Louis, and 100% in Richard-Toll zones.
Conclusion: Prior to this study, only the monovalent vaccine against AHSV serotype 9 was used on horses in the area. The polyvalent vaccine against eight types of AHSV was first used after our sampling. Our results reveal the presence of antibodies against AHSV 7, which suggests that Serotype 7 virus had been circulating prior to the mass vaccination. Chi-square test for the three serotypes showed that they are not independent. Based on these results we hypothesize that the vectors of AHSV 9 in the Senegal River delta may be the same vectors that introduced and spread AHSV 2 and AHSV 7. This work is the first description of AHSV 7 in Senegal.

19.097 Detection of Foot and Mouth Disease in Landi Dairy Colony, Pakistan, the world largest buffalo colony
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Purpose: Foot-and-mouth disease (FMD) is a highly infectious and contagious disease of cloven hoofed animals. The disease is endemic in Pakistan caused by Foot-and-mouth disease virus (FMDV) serotype O, A and Asia-1. Despite of regular vaccination, outbreak of the disease has become a regular event throughout the country every year. Determination of prevailing serotypes of the causal agent foot-and-mouth disease virus (FMDV) is now crucial need for strategic vaccination programme. The present research work was aimed to standardize a one step RT-PCR assay for the typing of foot-and-mouth disease virus serotypes prevalent among cattle in Landi Dairy Colony (LDC) to have an accurate knowledge of circulating virus strains in the country that will help to select more specific vaccines.

Methods & Materials: One step RT-PCR was successfully developed and standardized using the extracted RNA of reference FMDV (Type A, O and Asia 1) following adjustment of the concentration of the viral RNA of each serotype, volume of reaction mixture and thermal profile. One step RT-PCR was evaluated on 82 field samples (vesicular fluid, tongue epithelium and tissue from inter-digital space) of the years 2014 and 2015. Universal Primer Piars IF/IR were used for the primary diagnosis of FMDV and serotype specific Primer Piars were used for the serotype detection of FMDV.

Results: One Step RT-PCR was evaluated on 82 field samples (vesicular fluid, tongue epithelium and tissue from inter-digital space) of the years 2014 and 2015. Of the 82 field samples, 56 (68.29%) were found positive for FMDV. The RT-PCR successfully differentiated single as well as dual serotypes infection. The serotypes A, O and Asia 1 were confirmed in the samples of the year 2014 and only serotype O in samples of the year 2015. Higher detection rate was found in vesicular fluid (100%) followed by tongue epithelium (79.66%).

Conclusion: It may be concluded that the One step RT-PCR standardized in this study could be used for detection and differentiation of FMDV serotypes using field samples. For an effective and realisable FMD control program in Landi Dairy Colony, we suggest to introduce a twice annually mass vaccination of all buffaloes and cattle in the colony.

19.098 Pathogens in domestic cats and dogs in the Canary Islands, Spain
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Purpose: The aim of this work was to increase the knowledge about the range of pathogens affecting domestic cats and dogs in the Canary Islands, Spain, due to the actual scarcity of data. Another purpose of this study was to assess through immunological methods whether dogs in the Canary Islands are coming into contact with the nematode Angiostrongylus cantonensis.

Methods & Materials: Between 2014 and 2015, 264 dogs and 60 cats were attended in different veterinary clinics in Tenerife and Lanzarote, two out of the Canary Islands. Faeces samples were obtained from each individual to determine the presence of Cryptosporidium spp., Giardia spp., Toxoplasma spp. and Entamoeba spp. by morphological methods, whereas Giemsa-stained blood smears were occasionally analyzed for the presence of Babesia parasites. On the other hand, sera samples were obtained from 59 dogs belonging to Tenerife to carry out the detection of IgG antibodies against a 31-kDa glycoprotein of A. cantonensis by ELISA technique.

Results: The protozoa Giardia spp., Cryptosporidium spp., Cystoisospora spp. and Entamoeba spp., and the helminths Toxocara canis, Trichuris vulpis, Enterobius sp., Aelurostrongylus abstrusus, Dicrocoelium sp., Toxascaris leonina and Anclylostoma caninun
were found in domestic dogs. With regard to domestic cats, we found the protozoa 
*Giardia* spp., *Cryptosporidium* spp., *Entamoeba* spp., and *Toxoplasma gondii*, and the helminths 
*Toxocara cati* and *Aelurostrongyulus abstrusus*. *Giardia* spp. was the most prevalent pathogen 
in both domestic cats and dogs. The presence of *Cryptosporidium* spp. in both cats and dogs 
was occasionally confirmed by amplifying the 18S small subunit ribosomal RNA (18S rRNA) 
gene by using genus-specific primers. On the other hand, 11.89% of the dogs analyzed 
carried IgG antibodies against *A. cantonensis*. Finally, *Babesia canis vogeli* was identified in a 
dog by PCR and sequencing of the 18S rRNA gene.

**Conclusion:** The finding of pathogens with zoonotic potential in domestic cats and dogs in 
the Canary Islands highlights the necessity of assessing whether zoonotic transmission 
events from pets to humans are taking place in this archipelago. Pet owners should be also 
taught appropriate hygiene measures to prevent the transmission of some of these zoonotic 
pathogens.

19.099 Overwintering of epizootic hemorrhagic disease virus in white-tailed deer in 
Florida, USA: Unanticipated seroconversion and the case for alternative vectors

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**Purpose:** Hemorrhagic disease (HD) is a vector-borne disease of ruminants caused by two 
distinct but related viruses: epizootic hemorrhagic disease virus (EHDV) and bluetongue virus 
(BTV). HD is the most important viral disease of white-tailed deer in North America. In recent 
years, HD outbreaks have become more frequent, and HD has become a more global 
problem as the viruses have spread, causing infection in both domestic and wildlife hosts. 
The reasons for this incursion are not well understood but some hypotheses for this 
phenomenon include the expanding range of biting midge vectors (*Culicoides* spp.), 
potentially resulting in overwintering of these viruses, or the emergence of more virulent 
strains of these viruses.

**Methods & Materials:** To explore this problem in the subtropics (northwest Florida) we 
measured seroconversion to 3 endemic EHD viruses as well and BTV in a sentinel herd using 
well established serological methods. We also monitored the herd using a novel RT-qPCR 
assay for detection of all known EHDV serotypes. Simultaneously, we trapped *Culicoides* 
using CDC miniature light traps and by aspiration directly off of white-tailed deer enclosed 
in a 500 acre preserve.

**Results:** From January to March seroconversion to EHDV-1 and 6 occurred across the herd 
in all age groups of animals. The virus was transiently detected by molecular methods in 
hosts during this time period. Compared to midge abundance and diversity in the previous 
season (July-December), few species were present and none of established vectors were 
trapped in any trap or aspirated directly off of deer.

**Conclusion:** Transmission of these viruses appears to be year-round, even in the absence of 
“known” vectors. The ability of alternative vector species to transmit HD, besides those known 
to transmit these viruses in North America (*C. sonorensis*, *C. variipennis* and *C. insignis*), 
warrants further investigation.

19.100 Genetic disease susceptibility and surveillance system sensitivity: Classical 
scrapie

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**Purpose:** Susceptibility of sheep to classical scrapie (CS) is modulated by the PrP gene and 
can be grouped into five NSP Types, NSPI being most resistant and NSPV most susceptible. 
Surveillance is targeted on fallen stock (FS), i.e. dead or culled over 18-month-old animals. 
Testing of animals slaughtered for human consumption (SHC) is limited. Our aim was to 
assess the effect of genetic susceptibility on the surveillance system sensitivity (SSe) in 
Finland in 2008-2014. The SSe is the probability that at least one infected animal is 
detected if the population is infected at or above the design prevalence (*P*).

**Methods & Materials:** We estimated the SSe by scenario tree modelling. Nodes and input 
parameters are described in table 1. To account for variability and uncertainty, we fitted 
distributions to key parameters.

<table>
<thead>
<tr>
<th>Node</th>
<th>Branches</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSP Type</td>
<td>NSPI-V</td>
<td>NSP Type distribution of sheep in Finland based on breed PrP genotypes and breed distribution</td>
</tr>
<tr>
<td>Death type</td>
<td>SHC, FS</td>
<td>True SHC and FS population proportions</td>
</tr>
</tbody>
</table>
Infected | yes, no | Effective probability of infection (EPI) = adjusted risk \(^1\) (AR) x \(^2\) (P*)
Sampled | yes, no | Actual sampling numbers
Test result | pos, neg | Actual test results

Table 1. Nodes and input parameters for the model. \(^1\)AR is based on the estimated risk ratio (RR) of different NSP Types against the average risk in SHC in Great Britain (GB) (Arnold and Ortiz-Pelaez 2014) and adjusted for the NSP Type distribution in Finland. \(^2\)P* = 0.1% based on the OIE Terrestrial Animal Health Code.

Results: Cases in GB were concentrated to FS giving high RR’s in FS and susceptible NSP Types. When adjusted for Finland, the AR was highest for NSPV in FS. The SSE for Finland showed very little fluctuation in 2008-2014. Even at its lowest point in 2010, the mean was 99.72%. The representative SSE for the same year, i.e. without considering NSP Types and the added sensitivity in focusing the surveillance on FS, was 60.80%.

Conclusion: The Finnish sheep population is relatively sensitive to CS. Consideration of NSP Types increases the accuracy of the SSE. Genetic susceptibility factors can be of great value when developing/assessing surveillance.

19.101 Suspected case of West Nile Virus (WNV) in the territory of ASL Sassari (Sardinia) F. Sgarangella\(^1\), D. Marongiu\(^2\), G. Bitti\(^2\), S. Masala\(^2\), V. Floris\(^2\), B. Mossa\(^2\), P. Desini\(^2\)
\(^1\)Azienda Sanitaria Locale, Sassari, Italy, Italy, Italy, \(^2\)Azienda Sanitaria Locale, Sassari, Italy

Purpose: The purpose of this paper is to illustrate the health management of a suspect case of West Nile in the territory of ASL Sassari.

Methods & Materials: In Sardinia, eight wildlife rehabilitation centers transfer animals with serious illnesses to two centers of regional recovery (CRAS): Bonassai in Sassari and Monastir in Cagliari.

On April 4, 2015, three dead birds (two goshawks and one buzzard) and two live birds (one goshawk and one buzzard) found in the ASL of Nuoro, were transferred to Bonassai from the wildlife rehabilitation center of Olbia.

Both dead birds tested WNV-positive (RT-PCR). The National Reference Center for the Study of Exotic Diseases in Teramo confirmed the positive diagnosis of lineage 2 on September 21, 2015. The two live birds tested positive with a serum neutralization test. Although from September 4, 2015 to September 21, 2015 the two birds were not kept in isolation, biosecurity procedures were performed adhering to standard protocols typical for seropositive birds, as dictated by current legislation. The Local Crisis Unit decided to take two control samples, separated by 15 days, including blood, serum, and cloacal swabs of other birds introduced in the CRAS. They also set up two cages for catching insect vectors. Meanwhile hospitals, blood transfusions, and primary care providers were alerted in order to protect human health.

Results: Two buzzards of 25 sampled birds were positive by serum neutralization (Title 1:20 and 1:10), with no increase in their antibody titers. No birds or captured insects were WNV-positive when tested.

Conclusion: The two dead birds who were WNV-positive, had not stayed at the CRAS in Bonassai. The two live birds, though positive serologically, were not considered suspects by current legislation. It is possible, however, that in the period between the introduction of the animals and the control, the virus could have circulated. As a precaution, the serologically positive birds were treated as suspects, as evident by the activation of the described procedures. In the described case, a new procedure was adopted that involves the isolation of all incoming birds that come to the CRAS, pending the laboratory results for WND.

19.102 Accompaniment and Adherence to DOTS Treatments for Tuberculosis in 100 Patients
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Purpose: The success of DOTS depends on adherence to treatment for 6 months. TB cases to detect CAT, DOTS accept the urgency of establishing, but after finding, drop sick, do not observe the installation after 1mois.Conséquences resistance and failures and disease relapse affecting patient, parents and caregivers.

Our NGO - SHS & TBC, contributes in the context of adherence to TB treatment at the community level, through our relay (The ACCOT, meaning: Agent Community Charge of Compliance to treatment (under outpatient treatment
Methods & Materials: Our ACCOT, receive training and tools, move to the CAT which contains all the information on each patient, of course with the consent of the patient, confidence building between ACCOT and sick and ACCOT and CAT. Or the patient is free to come and take his medication or allow the ACCOT agent (Depends on the outstanding treatment plan) book at home. Main objective of the work of ACCOT is to observe regular intake, dates to get to CAT to receive his dose of anti TB drugs, clinical controls. Failure ACCOT the agent warns the CAT, measures taken on the behavior of the patient. So sick move will be followed in order to receive their doses.

Results: It should be noted:
- Our 40 ACCOT agents identified within the community, through local officials receive training and tools on the Observance of TB treatment approach Midfield Community.
- 100 Ill confirm the CAT, rely on treatments IDENTIFIED with the CAT are followed for 6 months, from July to December, 2015.
- Reduction of 98% of treatment abandonment by patients
- Our ACCOT agents, skills on TB, including Community Living TB prevention, motivation of patients, parents to visit the CAT in case of TB suspicion in family, community.

Conclusion: Reduction of mortality, morbidity
- Duplication of the action in other remote areas.
- Projection of scientific studies with patients in DOTS abandonment extracts positive measures

19.103 Pneumocystis jiroveci pneumonia the who and the where identifying the population at risk
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Purpose: Pneumocystis jiroveci is an opportunistic pathogen which can lead to life threatening respiratory failure. It has a documented mortality of between 5-20%. Historically it was almost exclusive to HIV patients however; an increase in immunosuppressive therapies has led to a reciprocal increase in the prevalence of Pneumocystis pneumonia (PJP) in this non-HIV population. Despite clear guidelines for PJP prophylaxis in HIV, there is a haphazard approach in other immunosuppressed populations. A growing body of evidence suggests that immunosuppressed patients are at an increased risk of PJP, but to what extent, or to whom that risk is greatest is not certain. Given this uncertainty we felt it prudent to review regional rates of PJP to develop a clearer understanding of the potential at risk population.

Methods & Materials: We audited a random cohort of 103 patients with Pneumocystis pneumonia over a 5 year period in a region in the United Kingdom. We collated information on potential risk factors, morbidity and mortality.

Results: The highest proportion of patients were cancer patients, however the most at risk population were rheumatology patients, with a 73% mortality rate in those who tested positive for PCP. Admission to ICU was 38% and mortality was 32%. Mortality was highest when prescribed three modalities of immunosuppression in combination; however prednisolone alone carries a mortality rate of 62%.

Conclusion: We feel that prophylaxis guidelines should be considered in these identified high risk groups, but much more study is required on the absolute risk, and on how prophylaxis should be approached.

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Purpose: What are the key attributes and performance indicators of measles surveillance system in Katsina State, Northwestern Nigeria?

Methods & Materials: The evaluation of measles surveillance system in Katsina state was conducted using the CDC’s updated (July 27, 2011) guidelines for public health systems evaluation which involved interview of stakeholders using pre-tested self administered questionnaires, secondary data analysis of measles surveillance data from Jan 2009 to Dec 2012 using microsoft excel, health mapper version 4.3.2 and epi info version 3.5 and review
of relevant documents such as guidelines for measles surveillance in Nigeria, CDC updated guidelines for evaluating public health surveillance systems etc.

**Results:** Majority (78%) of the stakeholders interviewed described the system as simple based on its operation and structure as it's integrated with other diseases and the disease has an easy case definition. The proportion of reported suspected measles cases from whom blood specimens were collected for the state for 2009, 2010, 2011 and 2012 were 88%, 81%, 98% and 96% respectively which were well above the global target of 80%. The Proportion of LGAs that have reported > 2 cases of suspected measles with blood specimen for 2009, 2010, 2011 and 2012 were 55%, 79%, 91% and 82% respectively. Only 2009 and 2010 figures were below the global target of 80%. Generally data was represented from all LGAs of the state but that of 2009 and 2010 have a fewer missing figures compared to of 2011 and 2012. The positive predictive values were 21%, 12%, 30% and 37% for 2009, 2010, 2011 and 2012 respectively.

**Conclusion:** Measles surveillance system is performing relatively well but there is need for improvement. The study highlighted that system was found to be simple, flexible, sensitive and acceptable but not very timely. It generates a fairly qualitative data with low representativeness and predictive value positive which can be attributed to the measles case definition.

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19.105 Reducing the impact of emerging infections through data sharing  
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**Purpose:** Data sharing has the potential to shift the paradigm of outbreak response in the public health, clinical and scientific communities. Increasing access to more data can address some of the key challenges of the limited, fragmented, poor quality data, which characterise emerging infections. To achieve this, we must design well-governed, equitable, collaborative data sharing models driven by the questions relevant to those directly affected, and ensure that the benefits of sharing are returned to them. We must also implement methodologies that maximize the usability of data to generate evidence.

**Methods & Materials:** A secure, flexible, technical architecture was developed based on lessons learned from other industries. Careful, centralised curation of varied types and sources of data has been applied to minimize overlapping resource investment and expedite implementation of evidence-generating analysis. We continue to work towards the establishment of appropriate governance models and benefits sharing for each disease community.

**Results:** Working with the research community addressing the emergence of antimalarial resistance, we have built a collaborative model of sharing data that has successfully pooled and analysed more than 80% of the clinical trials on critical antimalarials to deliver policy-changing evidence. This has served as the prototypic model for the development of new platforms for Ebola and Zika, with the aim of enabling sharing within a useful timeframe to affect the outbreak response. The varied technical and governance frameworks of the platforms assert that different types of data, and different communities of data generators, have different needs.

**Conclusion:** Failure to share data on emerging infections impedes outbreak response. In order to address this, investment must be made in sustainable infrastructure and governance for data sharing in advance of the outbreak. Data sharing has the potential to improve knowledge on many diseases, but resource limitations mean that efforts should be focused where they can have the greatest impact. The global threat of emerging infections puts these high on the priority list.

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19.106 Parasitic contamination of raw vegetables consumed in Asmara Eritrea  
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**Purpose:** Intestinal parasitic infection is one the factor responsible for food borne illness, it normally happens due to consumption of unclean, raw, or undercooked vegetables and it is a major public health problem in developing countries. The main purpose of this study is to evaluate the presence of parasitic contamination in vegetables which are consumed raw in Asmara, Eritrea.

**Methods & Materials:** A cross-sectional study was conducted to evaluate the level of parasitic contamination of raw vegetables sold in two local markets and produce in seven
farm fields of Asmara. 200 gm of each raw vegetables viz; lettuce, cabbage, carrot and girgir (local Name) were analyzed by using sedimentation method, and the stages of parasites were observed under a light microscope using ×10 and ×40 objectives. Statistical data was generated using Pearson chi-square test. A value of P < 0.05 was considered as statistically significant.

**Results:** Of, 205 vegetable samples, 117 (57.07%) were contaminated with one or more parasites, the most common parasit was *Ascaris lumbricoides* 61(29.7%), followed by *Giardia lamblia* 55(26.8%). Of, four vegetables, lettuce was the most contaminated, 42 (60.8%), followed by carrot 30 (60%), girgir 13 (56.5%) and cabbage 32 (50.7%). From 88 samples collected from two Markets, *Giardia lamblia* 32(36.4%), followed by *Ascaris lumbricoides* 11(12.5%) was dominant. 117 samples from seven farms showed higher presence of *Ascaris lumbricoides* 50(42.7%) followed by *Giardia lamblia* 23(19.7%), and they were statically significant with a P value of <0.001 and 0.008 respectively.

**Conclusion:** The outcome of this study has made us certain about the presence of parasites in vegetables. The most common parasite identified was *Giardia lamblia and Aascaris lumbricoides*. Among vegetables, lettuce was highly infested with parasitic contamination followed by carrot. This demonstration of parasites in raw vegetables which are frequently consumed by people of Asmara as part of their diet is alarming to their health. Health authorities must educate the farmers and retailers about the drastic implication of using faecally contaminated water and untreated human faeces as fertilizer for washing and growing vegetables, because *Giardia lamblia and Aascaris lumbricoides* are common where the above mentioned factors are in practice.


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**Purpose:** On November 16, 2015, media reported 06 individuals of a family presenting with diarrhea. A team was sent to Village Kotha Kalan, District Rawalpindi to identify the cause and propose control measures.

**Methods & Materials:** Active case finding was done. A case was defined as any resident of Kotha Kalan presenting with ≥ 3 loose stools within the past 24 hours with or without vomiting and fever since November 01, 2015. Age and sex-matched controls were identified from the same population. Laboratory and environmental assessments were carried out.

**Results:** A total of 55 cases were identified with median age of 5 years (range 9 months-60 years). Overall attack rate was 0.34%. The most severely affected age group was 1-14 years, n=42 (AR=5.9 per 1000). Visible clustering of cases around the water supply reservoirs was evident. Out of 55 cases, 51 reported drinking stored water (OR 9.3; CI 3.1-27.5; p-value 0.000), 18 had a history of contact with a case (OR 8.3; CI 3.0-22.6; p-value 0.000) and 48 used tap water for cleaning and washing (OR 5.06; CI 2.3-13.4; p-value 0.000) However frequent hand washing practices showed a protective effect. All water samples except the one from filtration plant revealed fecal coliforms. Moreover food (rice) samples were also positive for fecal coliforms. Environmental assessment revealed that water pipes had been replaced in late October 2015.

**Conclusion:** Contamination of stored water at the household level through soiled hands was the most probable cause of the outbreak with a component of person-to-person transmission. Use of tap water for hand washing could have been the source of contamination. Use of aqua tabs and cleaning of the water storage reservoirs/pipelines was recommended.

19.108 Environmental persistence of Human Rotavirus causing a large outbreak in Western Mindanao, Philippines, 2016

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**Purpose:** Enteric viruses are a leading cause of acute diarrhea outbreaks in the Philippines. In March 2016, a large diarrheal outbreak occurred in Zamboanga del Sur, affecting 1,538 cases with 6 reported deaths. Human specimens from the outbreak showed insignificant numbers of bacterial and parasitic pathogens were isolated in. To develop effective preventive measures, epidemiological information of viral agents must determined. This study aims to investigate the presence of three enteric viruses in stools and water samples collected during the Zamboanga outbreak.

**Methods & Materials:** A total of 106 stool and 10 water samples (approximately 1-L each) from water refilling stations, household water and deep wells were collected to determine the presence of three enteric viruses: group A rotavirus, norovirus and adenovirus by real-time
polymerase chain reaction. Water samples were concentrated by precipitation using polyethyleneglycol hydroextraction technique. Nucleotide sequencing and analysis using MEGA 6 software were performed to determine the prevailing genotypes on samples detected with rotavirus, norovirus and adenovirus.

**Results:** Enteric viruses were detected in 80 (75.5%) of the 106 cases of acute diarrhea. In 106 samples, rotavirus A was the most frequent virus identified in 65 (61.3%) cases, followed by norovirus in 5 (4.7%) cases, and adenovirus in 1 (0.9%) case. 9 (8.5%) out of the total specimens also showed mixed infection. Only rotavirus A was identified in 5 of the 10 water samples collected. G9P[8] and G3P[8] rotavirus genotypes were identified in both water and human samples.

**Conclusion:** Presence of G9 and G3 rotavirus genotypes in both water and human samples indicate the possible transmission of rotavirus from contaminated drinking water to humans. This finding corroborates the hypothesis that individuals act as potential reservoir for the spread of rotavirus in the environment. Such information is important for modeling transmission scenarios, identifying risk of food-borne and water-borne virus contamination, and targeting prevention and control efforts in reducing the spread of infection.

19.109 Ecology and surveillance of *Listeria monocytogenes* on dairy cattle farms

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**Purpose:** Dairy cattle farms serve as reservoirs for *Listeria monocytogenes* and dairy products are a common vehicle for human listeriosis. Understanding *L. monocytogenes* transmission in the dairy farm environment is a prerequisite for the development of control strategies against bulk tank milk contamination. Our aims were to investigate the ecology of *L. monocytogenes* on dairy farms and to examine the utility of in-line milk filter sampling as an economic alternative to bulk tank milk sampling for *L. monocytogenes* screening.

**Methods & Materials:** We investigated the ecology of *L. monocytogenes* on three Finnish dairy cattle farms in a longitudinal study from late 2013 to early 2016. During this period, we collected a total of 1746 samples from the farm environments, 240 milk filter samples and 207 bulk tank milk samples. *L. monocytogenes* were isolated using a standard two-step enrichment method and identified by PCR. Isolates were genotyped using pulsed-field gel electrophoresis (PFGE) with the restriction enzymes ApaI and Ascl and serogrouped using multiplex PCR.

**Results:** *L. monocytogenes* occurred in the bulk tank milk throughout the year in levels that were consistently small: ≤ 1 – 11 CFU/ml. *L. monocytogenes* was twice more prevalent in milk filters than composite bulk tank milk samples, but the prevalence in milk filters varied notably between farms. Milk filters were poor indicators of bulk tank milk *L. monocytogenes* contamination, with 50 % sensitivity and 60 % specificity. *L. monocytogenes* presented in the feces of healthy cattle throughout the year, with the prevalence peaking in late spring. In the barn environment, *L. monocytogenes* presented most frequently on floor surfaces, water troughs and feed tables. Each farm environment housed 2 – 3 predominant *L. monocytogenes* genotypes, which were wide-spread and isolated repeatedly for up to 24 months. All predominant genotypes belonged to serogroup 1/2a.

**Conclusion:** Our findings indicate that milk filter sampling cannot reliably replace bulk tank milk sampling for *L. monocytogenes* screening. Furthermore, persistent genotypes are responsible for the majority of *L. monocytogenes* contamination on dairy farms. Circulation of *Listeria* between the bovine gastrointestinal tract and the farm environment could account for the high prevalence of *L. monocytogenes* observed on cattle farms.

19.110 Biosynthesis of Ethylene gas by a range of bacteria as an alternative of Calcium Carbide for fruit ripening

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**Purpose:** To quantify and compare Ethylene gas production by a range of selected bacteria as an alternative of Calcium Carbide for artificial fruit ripening.

**Methods & Materials:** Bacteria were taken from different sources and were grown on their specific media at pH 7. Ethylene gas was detected by Ethylene specific analyzer (Drager, Germany).

**Results:** Ethylene gas production varied with the microorganism at pH 7 and the type of medium used for enrichment. Among all the species, *Listeria* produced Ethylene in highest amount, i.e. 19.5 ppm/125 ml of TSB, whereas *Salmonella* was found to be the minimum producer of Ethylene gas i.e. 2.0 ppm/125 ml of Luria broth.
**Conclusion:** Ethylene is a plant hormone that triggers the ripening of fruit which results in the transformation of complex sugars to the simpler forms, fruit softening, and degreening of the skin of the fruit. Both endogenous and exogenous ethylene can induce the ripening of fruits. Normally fruits produce ethylene that induces the natural process of maturation. This process is artificially accelerated by using different chemicals of which calcium carbide is the commonest. Calcium carbide in contact with moisture produces Acetylene gas which is an analogue of natural ripening hormone ethylene. Industrial grade calcium carbide also contains trace amounts of more toxic arsenic and phosphorous that converts the healthy fruits poisonous. Studies showed that bacteria also contribute in fruit ripening by producing Ethylene gas. Both pathogenic and nonpathogenic bacteria are capable of synthesizing Ethylene gas which is harmless to humans. As the concentrations of Ethylene gas produced by selected bacteria will not affect human health, the microbiological means is safer and can be used commercially on large scale for fruit ripening. On the other hand, by using Calcium Carbide which produces Acetylene gas, similar to Ethylene gas, has hazardous effects on fruits and ultimately human health. Inhalation of 33% or 35% of Acetylene gas can cause unconsciousness within 7 and 5 min, respectively, which may lead to death. These findings suggest that Calcium Carbide should not be used as an artificial fruit ripening agent and use of bacteria is safe and cheap.

19.111 In vitro detection and characterization of *Listeria monocytogenes* from raw milk samples

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**Purpose:** The study was designed to isolate, identify and characterize *Listeria monocytogenes* from raw milk samples sold in the region of Karachi – Pakistan.

**Methods & Materials:** A total of 25 samples were collected from different private dairy shops, private dairy companies and individual dairy firms. Isolation and Identification of *Listeria monocytogenes* were carried out according to official standard ISO procedure 11290-1.

**Results:** The overall incidence rate of *Listeria monocytogenes* in raw milk was 88% which indicated higher contamination. It was presented in 22 raw milk samples.

**Conclusion:** The result indicates that strict preventive measures should be adopted to ensure *Listeria monocytogenes* contamination free milk. So, we suggest that the general principle of food hygiene should still be enforced in order to minimize count of *Listeria monocytogenes* in milk and dairy products during handling, storage and manufacturing in traditional dairies. Control of the feeding cattle and milk pasteurization can also limit the contamination with *Listeria monocytogenes*.

19.112 Genetic diversity of *Campylobacter jejuni* and *Campylobacter coli* isolates from Austria

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**Purpose:** *Campylobacter* is the main cause of acute bacterial gastroenteritis in humans in the European Union. In Austria 6.259 cases of campylobacteriosis were reported in 2015. The aim of this study was to determine the genetic diversity of *Campylobacter jejuni* and *Campylobacter coli* isolates from Austria and to identify possible associations between multilocus sequence types and hosts (poultry, food and humans).

**Methods & Materials:** A total of 283 *Campylobacter* isolates (229 *C. jejuni* and 54 *C. coli*; 145 human samples, 83 broiler meat/offal samples, 54 poultry samples, and one environmental sample) collected in 2014 were characterized by *in silico* multilocus sequence typing extracted from whole genome sequence data using SeqSphere+ (Ridom, Münster, Germany). The MagnaPure Compact Nucleic Acid Isolation Kit I was used for automated genomic DNA isolation (Roche Diagnostics, Vienna, Austria). The NexteraXT Kit was used for library preparation and 2x300 basepair fragments were sequenced on a MiSeq (Illumina Inc., San Diego, CA, USA) to achieve at least a 70 fold average coverage. Raw reads were *de novo* assembled into a draft genome using Velvet version 1.1.04. Contigs were filtered for a minimum coverage of 5 and minimum length of 200 bp.

**Results:** The *C. jejuni* isolates had 74 different sequence types (STs) assignable to 21 clonal complexes (CCs) with the major CCs ST-21 CC (n=34), ST-354 CC (n=19), and ST-52 CC (n=18). 11 isolates had new STs and 45 STs were not assignable to CCs. The *C. coli* isolates had 30 different STs assignable to ST-628 CC (n=48), ST-1150 CC (n=2), and no complex
19.113 Environmental health factors associated with diarrhoea in the under five children in Mtendere, Lusaka, Zambia
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Purpose: To establishing environmental health factors associated with the prevalence of diarrhea diseases in Mtendere

Methods & Materials: An analytical community-based cross-sectional study was conducted among 274 randomly selected household with a child aged between 6-59 months old and having lived in the area for more than six months. A mixed method design was implored in which data was collected using questionnaires, Focus Group Discussion guide, observations and water sampling forms. Logistic regression was used to adjust odds ratios at 95% confidence interval. Chi square test was used to ascertain the significance of findings at p-value of <0.05.

Results: The prevalence of diarrhea among under five years of age in the study period was 37%. The most affected children were among 12 to 24 months old children. Contamination of water was more at household (74%) level than at sources (30%). The major factors associated with diarrhea were not washing hands with soap (p-Value 0.008), children from households with income higher than K2500 ($250) were 70% (p-Value 0.045) less likely to report diarrhea and equally children in households that stored drinking water in small mouth containers were 61% less likely to develop diarrhea (P-Value 0.002).

Conclusion: The findings have revealed a higher prevalence of diarrhea in the under five children in Mutendere than previously estimated. The major factors associated with the prevalence of diarrhea were poor water storage, inadequate hand washing and poverty. The increase in prevalence of diarrhea could also have been due to increased ambient temperatures during the El Nino period which coincided with the study period. Conserted efforts should be focused on water quality, water storage, hand washing and community development to reduce poverty at household level in order to reduce the problem of diarrhoea in Mtendere.

19.114 Rapid real-time PCR for the detection of Vibrio parahaemolyticus in seafood
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Purpose: Vibrio parahaemolyticus continue to be a major cause of food poisoning in seafood. The rapid detection of food-borne Vibrio parahaemolyticus is an important food safety tool. The purpose of this study was to develop the assay method for the rapid detection of Vibrio parahaemolyticus using a novel and ultrafast chip-type real time PCR system.

Methods & Materials: Three target genes(tdh, thl, trh) were selected to detect Vibrio parahaemolyticus. Primers and hydrolysis probes were designed totarget conserved regions in target genes and meet the requirements of a 5'-nuclease assay. Real time PCR condition was optimized on the NBS Labchip G2-4 system, a novel, ultrafast chip-type real-time PCR system. The primers and probes were evaluated against 89 isolates which were correctly identified by VITEK 2 systems(biomerieux). The limit of detection with cultured Vibrio parahaemolyticus was determined.

Results: The developed real-time PCR assay showed 100% inclusivity for the 65 V. parahaemolyticus strains tested and 100% exclusivity for 24 non-target bacterial strains. The assay was able to detect 2~20 gene copies and 20 CFU/mL in the frozen peeled prawns.

Conclusion: By using these target genes and NBS Labchip G2-4 system, the assay showed promising results for for fast and reliable detection of V. parahaemolyticus within 20 minutes.

19.115 Community-level risk factors for foodborne and waterborne disease in Gyounggi province, South Korea, 2015
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Purpose: Foodborne and waterborne disease (FWD) significantly affect public health worldwide. The Korea Centers for Disease Control (KCDC) estimated 438 FWD occur in 2015. Among them, 128 cases (31%) were reported in Gyeonggi province and it had been increased every year from 67 cases in 2012 to 128 cases in 2015. Thus, this study aimed to figure out the frequency of FWD and to analyze association factors using epidemiologic investigation report.

Methods & Materials: FWD is designated as a legal infectious disease in Korea which is run by self-reported system. If the affected number of people are between 2 and 6 (small group), investigators of public health center examine it. But if they are 7 or more (large group), professional investigators conduct it. Using the collected epidemiologic investigation reports, we studied; (1) the frequency of epidemic by infected size (small or large group) and the occurred places (meal service, restaurant, house, etc.), and (2) community-level risk factors (water supply and sewer service ratio, population density, farmland ratio, and the number of restaurants) which modeled using a Poisson distribution.

Results: Total 128 cases were reported and 93 cases (72.7%) occurred in small groups. Estimated places were usually restaurants [84 cases (65.6%)] and food services [34 cases (26.6%)]. The sources of infection were identified by 80.6% in large groups, but 37.1% in small groups. Moreover, the identification rate went down to 29.7% if it happened in a restaurant with a small number of people. In addition, the case of outbreaks by cities showed variations and the gap was bigger in small groups. Therefore, we conducted Poisson regression on small group cases by community-level to find out association factors. The result showed that a negative association with a percentage of sewered population and a positive association with the number of restaurants.

Conclusion: The study suggests that effort to increase the percentage of sewered population is associated with the decrease FWD burden, further political implementation of enhancing regulations on restaurant’s hygiene is needed not only to decrease the incidence rate but also to increase the detection rate of infection source.

19.116 Prevalence of acute hepatitis A virus and hepatitis E virus in urban cities of Sindh, Pakistan

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Purpose: The aim of this study was to determine the prevalence of HAV and HEV among population of five different cities of Sindh, Pakistan. To identify that which age group is at high risk of acquiring HAV & HEV. The objective of this study was to create awareness about transmission of HAV & HEV among general population. To determine that which area of urban cities of Sindh is suffering from HAV & HEV.

Methods & Materials: A cross-sectional study of 1-year duration was conducted in urban cities of Sindh, Pakistan. Samples were collected from viral infection suspected population of different areas of cities. Serum samples were analyzed for IgM anti-HAV and IgM anti-HEV for the detection of HAV and HEV, respectively by Enzyme Linked Immunosorbent Assay (ELISA) kits and then confirmed by PCR using specific primers.

Results: The seroprevalence of HAV and HEV positive patients were 60% and 20% respectively. There were 240 male samples and 260 female samples between the age group of 2 months to 30 years. It was found that young children from the age of 2 months to 10 years were the most affected population.

Conclusion: Based on the above data, it is identified that young population of Karachi, Hyderabad, Larkana, Sukkur, and Mirpurkhas is at the high risk of acquiring hepatitis A and hepatitis E virus due to poor sanitary conditions. This data will be helpful in planning for future vaccination and in improvement of hygiene.

19.117 The Use of Social Media and Business Reviews for Foodborne Illness Surveillance

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Purpose: Foodborne illness is an underreported global public health problem. In collaboration with public health departments in the United States, we developed a platform to enable local surveillance of foodborne illness reported on social media and restaurant review sites for supplementing traditional foodborne disease surveillance programs. In this talk, we will present the foodborne surveillance platform and discuss the potential impact of factors such as weather and socio-economic disparities.
Methods & Materials: Our foodborne illness surveillance database consists of 1.5 million foodservice reviews and approximately 742 million tweets. About 1-2% of tweets have a geocoordinate provided by users. We inferred the geo-coordinates of another 50% of tweets using the 'location' field from the Twitter user profile by querying the Google Maps API. For automated detection of foodborne illness reports, we first developed a list of keywords consisting of foodborne disease symptoms and disease names. Next, we used text matching to filter the reports that contained at least one of the keywords. We then used a supervised machine learning classifier - support vector machine classifier (SVM) - to extract relevant reports. We employed regression and geo-spatial techniques to explore the variation in reports relative to climate and socio-economic factors.

Results: The SVM classifier was evaluated using 6084 tweets. The classifier had an accuracy and precision of 85% and 82%, respectively. Similarly, we achieved a 91% accuracy, and a recall of 71% and 95% for “sick” and “junk” reviews, respectively based on 8068 manually labeled Yelp reviews. Next steps involve working with health departments to follow-up on these tweets and to determine if they warrant further investigation. Furthermore, the association between socio-economic variables and population adjusted review volume differed across the three US states considered. Additionally, cities with a high volume of reviews revealed significant seasonal variations with a single peak observed each year.

Conclusion: Real-time surveillance of foodborne illness reports can aid local public health departments to identify and limit the spread of foodborne disease outbreaks. In addition to monitoring reports of illness, this platform can also be used to respond to suspected foodborne illness reports and spur restaurant inspections to ensure food safety.

19.118 Biopreservative effects of lactic acid bacteria against foodborne pathogens
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Purpose: Lactic Acid Bacteria are naturally present in several foods. The presence of lactic acid bacteria in dairy products has long been well known but the recurrence of this distinct group of bacteria in fruits and vegetables has rarely been focused. Therefore, the presence of LAB in fruit, vegetables and meat were studied. Moreover, the study evaluated the efficacy of isolated lactic acid bacteria (LAB) as biocontrol agents against the foodborne human bacterial pathogens.

Methods & Materials: A total of 18 strains of bacteriocin producing lactic acid bacteria were isolated from 30 different food samples. The isolates were identified on the basis of conventional methods. The antagonistic activity of LAB strains were tested against food borne pathogens (Bacillus cereus, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Proteus sp. and Pseudomonas aeruginosa, Staphylococcus aureus) by agar well diffusion method.

Results: The isolates were identified as Lactococcus, Pediococcus and Leuconostoc. All the isolates of lactic acid bacteria were found effective against gram positive bacteria while few isolates were effective against gram negative bacteria.

Conclusion: The result suggests that LAB could be used as a natural bio preservative agent to prevent food spoilage and can also be used as a bio protective agent to treat various microbial infections.

19.119 Whole-genome sequencing analysis of Listeria monocytogenes isolated in South Africa
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Purpose: There is growing concern about the increasing prevalence of Listeria monocytogenes associated with foodborne outbreaks. Data concerning the prevalence and epidemiology of L. monocytogenes in South Africa are lacking. To prevent, investigate and control Listeria outbreaks, molecular epidemiological data are critical for understanding strain relatedness and defining source attribution. This present study describes the first whole-genome sequencing data for L. monocytogenes isolates from South Africa.

Methods & Materials: A cluster of human cases of L. monocytogenes was recently reported from the Western Cape Province (WCP) of South Africa. This initiated our inaugural whole-genome sequencing (WGS) analysis of L. monocytogenes. To date, 11 isolates have been analyzed; WCP [n=9], Gauteng Province (GP) [n=1] and Eastern Cape Province (ECP) [n=1]. For WGS analysis, raw sequencing data generated on Illumina MiSeq equipment (2 x 300
paired-end sequencing runs) was analyzed using tools available in the CLC Genomics Workbench Software; trimmed reads were assembled using the ‘De novo Assembly Tool’. Assembled WGS data was analyzed using bioinformatics tools and on-line analysis pipelines available at the Center for Genomic Epidemiology (CGE), Technical University of Denmark (http://cge.cbs.dtu.dk/services/).

**Results:** Single nucleotide polymorphism (SNP) analysis separated our 11 isolates into 6 branches of a SNP phylogenetic tree. In particular, SNP analysis determined that the cluster of WCP isolates was not the result of a single strain, but rather that several strains were involved. Multi-locus sequence typing (MLST) data concurred with SNP results in differentiating the isolates. MLST subtype data were as follows: ST6 (n=4, WCP), ST1 (n=2, WCP), ST876 (n=2, WCP), ST54 (n=1, WCP), ST3 (n=1, GP), ST820 (n=1, ECP). In particular, our largest group was associated with MLST subtype ST6, a subtype commonly associated with unfavourable outcomes in patients.

**Conclusion:** Analysis of WGS data using bioinformatics tools and on-line analysis pipelines at the CGE provided a single, rapid and cost-effective approach to investigate the molecular epidemiology of *L. monocytogenes*. This study has initiated a larger surveillance project for *L. monocytogenes* in South Africa. In future, all South African *L. monocytogenes* isolates will routinely be investigated using WGS.

19.120 *Maternal Cytomegalovirus (CMV) seroprevalence and primary CMV infection during pregnancy*

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**Purpose:** Primary CMV infection during pregnancy is a serious threat to the fetus. Although vertical transmission is likely to occur as a result of maternal infection, the rate of permanent sequelae is higher among infants born to mothers with primary CMV infection. Diagnosis of CMV infection among healthy adults generally relies on serologic testing. IgG-avidity may provide information on a window of time during which primary infection may have occurred. In this study we present a maternal CMV seroprevalence and primary CMV infection among pregnant women visited for evaluation of pregnancy and health care to an antenatal clinic in a tertiary care hospital in the capital city of Sri Lanka.

**Methods & Materials:** This study included 384 pregnant women at their 2nd trimester presented during 6 months period. Pregnant mothers found with underlying immunocompromise states were not recruited. Ethical clearance was granted from a recognized local body. Basic information such as age, parity or any associated medical conditions was noted alone with the blood sample taken under aseptic venipuncture, and transported with-in 6 hours to the testing laboratory, at virology reference center. All samples were tested for CMV-IgG and positive IgG samples were further analyzed using CMV-avidity assay to catch mothers having low-avidity, which signaling the associate risk. CMV-IgM test was carried out to confirm recent CMV infection on the samples negative for CMV-IgG assay and samples with low-avidity levels. All 3 sero-markers (IgG, IgG-avidity, and IgM) were tested using ELISA method (Commercial Enzyme Linked Immunosobent Assay) according to the manufacturer’s recommendations.

**Results:** A total of 384 mothers were tested for CMV-IgG and 358(93%) positive, while 26 negative. Only 01(0.3%) mother showed low-avidity and the high-avidity was showed-up among 357. Of the 27 mothers tested for IgM except one all the other negative for IgM.

**Conclusion:** The study reveals maternal CMV-IgG seroprevalence was 93% and primary CMV infection was considerably low. Data still convinced CMV infection associated risk factor is quite high as notably reasonable (7%) population is seronegative and indicated possibility of transmission in this kind of setting.

19.121 *Laboratory confirmed clinically suspected herpes encephalitis*

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**Purpose:** Herpes simplex virus type 1(HSV-1), type 2 (HSV-2) and varicella-zoster virus (VZV) infection cause trivial muco-cutaneous lesions and severe encephalitis in different population groups. Since specific antiviral drugs are available for these infections, sensitive laboratory test for identification of these viruses is clinically significant. In this study we present an analysis of clinically suspected and laboratory confirmed alpha herpes encephalitis at National Viral diagnostic Center, Sri Lanka.
Methods & Materials: This retrospective study included clinically encephalitis suspected samples from August 2015 through June 2016. Total of 353 CSF samples of adults and children referred to National Viral diagnostic laboratory had been tested from both immune-competent and immune-compromised patients. For molecular diagnosis DNA was extracted using a QIAamp Viral DNA extraction kit. The Real-Star Multiplex real-time PCR kit that includes primers, probes and enzymes was used to carry out the assay to determine the viral DNA. Test performed on Applied Biosystems 7500 platform, and results were analyzed. Patients were diagnosed as HSV1, HSV2, or VZV if they were positive with respective DNA. Samples were reviewed with sample quality, clinical profile and with epidemiological data.

Results: A total of 14(4%) CSF samples were positive and 339(96%) were negative for both HSV and VZV. Of the positives 8(56%) were positive for VZV, while 3(22 %) for HSV2 and 3 for (22 %) HSV1 infection. This positive group consisted of neonates (4%), infants (14%), children1-12yers (26%), and patients above 12 years (56%), also 13(93%) immune-competent and 1(7%) immune-compromised. Of the positives 70% obtained before starting the acyclovir or early course (1-3days) of treatment and 60% agreed with sample transportation policy.

Conclusion: This work convinced the multiplex Real-time PCR approach allowed simultaneous detection and genotyping of alpha-herpes viruses in CSF at any age in immune-competent and immune-compromised population. Results witnessed VZV and HSV are possible causes for encephalitis and demonstrated the VZV/HSV could lead to encephalitis in immune-competent population as well. The data suggested HSV-2 should be considered among the possible causes of encephalitis. Findings also appreciated timely collected and appropriately transported samples can maximize the yield.

19.122 Association between Trichomonas vaginalis infection and preterm labor among pregnant women in Ardabil, Iran

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Purpose: Trichomoniasis is one of the most common sexually transmitted infections in humans. The causative agent for this infection is a flagellated protozoan parasite called Trichomonas vaginalis (TV). Trichomonas vaginalis is capable of causing severe vaginitis and cervicitis, and is also linked with abnormal pregnancy outcomes, and cervical cancers. The aim of this study was to determine the probable association between TV infection and preterm labor among pregnant women in Ardabil (Northwest of Iran).

Methods & Materials: In this prospective, cross-sectional and descriptive study, two hundred women with precedent of preterm labor and two hundred non-preterm labor women who referred to Gynaecology and Obstetric unit at Alavi Hospital in Ardabil during 6 months (2016) were selected. Vaginal samples were collected from total of 400 women and examined with wet smear, staining with Giemsa and Diamond culture medium for detecting TV.

Demographic information was also obtained from voluntarily completed questionnaires. The data analysis was done using SPSS software version 23 with the chi-square and T-student tests.

Results: The prevalence of TV infection among 200 preterm labor women was reported 5% for Diamond media (gold standard test), while the rate of this infection among 200 non-preterm labor women was 1%. There was statistically significant association between the TV infection and preterm labor (sig. = 0.0062, p< 0.01).

Conclusion: Considering the main result of this study (association between TV infection and preterm labor) we recommend that women must be examined for trichomoniasis before pregnancy and the infection must be monitored and followed up during pregnancy.

19.123 Seroprevalence of Bartonella henselae and Bartonella quintana infections in children from central and northern Jordan

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Purpose: To investigate the prevalence of antibodies to Bartonella henselae (B. henselae) and Bartonella quintana (B. quintana) among children from central and northern Jordan.

Methods & Materials: Sera from 482 children were randomly collected from referenced governmental hospitals in the central and northern parts of Jordan during the period between January 2001 to March 2003. An indirect immunofluorescent assay was used to determine serum antibody titers to B. henselae and B. quintana. Sera that were reactive at a dilution >1.64 were considered positive. In addition, blood from 20 cats belonged to children with high B. henselae titers were tested using polymerase chain reaction.
**Results:** Out of the 482 serum samples examined, 53 (11%) and 20 (4.1%) had positive antibody titers for *B. henselae* and *B. quintana*. *Bartonella henselae* was isolated from 4 cats that belonged to 3 children with high antibody titers to *B. henselae*-IgG. The seroprevalence of IgG antibodies to *B. henselae* was significantly higher (*P*<0.05) in children aged 7-10-years than in younger or older ones. Having a cat in the household and having a history of cat scratches or bites were strongly associated (*P*<0.01) with seropositivity to *B. henselae*-specific IgG. Cats’ ownership and history of cat scratches or bites had no impact on the prevalence of *B. quintana*. Seropositivity to *B. henselae*-specific IgG was significantly higher (*P*<0.01) in children from northern Jordan than in children from central Jordan.

**Conclusion:** This study substantiates the presence of *B. henselae* in Jordan, documents the seroreactivity to 2 Bartonella antigens, and suggests that cat ownership and history of cat scratches or bites are important epidemiological risk factors for *B. henselae* infection in Jordan.

19.124 Prevalence and susceptibility profile of Bacterial agents of Pelvic inflammatory disease among hospital attendee in Bushenyi district Uganda

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**Purpose:** To determine the prevalence and antimicrobial activities of bacterial agents of Pelvic inflammatory disease in Bushenyi District Uganda.

**Methods & Materials:** Standard Microbiological analytical methods were used to assess the prevalence of Microbial pathogens among one hundred representative patients were adopted. Ethical clearance were sought and obtained from appropriate authorities and through participant’s informed consent.

**Results:** The investigation reveals 31.2% prevalence of bacterial etiology of PID among patients attending KIU hospital. *E. coli*, was were 100% prevalent in the samples collected from patients above 46 years of age. The age group 21-25 years has all microbes isolated including *Staphylococcus aureus*, *N. gonorrhoea*, *E. coli*, *Streptococcus species* and *Klebsiella species*. All bacterial isolates were susceptible to ofloxacin 30µg and Gentamycin 10µg with mean percentages of 89.5% and 80.3% respectively. 100% susceptibility was with *Staphylococcus aureus* and Streptococcus species were 100% susceptibility to Cefuroxime 30µg. The study showed that prevalence of microbes among PID cases was significantly dependent on age (*p*<0.01).

**Conclusion:** Pelvic inflammatory disease with polymicrobial etiology is highly prevalent among local women attending KIU hospital. Microbial etiologies of PID in KIU hospital, Ishaka are developing resistance to commonly used antimicrobial agents in Ishaka. Age was a significant factor that determined the prevalence of microbes among the population studied. The result is of public health significance and has policy implications for effective intervention

19.125 Identification of *Mycobacterium caprae* in a dairy farm in north-eastern Italy

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**Purpose:** Bovine tuberculosis (bTB) is a livestock notifiable zoonotic disease, which is globally subjected to eradication programmes. The north-eastern region of Veneto has been declared officially bTB free (OTF) since 2008 (Decision n. 2008/404/EC). However, active and passive surveillance measures are still implemented due to the potential risk for veterinary and public health. In September 2015, 24 out of 69 bovines from a dairy farm tested bTB positive (34.78%); 16 animals in the holding did not originated from Italy. The present work summarizes the outcome of the investigations carried out within the outbreak.

**Methods & Materials:** Tuberculin skin tests (TST) were performed accordingly to OIE. All the cattle in the farm were slaughtered, and retropharyngeal, tracheobronchial, mediastinal, mandibular, mesenteric, hepatic, and supramammary lymph nodes were collected and submitted to the Diagnostic Laboratory of IZSVe for pathological and histological analyses. Organ samples were delivered to the National Reference Laboratory (NRL - IZSLER) for bacteriological cultivation and PCR. The farm owner and his family were tested for tuberculosis: five people through TST, and two by thorax x-ray scans.
Results: Twenty-four lactating cows tested positive to TST, and at the slaughterhouse 24 animals showed macroscopic lesions. Five TST-positive animals did not present any visible lesion, whereas five TST-negative cattle presented lesions consistent with bTB. The Ziehl-Neelsen method revealed six positive histological samples. The NRL identified *Mycobacterium caprae* through molecular typing. No positive results were obtained on humans.

Conclusion: Despite Veneto region is officially free of bTB, *M. bovis* is occasionally detected in cattle imported from non-OTF areas. The genotype of *M. caprae* identified (Allgau type) is not actively circulating in Italy, being more commonly reported in Central-Eastern Europe. The epidemiological investigation indicated the introduction of 16 cows from neighbouring Countries in previous years, suggesting the role of movements of infected animals in the introduction of bTB in OTF regions. The outbreak detection stressed the need to keep a high alert level, as the rapid spread of *M. caprae* throughout Europe is causing great concern for veterinary and public health.

19.126 The incidence of Urinary Schistosomiasis among the people of Ohaukwu L.G.A. of Ebonyi state Nigeria

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Purpose: The essence of the study is to know the incidence of urinary schistosomiasis among the people of Ohaukwu L.G.A. of Ebonyi state. The knowledge of the disease is very poor among the people, this is due to the fact that not much research work has been in that area.

Methods & Materials: The prevalence study of urinary schistosomiasis involving 2,468 persons in nine villages of Ohaukwu Local Government Area of Ebonyi state was carried out between October 2002 and May 2003.10ml of urine samples were collected from individuals. The study was carried out in rural communities in the study area. Their natural source of water supply are the rivers, streams, ponds and irrigated farms. A simple random technique was used to select the names of villages. Well structured questionnaires were used to gather information on their knowledge about the infection.

Results: Of the sample subjects 1,215(49.2%) were positive for *Schistosoma haematobium* ova. Prevalence of the disease varied amongst villages but it was not statistically significant (p>0.05). Age differential showed a gradual increase from the less than five years old and reaching a peak in the 16-20 years of age. 575(53.2%) males were infected while 640(46.8%) females were positive for the *Schistosoma haematobium* ova in their urine. Intensity of infection in both sexes increased with increase in prevalence and had similar pattern of decrease. Altogether, 34.9% males and 29.7% females had low egg count below 50eggs/10ml of urine while males(65.1%) and females(70.3%) had high egg counts. Incidence study showed that transmission level is highest in dry season.

Conclusion: The study was able to know the prevalence of urinary schistosomiasis among Ohaukwu people. It is important to access the role of snail intermediate hosts in disease transmission for effective control measure. The result of the present study indicates high level of *Schistosoma haematobium* among the people which shows lack of proper health education to the people. Consequently, elimination of *Schistosoma haematobium* from Ohaukwu L.G.A may be difficult without controlling the snail population. Regular intake of praziquantel can also lower the disease distribution in the area.

19.127 The most common epidemiological, clinical and laboratory characteristics of patients with anthrax in Armenia

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Purpose: *Bacillus anthracis* is an especially dangerous pathogen, which can be used in biological warfare. The bacterium causes anthrax in susceptible hosts. Anthrax is endemic to Armenia. Our goal is to describe the most common epidemiological, clinical and laboratory characteristics of anthrax in patients hospitalized in “Nork” republican hospital from 2000-2015.

Methods & Materials: We used the medical charts of patients with anthrax admitted to “Nork” hospital during the period 2000-2015. The total number of patients with anthrax during that period was 20. The SPSS program was used to analyze the data.
**Results:** All patients had the cutaneous form of anthrax. Of these, 75% were males with a mean age of 46±17 years old while 85% were residents of rural areas. Livestock exposure was recorded in 80%, agricultural activities in 50%, 65% were involved in animal slaughter. The mean duration of hospitalization was 9±7 days. Main clinical symptoms were fatigue (55%), lymphadenitis (55%), fever (50%), pallor (10%), sweating (5%), headache (5%) and hepatomegaly (5%). The vast majority of patients (95%) had ulcers and carbuncles and only one had edema of the skin; these elements were located on upper extremities in 19 patients and on the face in 1 patient. Most patients (85%) received antibiotics, mainly ciprofloxacin or penicillin. In 75% of cases (15/20), bacterial culture was used for laboratory diagnosis, although culture results were positive for just 40% (8 patients) patients. In all other cases (culture-negative and no culture) PCR was used; all these results were positive. For the CBC, leukocytosis was observed in 15% of patients while ESR was elevated in 40% of cases and one case of anemia was recorded. The urine test showed 25% of patients with elevation of leukocytes.

**Conclusion:** Males living in rural areas who work with livestock are at higher risk for anthrax than urban dwellers. Skin lesions are primarily located on the upper extremities. Fever is not always present. Patients with anthrax symptoms but negative culture results should be tested with other diagnostic methods.

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**19.128 Preparing for Zika virus in Houston, Texas: A comprehensive, city-wide approach**

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**Purpose:** To present a comprehensive approach to preventing the spread of Zika to an at-risk, large municipality in the United States.

**Methods & Materials:** The Office of Surveillance and Public Health Preparedness (OSPHP) led efforts among 10 City of Houston departments (such as Public Works, Solid Waste Management, and Parks & Recreation) to: develop preparedness plans; implement an Incident Command System; ensure situation awareness; increase community awareness; reduce transmission opportunities; and provide direct interventions to reduce the threat of Zika. Houston’s action plan was built for three phases: 1) travel-associated cases identified in Houston; 2) limited local transmission of the virus; and 3) widespread local transmission in Houston and the region.

**Results:** The Houston Health Department played a pivotal role in preparing Houston and preventing the virus from being transmitted locally. Beginning in January, the OSPHP convened local stakeholders – researchers, public health authorities, petrochemical representatives, and others – to prepare cohesive, collaborative plans across the Southeast Texas region. Following this meeting, the OSPHP developed and implemented marketing campaigns, prevention strategies, surveillance improvements, laboratory technology, and policy aimed at achieving the defined goals of the response. These actions presented safeguards for Houston while improving the city’s local situational awareness surrounding the Zika virus.

**Conclusion:** Response to emerging infectious diseases demands a comprehensive, systematic approach. The city of Houston realized early that this warranted a multi-sector, inter-department response in order to mitigate the risk of Zika in Houston.
International guidelines (Disk diffusion method) for ten recommended antibiotics in veterinary medicine.

**Results:** We found no difference in the nutritional content of the raw milk samples. However, we found that twelve milk samples were contaminated with *E. coli* and *S. aureus* resistant to multiple antibiotics mainly to oxytetracycline and erythromycin (73% of all isolates). Other *E. coli* strains were resistant to Amoxicillin (n=10, 83%) and multiple resistance to fosfomycin, oxytetracycline and gentamicin (n=8, 67%). None of the bacterial isolates were susceptible (except to enrofloxacin). Samples obtained from 1098 animals (spread over forty six livestock farms) were screened for bovine TB and brucellosis. Twenty-three were positive for *Brucella* sp., representing a 4.2% prevalence. We did not find any positive cases of bovine tuberculosis.

**Conclusion:** Our findings suggest that milk raw could be a source of exposure to multidrug resistant *E. coli* and *S. aureus* strains as a result of poor livestock practices in the Sumapaz region (Colombia). Training in good farming practices is key to improve sustainable food production and avoiding antibiotic-resistant foodborne disease outbreaks. Further studies to characterize the mechanisms of antibiotic resistance are necessary.

**19.130** Molecular characterization of multidrug-resistant *Mycobacterium tuberculosis* isolated from different hospitals in Kathmandu valley

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**Purpose:** Tuberculosis (TB) is one of the deadliest and common major infectious diseases in developing and industrialized countries. Global TB control efforts have been severely hampered by the lack of diagnostic tests that are rapid, accurate, simple to use and can be applied at the point of clinical care. The aim of this study is to identify multidrug-resistant *Mycobacterium tuberculosis* in the tuberculosis patients through genotyping.

**Methods & Materials:** A total of 238 isolates from Kathmandu valley were tested for drug resistance. Extracted DNA was processed for Multiplex Allele Specific Polymerase Chain Reaction (MAS-PCR) for the detection of TB by using MPB64 and IS6110 primers and later mutation in katG and rpoB was detected using specific primers for drug resistance patterns.

**Results:** Out of 238 suspected cases, MAS-PCR was found to be positive for 35 (14.70%) isolates. Among 35 positive isolates, rpoB526 mutation and katG315 mutation was found in 5 (14.29%) and 3 (8.57%) isolates respectively. Two (5.71%) isolates showed resistance to both rpoB and katG confirming the multidrug resistant (MDR) tuberculosis.

**Conclusion:** The use of these assays in the clinical setting would significantly reduce the time to diagnosis of MDR tuberculosis, enabling the administration of appropriate treatment regimens at the outset of therapy and to estimate the economic and disease burden of tuberculosis which is essential to inform health policy, increase disease awareness and assess the impact of tuberculosis control technologies.

**19.131** Estimating variation within and between MLST types of S.aureus based on wgMLST

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**Purpose:** MLST as a genotyping method based on variation in internal fragments of seven housekeeping genes has been extensively used for study of evolution and population dynamics among *S. aureus*. The knowledge about genetic distances of genomes within and between MLST types can be useful for understanding the population structure estimated on MLST. The aim of this study was to explore the variation within/between MLST types of global collection of *S.aureus* using wgMLST.

**Methods & Materials:** 6645 whole genomes of *S.aureus* were downloaded from GenBank and analyzed with SeqSphere (Ridom) to determine MLST and wgMLST types. Loci with more than 10% missing data were excluded from analysis. As a result, eight MLST types with 5591 samples of *S.aureus* were used to estimate genetic distance within and between ST based on wgMLST data (2103 loci) calculated with Nei formula (M.Nei, 1972).

**Results:** Eight MLST types including 5591 *S.aureus* have been analyzed with wgMLST data to estimate the degree of variation within and between sequence types. Results of percentage difference within and between ST are presented in Table 1 and 2, respectively. Table1. Variation within ST based on wgMLST(%)
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<th>Mean</th>
<th>Min-Max</th>
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<tr>
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<td>0-17.4</td>
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<tr>
<td>ST8(n=1392)</td>
<td>3.9</td>
<td>0-21.0</td>
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<tr>
<td>ST22(n=987)</td>
<td>1.4</td>
<td>0-12.7</td>
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<tr>
<td>ST398(n=632)</td>
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<td>ST105(n=534)</td>
<td>1.3</td>
<td>0-5.9</td>
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<td>ST36(n=161)</td>
<td>0.5</td>
<td>0-2.7</td>
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<tr>
<td>ST239(n=128)</td>
<td>6.8</td>
<td>0-15.8</td>
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<tr>
<td>ST609(n=84)</td>
<td>0.1</td>
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Table 2. Variation between ST based on wgMSLT(%) 

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**Conclusion**: Analysis of global collection *S. aureus* isolated from different sources and places during decades demonstrated high genome difference between MLST types with many (6 out 7) differing alleles, while genetic variation within and between closest ST’s can be overlapped. It means impossible to find appropriate cut-off or rule in MST for clustering *S. aureus* based on wgMLST data to differentiate ST with single locus variants adequately, at least in some cases.

19.132 Common intestinal infectious diseases in the Republic of Armenia

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**Purpose**: The group of common intestinal infectious diseases (dysentery, yersiniosis, acute intestinal infections with unknown etiology, gastroenterocolitis with known etiology, enterovirus and rotavirus infections, nutritional toxicoinfections caused by staphylococci) are very common in the world, particularly in developing countries. The WHO estimates that about two million children worldwide die each year from diseases that cause diarrhea. This study was conducted to reveal the epidemiological situation in Armenia and to come up with the conclusions about the measures against the further development of the spread.

**Methods & Materials**: Data from the National Epidemiological Routine Surveillance of 2005-2015 were analyzed. Questionnaires, interviews, and field observations were used to assess the epidemiological situation and to find out the probable source and transmission routes for the infections.

**Results**: The incidence of common intestinal infectious diseases per 100,000 population from 2005 to 2015 was as follows: 2005: 147.6, 2006: 165.6, 2007: 167.9, 2008: 178.7, 2009: 175.2, 2010: 263.3, 2011: 242.4, 2012: 255.5, 2013: 272.4, 2014: 312.6 and 2015: 407.1. About 80% of the total cases were registered among children, which made up 1663.2 per 100,000 and which was 1.33 times higher than 2014. More than half of the cases were associated with contaminated food and in almost all foodborne cases, the cause of the infection was the incorrect preservation of food and lack of maintaining hygienic and anti-epidemic rules by vendors as well as by the consumers.

**Conclusion**: Sanitary and epidemiological control over food safety must be strengthened. Requirements for food safety should be based on evidence, taking into account relevant standards. The Law of the Republic of Armenia on food safety should be enforced. Awareness-raising programs should be conducted.
Effects of some amphizoic amoeba spread in human environments - new incidences of the emerging threat with the vision-threatening disease of public health significance

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Purpose: Some strains of free-living amoebae, ubiquitous in various natural and man-made environments, are causative agents of Acanthamoeba keratitis (AK), the vision-threatening disease mainly related to improper contact lens hygiene. Currently, AK incidences occur with increasing frequency in various parts of the world, also in Poland. A corneal epithelial injury and exposure of eye to water containing amoeba trofozoites or cysts are apart from contact lens wear other factors predisposing to AK. Clinical symptoms are similar to occurring in viral, fungal or bacterial keratitis; cysts of amoebae are exceptional resistance to chemicals thus the disease is often misdiagnosed and difficult therapeutically. Our studies have been performed for monitoring of in vitro dynamics of the new isolated corneal Acanthamoeba strains in regard to intensity of AK symptoms and management efficacy.

Methods & Materials: The slit-lamp and in vivo confocal microscopy were applied for clinical diagnosis. Corneal scraping material deriving from 25 patients with eye inflammations, contact lens wearers, suspected of AK were examined microbiologically, by culture and PCR technique. Changes in overall number of amoebae, trofozoites/ cysts proportion, status of amoeba morphology and survival time in cultures were monitored; all assays were repeated on twice, results analyzed statistically.

Results: Mixed bacterial-fungal-amoebic infections, with Pseudomonas aeruginosa, Fusarium spp, Acanthamoeba spp. in four corneal isolates and amoebic only infections in six isolates were detected. All Acanthamoeba sequenced isolates showed 98-100% homology with those available in GenBank belonging to the T4 genotype. The highest overall number of amoebae, their intensive multiplication and long, more than 20 month surviving time in culture medium indicating the strong strain viability were revealed for six strains deriving from severe AK incidences.

Conclusion: AK is serious medical problem as emerging threats of the public health. Despite advances in pharmacotherapy, treatment is often unsuccessful due to diagnostic mistakes delaying appropriate therapy and resulting in serious course of this vision-threatening disease. In vitro monitoring of dynamics/surviving time of cultivated Acanthamoeba strains isolated from affected eyes in relation to intensity of AK course may be hopeful tool for proper diagnosis and prognosis of management efficacy.

The growth of infection of Helicobacter pylori (Hp) and decreased immunity: Alarming recent trend

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Purpose: Helicobacter pylori (Hp) infection causes great social and economic damage due to high frequency in population, the high incidence of gastritis, gastric ulcer and duodenal ulcer, and gastric cancer. The goal: estimation of results of long-term monitoring of Hp-infection.

Methods & Materials: We summarized the results of 15-years prospective study of the incidence, dynamics and significance of circulating LPS/O-antigens, high molecular weight proteins, including CagA, and VacA Hp in biological fluids (feces, saliva, blood) of patients with acute intestinal diseases (more than 20,000 samples from 14,000 patients) and asymptomatic volunteers (23 persons, 8000 samples). Identifying Hp markers were carried out in reaction of coagglutination (RCA) on slides and on immunological plates with specially prepared test systems in accordance with the author's patents.

Results: It has been found the features of circulation of antigens and immune response of the organism, reflecting their interaction in tandem parasite-host, risks and prognosis of possible exacerbations during long Hp-persistence in the body. For the first time it was found a significant rise (by 17-41%) of the frequency of detection of LPS/O-antigen in feces, that is a marker of Hp multiplication in the stomach, more pronounced cyclical changes in the virulence of the Hp (CagA, VacA), which correlate with solar activity (Wolf number). Simultaneously the frequency of virulence antigens (LPS/O,
VacA, CagA) as a part of circulating IgG-immune complexes decreased in the blood and saliva, suggesting a decline of antitoxic Hp-immunity. The most pronounced abnormalities observed in 2015 and in the first half of 2016, which creates a dangerous situation of the risk of exacerbations helicobacteriosis and Hp-associated serious diseases (peptic ulcer disease, tumors of the gastrointestinal tract).

**Conclusion:** Taking into account the previously obtained data by other authors on increasing risk of occurrence of stomach cancer and of mortality with an increase Hp-infection in population, our findings about the rise of Hp pathogenicity factors in circulation and a sharp decrease in immunity in the last years indicate a serious situation with Hp infection and the need to continue monitoring of Helicobacter pylori and Hp-associated diseases.

19.135 Patient sharing and the interplay between acute and long-term care C. difficile incidence in the United States veterans health administration: A retrospective cohort study of 169 facilities

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**Purpose:** Although most acute and long-term care facilities in the same region are coupled by patient sharing and many experience high rates of C. difficile infection (CDI), the inter-facility spread of C. difficile is understudied. Our objective was to study the determinants of CDI incidence across acute care (AC) and long term care (LTC) facilities, with a specific interest in the role of patient sharing.

**Methods & Materials:** We conducted a retrospective cohort study from 2006-2012 across the Veterans Affairs healthcare system. Our outcome was laboratory-identified C. difficile infection, defined as a positive lab test in a patient having at least 3 days of facility exposure, and occurring at least 8 weeks from a previous positive test. Individual-level risk variables included age, antibiotic use, and history of acute or long-term care stay in the prior 56 days. Facility-level predictors included antibiotic use (days with therapy per 1,000 person-days), the proportion of persons with an acute or long-term care stay in prior 56 days, importation of CDI cases from AC, and importation of CDI cases from LTC per 10,000 person-days.

**Results:** 87 LTC and 82 AC facilities met our inclusion criteria. The incidence of CDI in LTC was 3.8 per 10,000 patient-days (n=6,766 cases) and was 17.9 per 10,000 patient-days (n=26,113 cases) in AC. In LTC facilities, importation of cases diagnosed in AC was common (median=75 per 10,000 patient-days). Comparatively, in AC facilities, importation of cases diagnosed in LTC was relatively uncommon (median=39, p<0.001). In bivariate weighted linear regression models, importation of AC cases was a strong predictor of increased CDI incidence in LTC (R²=0.62), but importation of cases from LTC was not as strong a predictor of AC rates (R²=0.21). Multivariate multilevel analyses confirmed the bivariate findings.

**Conclusion:** AC and LTC facilities are both impacted by importation of cases of C. difficile, though it appears that LTC facilities import substantially more C. difficile cases from AC settings compared to the converse. This research suggests that improved regional communication, and an inter-facility, coordinated approach to infection control, could help reduce CDI spread.

19.136 Seroprevalence of Toxoplasma gondii among childbearing women in Western Romania

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**Purpose:** Toxoplasmosis caused by a single-celled obligate intracellular parasite called Toxoplasma gondii, is an important zoonosis with medical and veterinary importance worldwide. The infection is usually asymptomatic in immunocompetent hosts, but may cause life-threatening consequences in immunocompromised patients and severe sequelae for the fetus. The aim of the present study was to evaluate the prevalence of Toxoplasma gondii antibodies among women of childbearing age in Western Romania.

**Methods & Materials:** Serum samples were tested for T. gondii IgG and IgM antibodies using Enzygnost® Assay (Siemens). We investigated sera of 553 consecutive women, aged 15-45 years, in whom laboratory tests were performed from May 2015 to May 2016 at “Pius Brinzeu” County Clinical Emergency Hospital in Timisoara, Romania.

**Results:** T. gondii IgG or IgM antibodies were demonstrated in 287 (51.89%) of 553 women. T. gondii IgG antibodies only were demonstrated in 282 (50.99%) women. In 5 (0.9%) women both T. gondii IgM and IgG antibodies were detected. High prevalence of T. gondii antibodies
has been observed in women of childbearing age in rural areas (67.38%) versus those from urban regions (45.93%) \( (p<0.001) \).

**Conclusion:** Our results suggest a high prevalence of *T. gondii* antibodies among women of childbearing age in Western Romania.

19.137 Epidemiology of diabetic foot infection in a tertiary care hospital in Beirut

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**Purpose:** Describe the epidemiology, demographics and clinical characteristics of patients with diabetic foot infections (DFI) in Lebanon as well as the microbiology and bacterial resistance in DFI foot ulcers, in order to help reach an optimal care in the healthcare establishments.

**Methods & Materials:** A retrospective study between January 2000 and Mars 2011 of 167 hospitalized DFI patients in a tertiary care hospital in Beirut.

**Results:** The average age was 66 years and males represented 73.65%. Duration of diabetes was 20 years with HbA1C >7 % in 79 % of the cases. 73.17 % of the patients had peripheral arteriopathy and 72.3 % peripheral neuropathy. 49.69 % had coronary problems, 48.67 % retinopathy and 47.65 % renal disease. Pseudomonas aeruginosa was the most frequently isolated bacteria from DFI (19.15 %), followed by Escherichia coli (11.91 %), Staphylococcus aureus (11.06 %), and of Enterococcus fecalis (11.06 %). Imipenem resistant P aeruginosa were 26%, ESBL-E coli 28.6% and MRSA 3.3%. Resistance to quinolones among enterobacteriaceae was 39%. Most prescribed antibiotics were: piperacillin/tazobactam, amoxicillin/clavulanic acid, and imipenme. Amputation was necessary in 36.3 % of the cases. The main risk factors of amputation were the infection itself, history of amputation and arteriopathy.

**Conclusion:** In the Lebanese population, the DFI has a male ascendancy, which affects badly controlled diabetic patients. Often, several complications of diabetes are present associated, in particular arteriopathy and peripheral neuropathy. Pseudomonas aeruginosa was the most frequently isolated bacteria. The amputation rate remains high with infection, arteriopathy and history of amputation as risk factors.


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**Purpose:** Tularemia is a bacterial zoonosis caused by *Francisella tularensis*, designated an especially dangerous pathogen. Inhalation of as few as ten organisms can cause disease. Tularemia is endemic to Armenia.

**Methods & Materials:** We have conducted a retrospective descriptive study of epidemiologic, clinical, diagnostic and treatment aspects of tularemia in Armenia among patients hospitalized in “NORK” Infectious Hospital from 2000–2015. Diagnosis was confirmed by an agglutination reaction and the clinical-epidemiology history of the patients.

**Results:** From 2000-2015 a total of 57 cases of tularemia were reported in Armenia. Of these, 84.2% were male and 78.9% were from rural areas. Distribution of age groups were: ≤18–24.6%, 19–30–54.4%, 31–44–14.01%, 45–7.0%. The most common symptoms include: fatigue (n=52, 91%), fever (n=45, 78.9%), pharyngitis (n=40, 70.2%), headache (n=27, 47.4%), hepatomegaly (n=5, 8.8%), and splenomegaly (n=2, 3.5%). The average hospitalization stay was 35.7 days. The most frequent clinical forms were bubonic (n=28, 49.1%), oropharyngeal (n=21, 36.9%) and oculoglandular (n=8, 14%). In all patients enlargement of the lymph nodes was reported, most frequently the cervical ones (n=52, 91.2%). Ultrasound imaging showed hepatosplenomegaly in three cases (5.2%). Antibiotics were given prior to admission for 26 (45.6%) patients and during hospitalization for 54 (94.8%). Twenty-three (40.4%) received monotherapy while 31 (54.4%) received combined therapy. Diagnosis was based on agglutination reaction in most cases (n=50, 87.7%). For seven patients, diagnosis was made based on only clinical-epidemiological history without laboratory confirmation (n=7, 12.3%). Surgical treatment was used for 8.8% patients. Presence of rodents at household was mentioned by 14 (24.6%), while four (7%) noted a vector bite and three (5.3%) had contact with rodents. Over half (56.2%) had centralized water.

**Conclusion:** Most of the patients were male and from rural areas. People from 19-30 years of age are more likely to contract tularemia. Approximately half of the patients had the bubonic form, mainly cervical. Other potential risk factors that might explain a long
hospitalization (e.g. suppressed immune system, treatment failure etc.), should be a topic for future investigations.

19.139 Detection of Rotavirus antigen among Pigs in Pig-Raising Communities and Institutional piggeries in Zaria and Environs, Kaduna State, Nigeria

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Purpose: This study was carried to detect rotavirus antigen and determine the risk factors associated with rotavirus infection among pigs in pig raising communities and institutional piggeries in Zaria and environs, Nigeria.

Methods & Materials: A total of 376 faecal samples from pigs of all ages were collected by purposive sampling from back yard and institutional piggeries. The samples were analysed using commercially available ELISA kit: BioK 343/2, for the antigenic diagnosis of rotavirus in porcine faeces.

Results: The overall prevalence of rotavirus antigen in pigs was 9.8% (37/376). There was a significant association between breed (p<0.05) (OR= 2.927, 95% CI=1.288-6.653) and rotavirus infection. Viral shedding was highest in the cross 15(15.5%), and lowest in the local breeds of pigs 11(5.9%). Farm type had no significant association with rotavirus. Rotavirus detection rate was higher in the institutional farms (12.0%). There was a slightly higher prevalence in the young animals than the adults with rates of 21(10.4%) and 16(9.1%) respectively.

Conclusion: This study provides for the first time data on the prevalence and risk factors of rotavirus infection in pigs in pig raising communities and institutional farms in Zaria and environs, Kaduna state, Nigeria

19.140 Whole transcriptome analysis to elucidate the role of M. tuberculosis curli pili (MTP) on host gene regulation in a pulmonary epithelial cell model

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Purpose: Bacterial adhesins, including Mycobacterium tuberculosis curli pili (MTP), are the first point of contact with host cells. MTP bind laminin and are viewed as bacterial virulence factors due to their key role in pathogenesis. In this study, we performed global transcriptomics in a pulmonary epithelial cell model to investigate the role of MTP as a potential biomarker for TB diagnostics and vaccine and drug development.

Methods & Materials: RNA was extracted from A549 human alveolar epithelial cells infected with M.tuberculosis wild type (WT) and mtp-knockout mutant strains 4 hours post-infection and sequenced using the Illumina HiSeq RNA Sequencing Platform. Sequencing reads were aligned to the human reference genome (Homo sapiens UCSC hg38) using TopHat and differential gene expression profiles were generated using Cuffdiff.

Results: The two strains induced different gene expression levels in epithelial cells. The WT and Δmtp- strains uniquely induced 307 (89 up-regulated and 218 down-regulated) and 115 (42 up-regulated and 73 down-regulated) genes respectively while 610 (345 up-regulated and 265 down-regulated) genes were induced by both strains. The wild type strain caused up-regulation genes involved in the complement system, activation of host cell receptors, toll-like receptor signalling, STAT response, cytokine production and tumor necrosis factor signalling in A549 pulmonary epithelial cells. The wild type strain also induced the enrichment of immune response-related canonical pathways, networks and upstream regulators.

Conclusion: Our gene ontology, canonical pathway and network analyses strongly suggest that M.tuberculosis invades epithelial cells with the aid of one of its major adhesins, MTP, which induces differential host gene expression leading to the host immune response against the pathogen. These findings add to the growing evidence that M.tuberculosis uses MTP as one of its major invasins and virulence factors during the infection of host cells. Our data confirms that epithelial cells play an important role in host immune response to infections. The development of rapid antimicrobial drugs that target MTP would play a huge role in preventing TB infections as these structures are mainly involved in adherence and invasion of host cells, suggesting that they could be potential biomarkers for TB vaccines and therapeutics.
Evaluation of pet animals involved in assisted interventions (AAI) as potential carriers of bacteria resistant to antimicrobials: Preliminary data

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Purpose: The attention paid to pets involved in Animal-Assisted Interventions (AAI) for their therapeutic benefits to humans is increasing worldwide. Their close contact with vulnerable categories prompts the consideration of the transmission of zoonotic pathogens between animals and patients. This study is aimed to verify the health conditions of pets used for AAI and to evaluate the potential spread of pathogens to humans, especially those causing severe hospital and community acquired-infections (HAI) such as extended-spectrum cephalosporin and carbapenem resistant Enterobacteriaceae, other Gram-negative multidrug-resistant bacteria (such as Pseudomonas aeruginosa, Acinetobacter baumannii, erythromycin and ciprofloxacin resistant Campylobacter spp., e.g.), methicillin-resistant Staphylococcus spp., vancomycin-resistant Enterococcus spp. and toxigenic Clostridium difficile.

Methods & Materials: Clinical examination of 65 animals (42 dogs, 22 donkeys and 1 horse, owned by 19 different therapists/handlers) involved in AAI was assessed. Faecal, perineal and oral/nasal samples were processed following classical microbiological procedures. The phenotypic antimicrobial-resistance profiles were determined by selective/chromogenic media supplemented with specific drugs or by the disk diffusion method according to the CLSI guidelines.

Results: The 65 animals tested were all in good health and none carried multidrug-resistant Acinetobacter baumannii, Pseudomonas aeruginosa and Klebsiella spp. nor Salmonella spp. Of the 42 dog sampled, 3 (7%) were positive to thermophilic Campylobacter spp., 1 (2%) to extended-spectrum cephalosporin-resistant E. coli, 6 (14%) to vancomycin-resistant Enterococcus spp., 3 (7%) to methicillin-resistant Staphylococcus spp., 1 (2%) to multidrug-resistant Stenotrophomonas maltophilia and 1 (2%) to C. difficile, respectively. Lastly, 1 (5%) donkey was positive to methicillin-resistant Staphylococcus spp..

Conclusion: These preliminary data highlight the good health conditions of the animals tested. Only few pets carried potentially zoonotic antimicrobial-resistant human pathogens. These data confirm that HAI are mainly associated with the spread of pathogens through person-to-person transmission and medical devices contaminations. However, our findings suggest that the presence of potential pathogens with resistant traits is not irrelevant and should be evaluated especially in animals in close-contact with immunocompromised/diseased persons. The lack of guidelines and of a systematic surveillance on pets involved in AAI as possible asymptomatic carriers of antimicrobial-resistant pathogens, poses the need to investigate their role in the transmission of these zoonoses to humans.

Seropositivity of delta hepatitis in patients with positive hepatitis B surface antigen, between 2011 and 2016

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Purpose: Hepatitis delta virus is defective RNA virus that requires hepatitis B virus to cause infection. Co-infection and superinfection are two forms of HDV according to the association with HBV. The prevalence of HDV infection is less than HBV infection but the clinical feature caused by HDV infection is more severe.

Methods & Materials: This study included a total of 1131 HBsAg positive serum samples which have sent to Microbiology Department of Trakya University Health Center for Medical Research and Practice in Edirne, between 2011 and 2016. Delta antibodies in HBsAg positive 1131 serum samples were studied by methods micro-ELISA (Diagnostic Bioprobes, Italy) HBsAg, HBeAg, anti-HBe were studied by chemiluminescence assay (Roche Diagnostics, USA). The data of patients were evaluated, retrospectively.

Results: Twenty eight of 1131 HbsAg positive serum were identified as positive in terms of delta antibodies. The ratio of HDV in HBsAg positive patients was found to be 2.47%. Twenty seven and one out of twenty eight patients were determined as superinfection and co-infection, respectively.

Conclusion: Our country is located in an intermediate endemic area for HDV. Although, seropositivity of HDV in this region has found a lower than the other regions of Turkey but also especially chronic HBV patients should be screened for HDV.
19.143 Frequency of HbeAg mutants in blood samples of Pakistani patients clinically suspected for Hepatitis B virus infection using real-time PCR

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Purpose: Hepatitis B virus can cause lifelong infection leading to cirrhosis, liver cancer and liver failure. Hepatitis B virus infection is a global health problem with 350 million disease carriers worldwide. In Pakistan, this number is estimated at be 7 million with a reporting rate of 5%. The objective was to identify correlation between HBV DNA and Hepatitis B envelope Antigen (HbeAg) in blood samples referred for Hepatitis B.

Methods & Materials: This study was conducted in the Molecular Pathology Section of the Aga Khan University Hospital. Hepatitis B virus DNA was examined on randomly selected samples. Serum samples (n-257) were analyzed by RoboGene Hepatitis B virus (HBV) Quantification Kit (Roboscreen, Germany) using Rotorgene (Real-time system). HBV serology (HbeAG) was performed on Axsym system (Abbot Laboratories, USA).

Results: Of 257 patients, 207 were males and 50 were females. The ages of patients were distributed in three groups, 0-20 years (32%), 21-40 years (51%) and 40+ years (17%). 82% of patients were positive for both Hepatitis B DNA by PCR and HbeAg. Whereas, 18% of patients were positive for HBV DNA but non-reactive for HbeAG.

Conclusion: Real-time PCR is more sensitive and also cost effective assay for the early detection in Hepatitis B Virus in patients with low viremia. It is also helpful in the identification of active HBV replication in samples carrying HbeAG mutants.

19.144 Microbiological study of Clostridium difficile infection in hospitalized patients

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Purpose: C. difficile infection (CDI) is associated with an array of clinical manifestations, ranging from asymptomatic colonization to mild diarrhea or more severe pseudomembranous colitis that may progress to toxic megacolon, intestinal perforation, sepsis and death. The aim of this study was to determine the incidence of C. difficile infection among children with hospital acquired diarrhea and to evaluate different microbiological methods for diagnosing CDI.

Methods & Materials: The study was conducted among children with healthcare-associated diarrhea who were inpatients at a university hospital in Cairo between May 2013 and June 2015. CDI was defined as the presence of diarrhea (three or more stools in 24 h), a stool culture positive for toxigenic C. difficile (TC) and a positive stool toxin assay (Enzyme Immunoassay EIA; or Cytotoxicity assay CTA). Healthcare-associated diarrhea was defined as symptom onset >48 h after admission to hospital.

All stool specimens (465) were processed by TC, only specimens showed positive results were subjected to other methods (CTA and EIA).

Results: C. difficile was isolated in 51 of 465 (11%) stool specimens. Of the 51 C. difficile isolates, 51 (100%) gave positive result by TC, 44 (86.3%) gave positive result by EIA and 50 (98%) gave positive result by CTA. All patients in this study were under antibiotic therapy. Antimicrobial classes with broad-spectrum activity, including 4th generation cephalosporins (P = 0.001), aminoglycosides (P < 0.001), levofloxacin (P = 0.032), meropenems (P = 0.012) and vancomycin (P = 0.002), were associated with CDI. The majority of patients received aggressive antibiotic therapy with more than one antibiotic during the hospitalization period (P < 0.001).

Conclusion: This study showed that antibiotic therapy remains the main risk factor for developing CDI in children. We found that the toxin EIA test is not suitable as a stand-alone test because of low sensitivity. CTA was found to be better correlated to clinical outcome compared with the TC method. We propose the use of TC as a gold standard for detection of toxigenic C. difficile, as it provides high sensitivity and specificity.

19.145 Mtb Acetyltransferase reduces the oxidative stress response and increases intracellular persistence via peroxisomal biogenesis

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**Purpose:** Characterizing a *Mtb* glycoprotein and its potential role in intracellular persistence by modulating ROS formation with the help of peroxisomal biogenesis.

**Methods & Materials:** *M. smegmatis* mc2155 and *Mtb* H37Rv were used in this study. Cloning and expression was performed in pSMT3 shuttle vector. Invasion assay in HeLa cells. Survival assay, autophagy, oxidative stress response, immunostaining by fluorescence microscope and Western blot analysis were done in macrophages.

**Results:** To identify novel *Mtb* glycoproteins, we employed a multi-lectin system to capture glycoproteins from purified *Mtb* cell wall and identified them by mass spectrometry analysis. This approach successfully identified a novel protein as putative acetyltransferase (ACTase). Recombinant *M. smegmatis* expressing the ACTase (*MsmACTase*) showed increased invasion in human epithelial cells and survival in mouse macrophages. Further studies revealed that increased intracellular bacillary burden was a result of inhibition of autophagy and ROS production in *MsmACTase* infected macrophages when compared with *Msm* wild-type and vector control (pSMT3) strains. Subsequent mechanistic studies showed that decreased ROS production was due to over expression of ROS scavenging organelle peroxisomes (PMP70, Pex5 and Pex19). Also more peroxisome formation lead to peroxisomal β-fatty acid oxidation by increased expression of ACOX-1, ACCA-1, Uox, MFE-2 in *MsmACTase* which helped the bacteria to survive under nutritional deprived conditions and could be correlated with more intracellular persistence. The same phenotypes were validated in case of H37Rv where expression of ACTase was depleted. Decreased expressions of peroxisomal markers were observed at 24, 48 and 72h post infection in mammalian cells.

**Conclusion:** We have shown that acetyltransferase gene (ACTase) of *Mtb* expressed in *M. smegmatis* aid intracellular mycobacterial survival through inhibition of autophagy and oxidative stress responses in macrophages. The present study reports that *MsmACTase* scavenges ROS due to over expression of peroxisome followed by peroxisomal β-fatty acid oxidation which provides insights a new mechanism how the pathogen surpass the host defense in *Mtb* infection. The above findings may lead to identification of a potential drug target for the antmycobacterial therapy.

19.146 Point of care urine lipoarabinomannan antigen detection for diagnosis of tuberculosis in Pediatric population

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**Purpose:** Due to pauci-bacillary nature of disease, difficulties in obtaining good quality specimen, low sensitivity of smear-microscopy and poor access to culture; rapid diagnosis of tuberculosis in children is challenging. To expedite diagnosis of pediatric tuberculosis, we evaluated Urine Lipoarabinomannan antigen (LAM) detection for its diagnostic accuracy. Young children with pulmonary tuberculosis develop disseminated disease more frequently than adults, which may increase the chances of mycobacterial antigen secretion.

**Methods & Materials:** Based on microbiological and clinical findings, 381 children including 280 and 101 of suspected pulmonary tuberculosis (PTB) and suspected tubercular lymphadenitis (LNTB) were categorized as Confirmed, sixty eight (17.9%) & 25 (24.7%); Probable, 66 (23.5%) & 20 (19.8%); Possible, four (1.4%) & 13 (12.8%); and No-TB, 142 (50.7%) & 43 (42.5%) respectively. In case of PTB, either expectorated sputum or induced sputum were taken while fine needle aspiration cytology was collected in LNTB. Samples were subjected to Ziehl-Neelsen stain, MGIT-960 culture and GeneXpert. For LAM antigen detection from urine, lateral-flow assay was performed as per manufacturer’s instructions.

**Results:** Among the PTB and LNTB cases, Eight (2.8%) and three children (2.9%) were positive by smear, 51 (18.2%) and 23 (22.7%) by GeneXpert, 48 (17.1%) and 9 (8.9%) by culture respectively. GeneXpert detected 20 and 16 cases more than culture in PTB & LNTB cases respectively and it detected 31 of 48 culture confirmed PTB cases (sensitivity 74%, specificity 91%) and 7 of 9 culture confirmed LNTB cases (sensitivity 82%, specificity 82%). On analysis of LAM assay with microbiological confirmation (either culture or GeneXpert Positive), sensitivity was 79% & 80%, specificity 71% & 67%, PPV 55% & 52%, NPV 89% & 88% in PTB & LNTB cases respectively. On adding the cases which were started with anti-tubercular therapy irrespective of microbiological confirmation, specificity of LAM assay improved to 92% & 93% and PPV to 93% & 92% in PTB & LNTB cases respectively.

**Conclusion:** GeneXpert assay & Urinary LAM antigen detection led to early detection of TB cases. Nevertheless, neither negative LAM antigen test completely rule out TB nor positive LAM test fully indicates towards tuberculosis. But these limitations were circumvented by good clinical, radiological & histopathological correlation which is needed for treatment initiation and culture continues to be gold standard.
Purpose: Cystic echinococcosis (CE) is a worldwide health problem. Humans are infected through ingestion of eggs in contaminated food, water, soil, or direct contact with dogs. Most cases remain asymptomatic. From 1970-1980 serological studies in Armenia showed 4.9% IgG positivity in rural populations and 2.3% in urban populations. The highest rates of surgical remediation for CE were reported in the Aragacotn region, where this study was conducted.

Methods & Materials: We conducted a cross-sectional systematic sampling in six villages (n=6000) encompassing 384 households. In each household one adult (> 18 y) was selected. The survey data were analyzed by EpiInfo. Detection was accomplished using the IgG antigen-strip (D-3356). The survey had 384 respondents.

Results: While most Yezidis (81%) have dogs (19% domestic/81% guard), only 48% of Armenians keep them (61% domestic/39% guard). Armenians were more educated with 63% having secondary or higher levels of education compared to 15% of Yezidis. They are also more knowledgeable of Echinococcosis (37% vs 6%) (RR 0.16 CI 0.06-0.43). However, 36% of Yezidis regularly deworm their dogs compared with just 16% Armenians (RR 2.27 CI 1.4-3.8). The use of personal protective equipment is more common among Yezidis (50%) than Armenians (44%). Being female is also protective (RR 0.27 CI 0.17-0.42). Using PPE during work: 50% of Yezidis, 44% of Armenians and sex (female) is a protective factor (RR 0.27 CI 0.17-0.42). Laboratory results showed 13 cases with IgG positivity (3.4% CI 1.6-5.2). Dog ownership is associated with an increased risk of being seropositive (RR 10.6 CI 1.4-80.6). Ownership of domestic dogs is associated with a greater risk of seropositivity for CE more than ownership of working dogs (RR 11.1 CI 1.5-84.4).

Conclusion: Armenians are more educated and are more aware of the transmission and prevention of Echinococcosis than Yezidis, but Yezidis practice more protective behavior. Owning dogs, especially small domestic dogs, was a risk factor. Awareness of the disease and adequate behavior of people while treating animals can reduce the burden of Echinococcosis.

Purpose: Leptospirosis is a water borne disease of humans transmitted through animals and distributed worldwide. The disease is characterized by high grade fever, headache, arthralgia, myalgia, meningitis followed by jaundice (icterus) and renal failure. The occupational groups like veterinarians, butchers, sewer workers, farmers working in the rice paddy fields, hunters, fishermen soldiers and all others working in water are at the highest risk in contracting the disease. The burden of disease is attributable to the countries where workforce is working in contaminated water.

Methods & Materials: Through 2 stage random sampling from 5 rice cultivating districts 125 subjects were selected, i.e 25 subjects from each union council involved in the rice cultivation and 25 subjects who were not involved in the rice cultivation were selected randomly from each union council. 5 ml blood serum was collected and tested against each of the five antigens against the serovars i.e. Leptospira interrogans var canicola, grippotyphosa, hardgo, icterohaemorrhagiae and Pomona by the slide macro agglutination test (SMAT). The results were interpreted as negative in the absence of no agglutination, followed by mild agglutination and strong agglutination.

Results: A total of 125 blood serum/samples of farmers involved in rice cultivation for the last ten years or a more from five districts of Punjab, were subject to (SMAT) test. 21(17%) farmers were found negative whereas 65(52%) farmers were mildly positive and 39(31%) were found strongly positive. The control and matched comparative group of subjects (N=125) who were never involved in the cultivation of rice during their past life, were selected from the same village from where the rice cultivators were selected and were subject to SMAT test. As a result, 72(58%) farmers were found sero-negative (p<0.05), 50(40%) farmers were mildly positive (p<0.05) and only 2(0%) were strongly sero-positive (p<0.05).

Conclusion: This study sufficiently establishes the existence of leptospirosis in Punjab, Pakistan. However, it would be essential to carry out wider scale study to know the magnitude of the problem so that suitable diagnosis, treatment and preventive strategies could be adopted to control its incidence and to improve health status of workforce.
19.149  Seroprevalence of toxoplasma gondii and hepatitis b virus infection among pregnant women attending antenatal clinic in selected health institutes of Jigjiga east Ethiopia

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Purpose: Hepatitis B Virus and Toxoplasma gondii infections in pregnancy result in a number of adverse outcomes including vertical transmission to the fetus which may have a wide spectrum of clinical diseases and neonatal death. However, HBV and Toxoplasma gondii infections remain a global public health problem; data on co-morbidities of these among pregnant women in Jigjiga, Ethiopia are scarce. Thus, the aim of this study was to determine the sero-prevalence of HBV and Toxoplasma gondii infections and associated factors among pregnant women attending ANC in the selected health institutes of Jigjiga, East Ethiopia.

Methods & Materials: A cross-sectional study was undertaken from September to January, 2013. Structured interviews were used to collect socio-demographic and obstetric data. Sera against HBsAg were screened by using HEPALISA HBsAg ELISA kits. Toxoplasma gondii infection were detected by a commercially available latex agglutination Huma Tex TOXO test kits. Data were analyzed using SPSS-19, computer software package and were summarized by descriptive statistics and binary logistic regression. Odds ratio (OR) and 95% confidence intervals (CI) were calculated.

Results: Of the 301 pregnant women studied, reactive HBsAg was noted in 18 (6%) and sero-prevalence of Toxoplasma gondii was 105 (34.9%). The prevalence of HBV and Toxoplasma gondii co-infection was 11(10.5%). None of the risk factors assessed were significantly associated with HBsAg positivity however women with toxoplasmosis were more likely to be showed eye disease.

Conclusion: Our finding showed that HBV and Toxoplasma godni infections are still important public health concerns among pregnant women in the Jigjiga area. Hence, we recommend that all pregnant women ought to be routinely screened for HBV and Toxoplasma gondii infection accompanied with health education during their antenatal visits.

19.150  Do coinfection patients with HIV/HCV respond differently to new hepatitis C treatments than HCV monoinfected patients? Data from HAVACS and the Atlanta Veterans Affairs Medical Center

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Purpose: New treatments for hepatitis C virus (HCV) using direct antivirals are providing longer sustained virologic response (SVR) rates than previous HCV treatments. Studies of previous HCV treatments found a difference in SVR between HCV monoinfected patients and those with HCV and HIV coinfection. Using data from the Atlanta VA Medical Center, where drug access issues are ameliorated, we compared treatment related outcomes in coinfected and monoinfected persons who had received a prescription of ledipasvir/sofosbuvir (LDV/SOF).

Methods & Materials: We examined clinical and demographic data for all HIV/HCV coinfected patients being treated with LDV/SOF for their HCV infection and compared them to all treated HCV monoinfected patients seen between January 2015-March 2016. Chi-square, Fischer’s exact tests, and one-way analysis of variance were used to compare characteristics across groups.

Results: A total of 351 patients were treated with LDV/SOF between January 2015- March 2016. Thirty-one were coinfected with HIV and 320 were monoinfected with HCV. Demographic characteristics were similar between these two groups other than race. Eight-seven percent of HCV/HIV patients were black compared to 65% of the HCV-monoinfected patients (p=0.0200). Treatment completion rates were high in both groups; 1 coinfected and 4 monoinfected patients discontinuing LDV/SOF during treatment (p=0.4526). Though adherence rates were quite high for both groups, HIV/HCV coinfected patients had better adherence (100% vs 83.1%, p=0.0168). Despite starting treatment with slightly higher HCV viral load levels (1,860,000 vs. 1,640,000 IU/mL), all HIV/HCV and HCV monoinfected patients had 100% virologic response by 12 weeks of treatment. Of those who completed therapy, virologic relapse was seen in 3 (12%) HIV/HCV patients and in 10 (5%) monoinfected patients (p=0.1483).

Conclusion: We found very low rates of LDV/SOF discontinuation and high rates of adherence and 4 and 12 week virologic response rates regardless of HIV status. However, we did find a higher-than-expected rate of HCV virologic relapse in the HIV/HCV patients. Though this finding was not statistically significant, it is clinically concerning and will require further study.
19.151 Clinical, laboratory and Imaging characteristics in patients with Neurological deficits in Tuberculous meningitis and its effect on patient outcome

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Purpose: Focal neurological deficits occur in 15–57% of patients with Tuberculous meningitis and are important determinants of patient outcome. This study was performed to study the clinical, laboratory and imaging characteristics in patients with neurological deficits in Tuberculous meningitis and to assess its impact on patient outcome at one year.

Methods & Materials: A total of 209 patients, diagnosed to have Tuberculous meningitis by consensus case definition were prospectively recruited, evaluated and followed for 1 year. These were grouped into patients with and without neurological deficits. Demographic, clinical, laboratory and neuroimaging findings were compared between the two groups and analyzed for significance by chi-square test. Multinomial logistic regression was done to assess patient outcome at 1 year.

Results: Any neurological deficit was present in 92 (44%) patients of Tuberculous meningitis. Motor deficit was seen in 68 (32.5%), cranial nerve palsy in 62 (29.6%) and cerebellar signs in 17 (8%) alone or in combination. The neurological deficits were significantly associated with advanced stage of disease, presence of seizures, multi drug resistance, basal meningeal enhancement, hydrocephalus and infarcts. At 1 year follow up, 26 (28.25%) patients with any deficit had died, 44 (47.8%) patients had residual neurological sequelae whereas 22 (24%) patients recovered. Basal meningeal enhancement and infarcts in the distribution of middle cerebral artery were significantly associated with poor prognosis.

Conclusion: Neurological deficits occurred in 44% of patients with Tuberculous meningitis and were significantly associated with death and residual neurological sequelae in comparison to recovery at 1 year. The possibility of cerebral infarction, basal meningeal enhancement must be considered while treating patients with TB meningitis who develop focal neurological signs as these patients are associated with worst prognosis.

19.152 Risk of West Nile virus among horses in Qatar: A preliminary study

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Purpose: 1. To determine the prevalence of exposure to West Nile virus (WNV) among horses in the State of Qatar.
2. To identify the factors that are likely to associate with seroconversion to WNV.

Methods & Materials: 1. Conduction of cross-sectional epidemiological study on possible exposure of Qatari horses to WNV through:
   a. Collection of serum samples from random number of horses reside in different localities in the State of Qatar.
   b. Screening of the samples for anti-WNV IgM Ab and anti-WNV prE Ab applying enzyme-linked immunosorbent assay (ELISA) using the commercial kits (ID-Vet, France).
   c. Collection of data hypothesized to associate with likelihood of seroconversion to WNV.
   d. Evaluation of the significance of association between each of the putative risk factors and the likelihood of seroconversion to WNV using the logistic regression statistic test.

Results: 1. Out of the totally collected 260 serum samples from horses reside at six different location 23% of the horses showed evidence of seroconversion to WNV.
2. None of the horses that were sampled had been vaccinated against WNV disease nor showed clinical symptoms at the time of sampling.

Conclusion: The study demonstrated for the first time that horses in Qatar have been exposed to WNV hinted at the possibility that the virus might be endemic in the country and has potential to pose risk to human public health.

19.153 Structural-Functional polymorphism of HBX, a potential oncogene of HBV

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Purpose: HBV is one of the leading etiological agents of hepatitis around the globe. The chronic HBV infections are also associated with the incidence of hepatocellular carcinoma (HCC). In this regard, a viral protein HBx is arguably considered to be major protein that underpins the incidence of HBV mediated HCC.

Methods & Materials: In total 250 whole genome sequences of all known genotypes of HBV and HBx were collected from the NCBI database and subjected to multiple sequence
alignment. The phylogenetic tree was reconstructed using the maximum likelihood method, employing 1000 bootstrap replicates. One representative sequence was subjected to develop the molecular model using iterative threading and homology modelling. The models were subjected to structural and thermodynamic refinements and used to develop the potential dimers on the basis of geometrical complementarity. Structural models showed significant alterations in the regions that lead to homodimerisation of HBx molecule especially at the spatial positioning of the side chains of residues involved in the formation of intermolecular complex. This suggests the variations in the dimerisation capabilities between different HBx and consequently their associated functions. However, the dimer interaction region showed a region which could further be exploited for designing disruptor/small inhibitor. Additionally, all sequences were predicted for the post translational modifications by 14 different kinases to implicate their biological functions.

**Results:** Structurally, most part of HBx protein was found as intrinsically disorder providing rationale for its promiscuity for partner proteins ensuing multiple biological roles including carcinoma. Molecular modelling of the models shows considerable structural variations among the HBx protein of different genotypes implying varying role in the HBV associated hepatocellular carcinoma. This observation is further strengthen by variations in the post translational modification profile and conformation of dimerization. Post translation modification analysis showed kinases involved in the cell cycle regulation and apoptosis tends to modify most of the sequences of HBx pointing towards their role in oncogenesis.  

**Conclusion:** The present study provide first composite picture of the structural variations in the oncogene of HBV genotypes and hbx gene and their potential role in HCC. Augmented with the further studies the present investigation may elucidate the potential targets for therapeutic intervention.

19.154 Pneumococcal meningitis: Clinical aspects, bacterial profile and clinical course

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**Purpose:** Pneumococcal meningitis is the most common bacterial meningitis at all ages. They represent a large majority of cases that need intensive care treatment. Our objective was to report the prevalence and study the clinical aspects, the bacterial profile and the clinical course of pneumococcal meningitis.

**Methods & Materials:** Retrospective study including patients hospitalized with pneumococcal meningitis between January 2005 and December 2015. Pneumococcal meningitis was diagnosed by the identification of the pathogen on the direct specimen test of cerebrospinal fluid (CSF) and / or in CSF culture.

**Results:** Among 902 cases of meningitis all forms, we collected 71 cases of pneumococcal meningitis a prevalence of 7.8%. The average age of patients was 37 years. The male outnumbered the females with a sex ratio of 2.6. Nineteen patients had a history of head trauma and ten patients had at least one comorbidity. The average time of evolution of symptoms before hospitalization was 3 days. The main clinical signs were fever (89%), meningeal syndrome (77%) and consciousness impairment (59%). The brain scan showed sinusitis in 10 cases (14%). The CSF was blurred in 62 cases (87%). The average white cell count was 3361 cells / mm3. The chemical CSF study showed low glycorachie below 2 mmol / l (85%) and an elevated protein higher than 1 g / l (100%). All cases were confirmed either by The direct specimen test on CSF, culture or PCR. Twelve patients had associated bacteremia. The minimum inhibitory concentrations of penicillin G and ceftriaxone respectively ranged from 0.008 to 0.5 mg / l and 0.006 to 0.25 mg / l. A decreased sensitivity of pneumococcus to penicillin was isolated in 5 cases. All patients had received antibiotic therapy with third-generation cephalosporin associated with vancomycin in 6 cases and rifampicin in 4 cases. Steroids were prescribed in 17 cases (24%). The outcome was favorable in 64 cases (90%), 7 patients were transferred to the ICU for neurological worsening.

**Conclusion:** In this study the prevalence of pneumococcal meningitis was high. The incidence of pneumococcal diminished sensitivity to penicillin was low. The prognosis depends on instauring therapy.
Federal state level management of an imported Lassa fever case to North Rhine-Westphalia, Germany, March 2016

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Purpose: Lessons learned from a Federal state level regarding contact person management. One imported Lassa fever case in March 2016 from Togo to Germany involved 57 contact persons and 16 local public health departments in North Rhine-Westphalia (NRW). The patient was flown out to Cologne on 25 Feb with diagnosis of malaria and sepsis and died on 26 Feb. Lassa virus diagnosis on 9 March immediately set off a number of public health measures. This case was part of a WHO outbreak investigation with one secondary case in another German Federal state and one more case in Togo, who was repatriated to the US.

Methods & Materials: Contact persons of the index case were categorised, informed and followed up accordingly to their category of contact. An anonymous line list of contact persons was available on state level with the contacts' data being investigated on local level.

Results: Overall 57 contact persons were followed up in NRW. Of those 52 were health care personnel. Contact categories included zero high risk contacts, 33 persons with increased, 18 with moderate and 6 with low risk. Public health measurements such as domestic quarantine, measuring temperature twice daily and interrogation by the local public health department were implemented. Several teleconferences were held to exchange information between the health departments and other authorities involved. One major problem was the unreliableness of some contact persons, who did not adhere to communicated control measures and provoked a number of further contacts. Six contact persons developed mild symptoms consistent with a mild Lassa virus infection and were therefore biosafety level 3 isolated. All of whom were finally tested negative for Lassa virus.

Conclusion: Information between different authorities involved has to be shared rapidly and should be facilitated by a central coordination. It is mandatory to well-inform those affected, reinforced by a written order for the contact person from the authority in charge. Implementing public health measures requires sufficient staff on all levels to be able to meet such challenges. Even one imported case may have an impact on an international level. Rapid communication and cooperation at all levels is crucial.

Survey on the impact of the Ebola outbreak in West Africa on public health in North Rhine-Westphalia, Germany

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Purpose: The Ebola outbreak in West Africa has also been a challenge to other public health systems. North Rhine-Westphalia (NRW) is the largest state in Germany with a population of approximately 18 million people. Even though no imported cases were reported, many uncertainties and questions emerged in NRW regarding preparedness for such a case.

Methods & Materials: An online questionnaire via LamaPoll has been used to identify the workload and challenges among local public health departments in NRW, Germany, during the Ebola outbreak in West Africa. The survey was anonymous and voluntary.

Results: Ultimately 40 of 54 finished questionnaires were analysed. Of those 11 were completed in an urban district and 26 in a rural district in NRW (3 not stated). People that answered the questionnaires were mainly medical doctors (31), working with infectious disease public health for 15 years on average (range 1 to 36 years). Most local public health departments in NRW had to deal with the Ebola topic, but 23 of the participants did not report any probable Ebola case and 29 less than 5 suspected cases. Nevertheless almost all participants (39) stated they had a higher workload due to the Ebola outbreak. Although 26 participants stated to have purchased PPE in their public health office, only 19 have trained donning and doffing of PPE and mere 5 are planning to continue PPE training. Also 82% of participants think to be better prepared for suspected cases of highly pathogenic infectious diseases. However, only 4 participants are allowing enough time in their department for preparedness regarding such a case. Half the participants used the epidemics preparedness plan NRW and found it to be helpful, but 25% wished to receive relevant information sooner.

Conclusion: Emerging challenges during risk assessment of imported possible Ebola cases should be used to scrutinise and improve preparedness regarding suspected and probable cases of public health significance on all levels. The NRW Centre for Health focuses to support the local public health departments as specific and timely as possible. Central coordination and networking between all players involved are required to implement public health measures efficiently.
Evaluation of Acute Flaccid Paralysis (AFP) Surveillance system in Balochistan

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Purpose: The purpose of this evaluation is to identify the weaknesses and strengths of this system.

Methods & Materials: CDC’s updated guide lines for evaluation of public health surveillance system were followed and a descriptive study was carried out in March 2011 to evaluate the system’s functioning in 2010. Literature was studied, records were reviewed in provincial health department, stakeholders were identified and information was obtained through a designed questionnaire based on system attributes by conducting in depth interviews and focus group discussions with the stakeholders.

Results: AFP Surveillance is active meaning the staff visits hospitals frequently and also goes door to door to find cases. It is simple with an easily understandable case definition. System demonstrated its flexibility by incorporating information on measles and neonatal tetanus in 2009. Representativeness is average as system has limited integration with private sectors. All 30 districts submitted reports daily to the national level in the year 2010. However there is a gap in information sharing from district to provincial (sub-national) level. Ninety percent of the reports are correctly filled. Information is shared with national & international health authority through electronic system within 24 hours. System sensitivity is 100%, whereas PVP is 5.6%.

Conclusion: We recommend integration of AFP with private sectors and report sharing at provincial level. Data analysis at provincial level is necessary for policy making due to the security-compromised situation.

Effect of parasite diversity on the levels and quality of antibody responses to Plasmodium falciparum in an area of seasonal malaria transmission

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Purpose: Antigenic polymorphism has hampered the development of effective vaccines. Antibody responses against one allelic form of an antigen have been less effective against parasites expressing a different allele. Cross-reactive antibodies with broader in vitro inhibition capacity have been induced in animal models following immunization with an allelic cocktail and a similar phenomenon may occur in humans in malaria endemic areas following repeated parasite exposure, even though the exact parasite history may not be known. Individuals in these areas may concurrently carry multiple parasite clones at any given time, and this may influence antibody quality in these individuals. We therefore assessed the effect of parasite diversity on the levels and quality of antibody responses to P. falciparum in individuals living in an area of seasonal malaria transmission.

Methods & Materials: A total of 300 stored plasma samples from study participants at two sites, one in proximity to an irrigation dam and the other at least 20km away from the dam. Samples were collected twice, in the wet and dry seasons from individuals between ages 1-70 years. Indirect ELISA was used to determine the levels of IgG to AMA1, CSP and CelTOS in plasma samples. Competition ELISA was used to determine relative proportions of cross-reactive and strain-specific anti-AMA1 antibodies. Malaria parasites were detected in participant samples by both microscopy and molecular methods.

Results: High proportion of parasitaemic individuals at the dam site was observed during the dry season (p=0.0001). Overall MOI between wet and dry seasons were 1.76 and 1.46 (p=0.001). A similar trend was observed between seasons for the non-dam site (p=0.001). No difference was observed in parasitaemia between sites. Antibody levels to sporozoites antigens (CSP and CelTOS) were higher at the dam site compared to the non-dam site, irrespective of the season. Antibody specificities to multiple AMA1 alleles were observed at sites with MOI greater than 1 and specificity to only the 3D7 allele at sites with single infections.

Conclusion: Dam could influence seasonality of malaria transmission. Anti-CSP and anti-CelTOS antibodies could be used as transmission monitoring markers. And Malaria immunity could be due to wider breadth of antibody responses rather than high parasite burden.
Detection of viral RNA in tissues following plasma clearance from an Ebola virus infected patient

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\textbf{Purpose:} An unprecedented Ebola outbreak occurred in 2014-2015 in West Africa. A better understanding of the EBOV life cycle is fundamental to develop new countermeasures, as well as to fully comprehend the pathways of inter-human transmission. We have explored the possibility of viral persistence in different body fluids' samples obtained from a Health Care Worker (HCW) infected in Sierra Leone and treated at INMI L. Spallanzani, Italy. To evaluate whether the virus was in a replicative status or simply derived from blood spill over, we compared the trends of EBOV-specific negative sense genomic RNA (neg-RNA), positive sense RNA (pos-RNA) and total viral RNA in different clinical samples.

\textbf{Methods & Materials:} Clinical samples were inactivated in BSL4 facility and RNA was extracted with QiAamp Viral-RNA Mini-Kit (Qiagen). Quantification of total viral RNA was performed with the reference test Altona-FilovirusScreen Kit1.0 (Altona Diagnostics). To measure EBOV-specific negative sense genomic RNA (neg-RNA) and positive sense RNA (pos-RNA) [including both replication intermediate (cRNA) and messenger RNA (mRNA)], L-gene specific reverse or forward primers were used in the reverse transcription step.

\textbf{Results:} The clinical samples analyzed (sputum, nasopharyngeal swab, ocular swab, urine and plasma) show different trends in neg-RNA, pos-RNA and total viral RNA levels. In Nasopharyngeal swab, Ocular Swab and Urine, total viral RNA is the only one detectable, until Day 12, 5 and 15 after hospitalization, respectively. On the contrary, in plasma, total viral RNA, neg-RNA and pos-RNA levels simultaneously decreased, starting from day 3 and becoming undetectable at Day 6. In Sputum, pos-RNA levels decreased since Day 8, persisting at detectable levels up to Day 10, coherently with total viral RNA and neg-RNA levels, which start decreasing at Day 10 and become undetectable at Day 11.

\textbf{Conclusion:} The presence of viral RNA and replication markers (pos-RNA and neg-RNA) in sputum might be of relevance in future analysis regarding the persistence of the virus in the upper respiratory tract, even after viral clearance from plasma. These results should be taken under further investigation in order to better understand the role of the respiratory tract for possible involvement in viral shedding, viral replication site or as a viral reservoir.

19.159 Burden of Chagas disease related cardiomyopathy in Guyana

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\textbf{Purpose:} A descriptive study to fill a knowledge gap regarding Chagas disease cardiomyopathy prevalence in Guyana by testing for antibodies to Trypanosoma cruzi in patients throughout the country who are identified as having cardiac abnormalities on screening Echocardiogram (ECG) and echocardiography. This has public health significance as the chronic disease phase carries a high mortality rate due to its progression to dilated cardiomyopathy in 20-30\% of cases.

\textbf{Methods & Materials:} Health workers at Regional Health Centers recruit and screen patients that present with unexplained syncope, palpitations or overt/suspected heart failure. This is occurring throughout all 10 regions of Guyana. Consenting patients undergo screening with 12 lead ECG and Echocardiography. Patients with abnormal findings including: (1) AV conduction abnormality on ECG, (2) RBBB on ECG, or (3) echocardiographic evidence of left ventricular dysfunction, have blood drawn and the Standard Diagnostics Rapid Test for T. Cruzi (sensitivity 99.3\%; specificity 100\%) performed to determine if seropositive for antibodies to T. Cruzi. We anticipate screening 1200 patients, 300 at the Georgetown Public Hospital Corporation, and 100 patients per region in the outlying 9 regions. Data collection began in July 2016. Patients with a positive Test and their local physician are provided with test results, as well as education regarding Chagas positivity and management of cardiac disease as indicated by their individual results. All Chagas seropositive patients will also receive a referral to Vector Control Services, Ministry of Public Health of Guyana.
**Results:** Expected: The seroprevalence of *T. cruzi* in patients with cardiac abnormalities will be similar to that of other countries in the Guyana Shield where it is endemic. Chagas associated cardiac disease prevalence may represent a higher preventable burden of morbidity and mortality than previously identified.

**Conclusion:** Considering the prevalence (.5%-6.7%) and significant public health burden of Chagas disease in countries surrounding Guyana and the status of Chagas Disease as a neglected tropical disease, knowledge about its prevalence in the country is critical for public health planning and vector control. With knowledge about the rates of Chagas infection, resources could be allocated to improved prevention and earlier treatment leading to decreased morbidity and mortality.

19.161 Surveillance of Zika virus infection: The experience of an adult tertiary care hospital in Singapore

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**Purpose:** Singapore is vulnerable to the importation and transmission of Zika virus as it is a travel hub with the Aedes mosquito present. Tan Tock Seng Hospital, which serves as the national outbreak response centre in Singapore, set up a Zika surveillance system after the designation of Zika as a notifiable disease in January 2016. We report our findings and describe the first confirmed case of Zika in Singapore.

**Methods & Materials:** We conducted a retrospective review on all cases screened at the Emergency Department of Tan Tock Seng Hospital between 27 January and 8 June 2016.

**Results:** 11 patients (aged 18-55 years) were screened during the study period. 55% were female. All patients had travelled to Zika-affected areas within 2 weeks of symptom onset. Eight (73%) were treated as outpatients. Of these, four were tested negative for Zika using PCR (Real-Time Reverse-Transcription) on serum. One of them was positive for dengue NS1 antigen. Zika was not tested in remaining 4 due to incompatible clinical pictures. Three patients were admitted for viral illness; all were male Singapore residents (aged 37-48 years), and presented with fever, rash, and one of the following: arthralgia, myalgia, headache or non-purulent conjunctivitis. These patients presented to hospital between 2-7 days from symptoms onset. The first Zika patient in Singapore was detected in a private hospital and transferred to TTSH isolation facility at day 4 of illness. He had travelled to Brazil (Sao Paolo & Rio De Janeiro) and presented with fever, maculopapular rash, arthralgia and headache. Zika virus remained detectable in blood and urine at day 7 and day 9 from symptoms onset respectively. He made full recovery and was discharged after tested negative for Zika PCR in blood for 2 consecutive days. Zika virus was not detected in the urine on Day 16. Vector control was stepped up at the surrounding residential areas of the Zika case, active surveillance did not detect further transmission.

**Conclusion:** The threat of importation and on-going transmission of Zika remains in Singapore. As no specific treatment or vaccines exist for Zika presently, surveillance and vector control are vital to minimise the risk of spread.

19.162 Antitherpetic activity of two fullerene derivates in vitro and in vivo

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**Purpose:** The discovery of the fullerene with its capacity to bond different chemical groups leads to synthesis of new compounds with antiviral activity. Two new synthesized fullerene derivates were studied for their antiviral activity. The objective of the work was to study the antitherpetic activity of the water soluble and water insoluble fullerene derivate.

**Methods & Materials:** Compound No. 1 – fullerenepolyaminocapronic acid is insoluble in water, compound No. 2 – sodium salt fullerenanminocapronic acid is soluble in water. Antiviral activity of the compounds was studied in Vero cell culture infected with Herpes simplex virus, type 1 (HSV) and in swiss mice intracranialy infected with HSV. The compounds were administrated intraperionaly.

**Results:** Both compounds showed antitherpetic effect in vitro. For compound No. 1 ED₉₀ was 5.0 mg/ml at virus load 100 TCID₅₀ and ED₅₀= 20 mg/ml at 1000 TCID₅₀ . For compound No.2 ED50 was 5.3 mg/ml and ED₉₀ = 9.1mg/ml .

In vivo experiments the protective effect of the compound No.2 was 20% after 1dose (100 mg/kg) and increased up to 29.8% after 5 injection (500 mg/kg). The protective effect reached 41.0 % when total dose of the compound No.2 was 1000mg/kg (total). The compound No.1 - fullerenepolyaminocapronic acid - at the total dose of 1000mg/kg protected 83.3% infected mice versus 75.0% survived mice who received acyclovir (50.0
mg\(\text{r}g\)). Mean life span of infected mice was 12.5 days in control group, 17.5 days in the acyclovir group and 17.9 days in the group treated with the compound No.1.  

**Conclusion:** Both fullerene derivates showed antiviral effect and protected Vero cells infected with HSV. The fullerene-polyaminocapronic acid was more active then sodium salt fullerene-polyaminocapronic acid. But the later is soluble in water. High protective effect was observed in mice infected with HSV treated with fullerene-polyaminocapronic acid 200mg/kg daily (total 1000 mg/r/kg) – rate of survival was 83.3%.

19.163 Prevalence, risk factors and spatial distribution of *Ascaris lumbricoides* infection in Swaziland  

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**Purpose:** Ascariasis is a parasitic disease caused by *Ascaris lumbricoides*, a soil transmitted helminth, and poses a public health threat in developing countries including Swaziland. It infects approximately 1 billion people worldwide, and account for 60 000 deaths annually. The objective was to map the ascariasis burden in Swaziland to inform interventions as the country strives for elimination by 2020.  

**Methods & Materials:** The study was a cross sectional study focusing on primary school going children. A two-stage cluster sampling was done at the Inkundla level. A total of 14 299 children aged 10-14 years from 276 primary schools were enrolled in the study. Stool samples were collected in the morning using a plastic bottle for parasitological examination using the kato-katz procedure. Behavioral data was collected from all participating children. Poisson regression analysis models were fitted to investigate risk factors of ascariasis infection.  

**Results:** The prevalence of ascariasis was 4.11% (males 4.40% and females 3.82%). Crossing rivers (IRR 1.45; 95% CI 1.16-1.80) was a major risk factor for ascariasis infection. Fishing (IRR 1.51; 95% CI 1.16-1.96) was also associated with infection. Defecating in school toilet was a risk factor for infection as those not using it were 48% less likely to be infected (IRR 0.52; 95% CI 0.30-0.91). Increasing age was associated with increasing prevalence as those aged 13 years were 44% more likely to be infected compared to those aged 10 years (IRR 1.44; 95% CI 1.10-1.89). There were regional differences as those living in the Shiselweni region were 80% less likely to be infected compared to those living in the Hhohho region (IRR 0.20; 95% CI 0.14-0.28). Not living in the Highveld was also protective as those living in the plateau were 54% less likely to contract ascariasis (IRR 0.46; 95% CI 0.24-0.88).  

**Conclusion:** Despite the availability of toilets, toilet use was not protective, and in fact found to be a risk for infection which raises questions about their state of cleanliness. There is therefore a need to strengthen hygiene education in schools. Lastly prevention and control activities should be tailored to target different regions and ecological zones.

19.164 Exploring epidemics; A Smithsonian museum endeavor for the public  

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**Purpose:** To enhance the understanding of infectious disease epidemics by the general public. To raise awareness of the causes, appropriate responses, and interventional strategies to identify epidemics at the early stages, using the One Health model of human, animal, and environmental health.  

**Methods & Materials:** The Smithsonian Institution’s National Museum of Natural History in Washington, DC, with its 7 million national and international visitors each year, began work in 2015 on an exhibition and public programming initiative on epidemics caused by zoonotic viruses. The Museum and Smithsonian’s Global Health Program (SGHP), outside experts, and multidisciplinary stakeholders are working together to open the exhibition from early 2018 through early 2020, coinciding with the 100-year commemoration of the 1918-1920 Influenza pandemic. Key messages include the interconnectedness of human, animal, and environmental health, the similarities and differences between outbreaks, the multidisciplinary nature of outbreak responses, and socio-cultural impacts.  

**Results:** Epidemics, pan-epidemics, and pandemics in the exhibition include: HIV, Influenza, Ebola, ZIKA, MERS, SARS, Nipah, Hanta, and others. The opening section will introduce the One Health concept. A later section will focus on survivors and voices from the communities affected by the epidemics. Another will focus on current and likely future epidemics, in part facilitated by a ProMED ticker. Specimens from the Museum’s immense collections will be highlighted throughout the exhibition, as will the field and laboratory work of the
Trichinellosis in western Romania a 4 year retrospective study

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Purpose: Trichinellosis, also called trichinosis, is a zoonosis caused by Trichinella spp, a helminth that infects a very broad range of animals. Humans can get the infection by eating raw or undercooked meat with Trichinella larvae encysted in muscle tissues of domestic or wild animals. Symptoms may range from very mild to severe and are related to the number of larvae consumed in the meat. The aim of the present study was to evaluate the prevalence, symptoms and risk factors of human trichinellosis in Western Romania over a period of 4 years (2012-2016).

Methods & Materials: This retrospective study included 23 consecutive patients diagnosed with trichinellosis and hospitalized at “Victor Babeș” Infectious Diseases Hospital in Timișoara, Romania, between 01.01.2012 - 30.06.2016. Medical records of these patients were investigated to collect clinical, laboratory, epidemiological, and therapeutic data.

Results: The 23 patients ranged in age from 2 to 61 years (mean = 31.9 years), 12 (52.2%) were males. Fever (69.5%), myalgia (65.2%), weakness (43.4%) and eyelid edema (34.8%) were the most frequent symptoms. High levels of white blood cells were reported in 10 (43.5%) patients and eosinophil counts ≥ 4% were reported in 21 (91.3%). Wild boar meat was the source of infection in 4 (17.4%) patients and pork meat was the source of infection in 19 (82.6%). In 43.5% of the patients, the hospitalization period ranged between 10 and 17 days and required significant health care resources. Albendazole was the antiparasitic drug administered in 56.5% of the patients.

Conclusion: Trichinellosis still remains an important public health problem in Western Romania. Sanitary education programmes for both swine breeders and consumers and implementation of strict hygienic measures are strongly recommended.

Association of Human Leukocyte Antigen alleles and Cytomegalovirus disease after Kidney Transplantation

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Purpose: Cytomegalovirus (CMV) is an important cause of morbidity and mortality in transplant patients. With more patients undergoing transplants, along with the expanding indications for immune-modulating agents, the number of patients at risk for developing CMV disease is increasing.

The aim of our study was to analyze the association of CMV disease and particular HLA genotypes in recipients after transplantation.

Methods & Materials: This study included 674 kidney transplantations between January 2009 and December 2014 and it was performed in CIURT Cluj-Napoca. The patients were divided into two groups according to the presence of CMV disease. All recipients were serum CMV IgG positive (100%), but none of them was CMV IgM positive (0%).

Results: From a total of 674 patients, 416 patients were male and 258 patients were female. The range of patients was 4-74 years. Relation of each allele to CMV disease is expressed through an OR calculated in the context of all allele at each locus. The HLA alleles frequencies were determined in patients with CMV disease and recipients without CMV disease. The difference in HLA frequencies between these two groups was statistically significant.

Association analysis of HLA-A reveals the A36 allele (p<0.05) predispose to increased risk of CMV disease. The OR values was 18.50. HLA-B with greater allelic polymorphism has two
alleles predispose to CMV disease: B48 and B57 shown by p<0.05. Concerning HLA-DRB1, our results reveals one protective allele against developing CMV disease: DRB1*09, p<0.05, and OR < 1 and no alleles for this locus which give susceptibility to CMV disease.

**Conclusion:** We have concluded that a larger number of samples will be required to confirm our data: the association of HLA-A36, HLA-B48, HLA-B57 with CMV disease and HLA-DRB1*09 may be protective against CMV disease.


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**Purpose:** Tavush Marz is a semi-mountainous area bordering both Georgia and Azerbaijan. The population (~120000) is mainly involved in cattle breeding and planting crops. The first case of visceral leishmaniasis in Tavush marz after 30 years interruption was registered in 2001. Since then 1-5 cases have been reported annually. The rural residential areas do not have sewage systems and waste removal is irregular. Stray dogs and jackals are present.

**Methods & Materials:** Retrospective analyses were conducted using epidemiological investigation cards, infectious disease registers, and ambulatory cards to define the epidemiological geography and its peculiarities for visceral leishmaniasis in the marz.

**Results:** From 2001-June 2016, 24 cases of visceral leishmaniasis were reported with two resulting in death. The annual case reports peaked at five in 2009. The majority were registered in the northern part of the marz. The five cases reported in Noyemberyan town were the most in any single locale. Twenty-two (92%) cases occurred among children under the age of 5 years old with three (12.5%) of those occurring among children under the age of one. Diagnosis and treatment was performed in Yerevan. In only four cases (25%) did the healthcare providers of Tavush Marz suspect visceral leishmaniasis. For 25% of cases diagnosis and treatment was performed after at least one month after infection. The most delayed case was addressed two months after symptoms presented. In eighteen (75%) of cases the healthcare providers of the Marz did not follow the standard definitions for visceral leishmaniasis cases.

**Conclusion:** The northern part of Tavush Marz is endemic for leishmaniasis and there is a tendency to spread to the south. These cases were reported among children up to 5 years of age. Development of the capacities are required for diagnosing and treating visceral leishmaniasis in the Marz. An intersectoral program of visceral leishmaniasis prevention and elimination should be introduced.

19.168 Accidental sexual HIV exposure: Experience of an infectious diseases unit in Casablanca

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**Purpose:** The risk of sexually transmitted infections depends of pathogenic viral agent and the type of sexual exposure. The aim of the study is to describe the sociodemographic characteristics of the victims, the conditions of sexual exposure, the serological profile of patients and their evolution.

**Methods & Materials:** This is a retrospective study conducted in the infectious diseases unit from 2004 to 2014 which included 80 patients who had consulted for a sexual exposure (SE). Data were collected from patient files and electronic records.

**Results:** The number of SE was 9 in 2004 and has increased to reach 20 in 2012 and 30 in 2013. The mean age was 28 ± 18 years [9-82], with a sex ratio 3.2. Eighty five percent of patients were single and 11% married, 10% had a known virologic status. The average timeline between the accident and the treatment was 39 hours.

The exposure was vaginal (66%), anal (20%), orogenital (6%) or anogenital (8%). It was consensual intercourse in 74% of cases and a rape in 26%. The virologic status of the sexual partner was determined in 20% of the cases with a positive virologic HIV status in 1 case. A condom was used in 46% of the sexual intercourses, and the condom was ruptured in 44%. 99% of the exposed persons beneficiated from HIV rapid test that was negative. Only 2.5% of the partners had this test that was positive in one case and negative in the other. The post exposure prophylactic treatment was started in 91% of cases, 9% were passed due or did not require one.

HBV vaccine and a serologic monitoring of hepatitis B and C were offered to all patients. Unfortunately all exposed persons except one were lost-to follow up.
Conclusion: The risk of viral transmission during intercourse varies depending on the type of exposure and the source person. Determination of HIV status of both partners is critical, and the decision to start a post-exposure HIV exposure is reserved for emergency situations at high risk of transmission.

19.169 Spotty fever tick: About 259 cases

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**Purpose:** Rickettsiosis is a re-emerging infectious disease, potentially fatal and globally widespread. Tick-borne Rickettsiosis is a tick bite fever, which includes fever, rash and an inoculation tick-bite spot. The objective was To determine the epidemic, clinical profile, the response to treatment of this disease.

**Methods & Materials:** Retrospective study conducted at the infectious diseases department at UCH Ibn Rochd of Casablanca from May the first, 1992 to 31 December 2014, which included patients hospitalized for rickettsial disease. Data were collected from hospital records and analyzed on Excel.

**Results:** we collected 259 cases with a sex ratio of 2male/1female. The average age was 43.6 years (15-78ans). Clinical manifestations were dominated by the rash in 85%, fever in 93%. The inoculation tick-bite spot was found in 43% of cases. Neurological signs were one of the criteria for hospitalization, and found in 110 patients (42%), followed by liver damage in 78 patients (30%), and pulmonary damage in 66 patients (25%). A high white cells level was noted in 72% of patients and low plats rate in 49%. A CT scan was performed in 27% of cases, objectifying brain damage in 10% of cases, namely brain atrophy (n = 5), parietal encephalitis (n = 1) and capsulo-thalamic hypodensity (n = 1).

Lumbar puncture was performed on 28 patients as in 11%, meningitis was found in 46% of cases, and a high albuminorachie in 21% of cases. Five patients had a cardiovascular event, two dysrhythmia, one case of subendocardial ischemia and junctional tachycardia and another case of distal microangiopathy. The diagnosis was retained analyzing all epidemic, clinical and biological data.

The indirect immunofluorescence serology was performed in 93% of cases and positive in 30%. Doxycyclin represented the treatment of reference for 70% of hospitalized patients, 19% were put under phenicol, 9% under fluoroquinolone and 2% under two antibiotics. The outcome was favorable in 97% of cases (n = 250), 1% (n = 3) kept a residual neurologic signs type motor deficit, deafness and psychiatric disorder.

**Conclusion:** Rickettsial disease is poorly understood by practitioners, though it is easily diagnosed with good outcome when the antibiotics are instaured early.

19.170 Sero-prevalence of *Toxoplasma gondii* antibodies in slaughterhouse workers in Khartoum state, Sudan

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**Purpose:** This study was carried to determine prevalence of anti-*Toxoplasma* antibodies in Khartoum state slaughterhouse workers and evaluate the risk factors. *Toxoplasma gondii* is one of the most common parasites that cause infection to human and animals. *Toxoplasmosis* manifests as mild non specific infection or may be asymptomatic in immunocompetent humans. It may lead to cerebral toxoplasmosis in immunocompromised humans. Congenitally acquired toxoplasmosis can lead to abortion, stillbirth or mental retardation. A recent study of farmers in Khartoum identified potential risk factors for transmission of toxoplasmosis (Manal et.al.,2009). Khalil et.al.(2012) reported a prevalence of 43.6% in human in Khartoum state while Abdel Hmeed (1991) reported a prevalence of 41.7% in Gesira state. Slaughterhouse workers can acquire infection through wounds when they handle raw meat contaminated with *Toxoplasma gondii*.

**Methods & Materials:** Using a standard venepuncture technique, blood samples were collected from 315 workers at Khartoum slaughterhouses. The blood was transferred to labeled sterile tubes, immediately stored in upright position in a cool box. Samples were transported to the laboratory, stored overnight before serum separation. Sera were stored in aliquots of 200 µl at -20°C. IgG ELISA kit (DRG Instruments, GmbH, Germany) used to
detect anti- *Toxoplasma gondii* antibodies on the collected sera. The workers were divided into groups according to age; group one (23-30) year, group two (31-37) year and group three (above 38 year). Data were statistically analyzed using Chi square test, calculated with Excel 2007 (Microsoft).

**Results:** 137 Out of 315 serum samples were positive giving prevalence of 43.49%. More positive were detected in age group (26-30) year than the other groups (p=0.0001). Prevalence rate by sex was found to be higher in females than males (p<0.05).

**Conclusion:** 43.49% of the slaughterhouse workers in Khartoum were infected with *T. gondii*. The majority of farm managers in Sudan send animals with reproductive problems to slaughterhouses so the public health importance of the seropositive livestock animals needs to be reconsidered since 56.3% of the culled dairy cows were infertile (Elfadil 2014). Raising the awareness of slaughterhouse workers and farmers on toxoplasmosis may serve in control measures.


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**Purpose:** Naegleria fowleri is an amoeba that causes a rare and a life-threatening infection of the brain with CFR 97-100% (primary amoebic meningoencephalitis (PAM). Swimming in warm fresh water, and deep rinsing of the nose with infected water are the associated risk factors. In early September 2014, the media reported many PAM deaths in Karachi. The Department of Health deputed Field Epidemiology & Laboratory Training Program fellows to investigate the outbreak and provide recommendations for control.

**Methods & Materials:** PAM cases were identified (diagnosed by CSF and PCR) in Karachi through review of records. Three patients and families of the deceased patients were interviewed and geographical coordinates of last places of residence were recorded. Water supply routes, supply methods and water sources were investigated.

**Results:** Twenty three PAM cases (3 females) were found in Karachi. Case fatality rate was 100%. Age range was 4-67 years (mean: 30). All patients were diagnosed from May to October. Fifteen of 23(65.2%) patients lived in the 2/6 districts of Karachi in a geographical cluster with one water filtration plant (total 4 pumping plants in Karachi). Fourteen (60.8%) were using municipal water and 11(47.11%) regularly used water for rinsing the nasal passages during ablution. No chlorination records were found for the years 2012-2014 for the said pump.

**Conclusion:** This was the largest outbreak of Naegleria fowleri being reported in Pakistan. A poorly chlorinated water supply and warm temperatures may have caused this outbreak. After the start of the outbreak investigation and media coverage, the government initiated corrective measures including increased chlorination of water and an awareness campaign. In 2015 (July) only 02 out of 09 new cases arose from the previously effected districts.

19.172 The results of epizootiological monitoring of the natural foci for leptospirosis in Armenia

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**Purpose:** There are permanent natural foci for leptospirosis in all regions of the Republic of Armenia. Epizootiological monitoring of natural and agricultural foci is necessary to prevent human infection. The Laboratory of Particularly Dangerous Infections of NCDCP performs such monitoring, taking into consideration epidemiological and epizootic condition of these areas including the number of rodents.

**Methods & Materials:** The highly sensitive micro-agglutination reaction (MAR) of leptospira is used to detect infection in rodents. It allows both determination of the presence of antibodies and serotyping of the agent, thus allowing the opportunity for performing a targeted epidemiological search of the infection focus. MAR serotyping is enabled by the set of 12 serotypes of leptospira cultures grown for 6-10 days. The “squeezed drop” reaction is used to study samples of animal blood dried on filter paper.

**Results:** From 2006-2015 blood samples from two to three thousand rodents captured from all the Marzes of Armenia were examined with MAR. The number of rodent infections was reported by year. The rates of rodent positivity for leptospirosis in the MAR assay was 4% for 2006-2007, 9% for 2008-2009, 5-6% for 2009-2013, 10% for 2013 and 6-8% since 2013. All
twelve serotypes were observed during the study period with three serotypes being particularly high: *L. autumnalis* (15%), *L. hebdomadis* (12%) and *L. icterohaemorragiae* (9%).

**Conclusion:** These studies have helped to characterize the natural foci of leptospirosis in Armenia which is a potential threat for humans. Continued, regular epizootiological studies are necessary to monitor this situation.

19.173 Meningococcal meningitis: 71 cases


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**Purpose:** Invasive meningococcal disease represents a public health problem and a leading cause of morbidity and mortality worldwide. Until recently no universal vaccine existed against serogroup B, which explains most of the cases in setting like Morocco.

**Objectives:** Analyze the epidemiological, clinical course and outcome of meningococcal disease in Morocco.

**Methods & Materials:** Retrospective study of all patients, admitted to department of infectious disease in UHC of Casablanca, with confirmed meningococcal meningitis between January 2003 and June 2016. The data were collected from medical records.

**Results:** A total of 71 cases were identified during a study period. The frequency was four cases a year. The mean age was 28 years [15-70]. Fifty percent of patients were men. The average time of evolution of symptoms before hospitalization was three days [1 to 15 days]. 52 % of patients were diagnosed during the winter period and the contagion was found in 2 % of cases. The main of clinical symptoms were fever (100%), meningeal syndrome (42%), purpura (21%) and disorders of consciousness (24%). The cyto-chemical cerebro-spinal fluid analysis founded a median of protein and glucose levels 4,1 g/l and 0,28 g/l respectively. The average cell count was 3893 cells/mm³ [25-35000]. Smear or culture showed the meningococcal disease in 59 cases (83%). The polymerase chain reaction (PCR) was positive in 6 cases (10%). In twelve cases, the confirmation etiology was based on blood culture. All serogroup identified was type B. Patients had received antibiotic therapy; with third-generation cephalosporin (ceftriaxon) in 47 cases (66%) and Penicillin A in 24 cases (34%). Corticosteroid therapy was prescribed in eight cases (11%). The mean hospital length of stay was 15 days. The outcome was favorable in 68 cases (96%) and three patients (4%) died.

**Conclusion:** Serogroup B invasive meningococcal infection is frequent in Morocco. Inclusion of the meningococcal B vaccine in the national immunization program should be carefully considered.

19.174 Human rabies in Morocco

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**Purpose:** In Morocco, human rabies is regarded a public health problem. New cases are reported annually despite the establishment of the National Anti-rabies program. The aim of our study is to make an inventory on this dreaded anthropozoonosis.

**Methods & Materials:** This is a retrospective study conducted in the infectious diseases department at University Hospital Center HC Ibn Rochd of Casablanca, describing the epidemiological and clinical aspects of rabies encephalitis. Data were collected from the medical records of patients hospitalized from 2008 to 2015.

**Results:** Twenty-two confirmed cases of human rabies were reported, with an average of three cases per year. Seventy percent of the cases were reported in rural areas with a higher distribution in the province of El Jadida. The average age of patients was 46.7 years [15 -79] with a male predominance [17H / SF]. The dog was the animal involved in the majority of cases (95.4%) and the cat in one case. The bite is the only type of exposure in our series, preferentially the upper limbs (63.3%). Only four patients had a rabies vaccination, incomplete and late administered after exposure. No person bitten has received rabies immunoglobulin. The average incubation period was 85 days [30 to 240 days]. The majority of patients (81.8%) had the furious form of the disease, while the paralytic was occurred in 18. 2% of cases. Research of rabies antigen by direct immunofluorescence assay in biological fluids was performed in 18 patients, were positive in all cases. Encephalitis was fatal in all cases after mean hospital duration of 58 hours.

**Conclusion:** The rabies encephalitis is not yet eradicated in Morocco, hence the importance of awareness, ongoing training of health personnel and especially the control of dog rabies through animal health surveillance.
Purpose: Tetanus, though an eminently preventable disease still ranks a leading cause of death in Morocco as well as in other developing countries despite the implementation of an expanded program of vaccination.

Objectives: Determine the epidemiological and evolving profile of patients admitted to the service for tetanus.

Methods & Materials: This is a retrospective study conducted in the infectious diseases department at University Hospital Center of Casablanca, describing the epidemiological and clinical aspects of tetanus. Data were collected from the medical records of patients hospitalized from 2011 to 2015.

Results: During the study period, 13 patients were transferred to our department after an average duration of hospitalization of 18 days (1-37). The average age of patients was 82 years (28-97), 86% of them were over 70 years old and 75% were women. The occupation exposure justified 61.53% of cases in our series. The commonest portal of entry was the lower limb (92.3%). The vaccination status of patients is unknown. Tetanus prophylaxis was not prescribed after exposure. Tetanus was of moderate severity (n = 8) and severe (n = 5) according to the classification of Dakar. The treatment consisted of an antibiotic therapy with metronidazole (n: 10), and penicillin G (n: 3), associated to benzodiazepines (76.92%). The first dose of tetanus vaccine was administered in all patients during the hospitalization. The average length of hospitalisation was five days [2-15]. The outcome was favorable in 3 cases, marked by complications such as pulmonary disease (n = 6), bacteremia (n = 2), and urinary tract infection (n = 2). The organisms isolated were Enterobacter (n = 3), Klebsiella pneumoniae (n = 2), Pseudomonas aeruginosa (n = 2) and Escherichia coli (n = 1). Bedsores was the only decubitus complication observed in four patients. No deaths had occurred.

Conclusion: Tetanus remains a public health problem in Morocco. Thus, it is essential to check the vaccination status of patients and required to include reminders vaccine in the national immunization program.

Purpose: To determine the syphilis prevalence among HIV-infected adult followed up in the infectious diseases unit of Ibn Rochd University Hospital of Casablanca.

Methods & Materials: We conducted a retrospective study of all HIV-infected patients who had an active syphilis between January 2005 and September 2015. The data was collected from electronic records (NADIS* software).

Results: We found 135 patients with syphilis out of 3688 followed up during the period of our study: prevalence of 3.66%. The mean age was 39 years. The male gender is dominant 85M/50F, and 25% of the men are MSM. We found history of syphilis in 6 patients (4%), HBV co-infection in 18 cases (13%) and HCV co-infection in 5 cases (3%). The number of syphilis infection increased up to 2014 (from 3 to 28 cases/year). The diagnosis of the syphilis infection was made at the first check-up after HIV diagnosis (78%), or during the follow up (22%). The median CD4 was 224 cells/mm3 and the mean viral load was 5.82 log10 copies/ml. Sixty percent of the patients received an active antiretroviral treatment. The clinical forms were cutaneomucous secondary syphilis in 126 cases (93%), neurosyphilis in 6 cases (4%) and tertiary form in 3 patients (2 cases of syphilitic arthropathy, and 1 case of cerebral arteritis). The patients with cutaneomucous secondary syphilis were treated with a benzathine penicillin G, and those with tertiary form and neurosyphilis with penicillin G. The evolution was good in 95% of the cases.

Conclusion: This study showed an increase of cases of syphilis among HIV-infected patients. The risky sexual behaviour among patients shows the importance of education concerning the means to prevent the sexually transmitted infections.

Purpose: The HIV epidemic in Muslim republics has its social and cultural variables that largely influence the risk behaviors related to HIV-transmission. Due to the cultural and religious traditions the sexual life is considered a private matter and is very often a taboo topic
for discussion even with the doctors which makes it difficult to evaluate the dominant routes of spread of HIV-infection. In Kabardino-Balkarskya Republic with the population of 862050 people there are 881 HIV-infected (125 newly diagnosed which is 2-fold higher considering with the previous year).

The goal of the work was to study the epidemiological aspects of HIV-infection in Kabardino-Balkarskya Republic.

**Methods & Materials:** The survey was conducted among 62 HIV-individuals who attended the Republican AIDS Center and who gave their written consent to participate in the study. Epidemiological, clinical data was collected. The genetic analysis of pol-gene region was performed by ViroSeqHIV-1 kit.

**Results:** The majority of the individuals was male(69.4%); median age 30.9 years. Among the women median age was 23.5 years. The age group of 30-35 years being most prevalent among men and the age group 18-23 years most prevalent among women (60.5% and 47.3%, correspondingly). The most frequent HIV-1 genetic form was subtype A1(71.6%). Intravenous drug use was the dominant mode of HIV-transmission both among men and among women (74.4% and 63.6%, respectively). About 25.6% of male participants were infected via sexual route. The men refused to specify whether it was heterosexual or homosexual contact. However the relatively high proportion of HIV-1 subtype B(28.4%) indicates that there is a fairly broad stratum of HIV-infected males who have homosexual contacts. Among the female participants 10 women did not want to discuss the way they were infected and was excluded from the survey.

**Conclusion:** The parenteral route of HIV transmission is dominant in the territory of Kabardino-Balkarskya Republic unlike in the European part of the country. The majority of HIV-infected are young people of age 18-30 years which indicates the risk behavior among representatives of this age group and poor knowledge about modes of HIV transmission and prevention.

19.178 Immune responses induced in individuals with dual infection Tuberculosis (TB) and HIV during antiretroviral and TB therapy

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**Purpose:** Both Tuberculosis (TB) and HIV have profound effects on the immune system and are characterized by dysregulation in cytokine secretion which can activate virus replication and negatively regulate T-cell activation. Understanding the cytokine production in patients with dual infection HIV+TB may enhance understanding the factors that determine protective immunity or susceptibility to both infections. The goal of the work was to study the impact of HIV co-infection on plasma levels of cytokines in patients with TB before and after HAART/TB treatment.

**Methods & Materials:** For the study of cytokine profile (IFN-γ, TNF-α, IL-2, IL-4, IL-10) blood samples were taken before the treatment and afterwards each 30 days over the period of 8 months. Plasma levels were measured by commercial ELISA kits (sensitivity 0.5 pg/ml) following the manufacturer’s specifications. Each sample was run in duplicate. Mann-Whitney test was performed to check for the significance differences. P value < 0.05 was considered significant.

**Results:** A total of 135 individuals were enrolled in the study: 85 patients with dual infection HIV/TB and 50 patients with monoinfection TB. All patients were drug-naïve. The majority of the patients was men: 68.5% in HIV/TB group and 81.1% in TB group. The mean age for both groups was: men 35.7 years(27÷48) and women 38.6(29÷71) years. On the whole the plasma levels of pro- and –anti-inflammatory cytokines were much higher in TB patients than in HIV/TB patients (p<0.05). The median level of all studied cytokines was significantly decreased after treatment for patients with TB monoinfection. However for patients with dual infection HIV/TB there was no significant difference in cytokine expression before and after therapy.

| Cytokine, pg/ml | HIV/TB (n=85) Before | | HIV/TB (n=85) After | | TB (n=50) Before | | TB (n=50) After |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| IFN-γ           | 13.25(0÷60)     | 4.35(0÷50)      | 25.5(11,9÷43,6) | 7.5(0÷11,6)     |
| TNF-α           | 5.1(0÷20)       | 4.65(0-12.5)    | 5.88(4,7÷6,8)   | 3.07(0÷5.5)     |
In 2015, one in 3 HIV deaths was due to TB. The timely detection and treatment of TB in HIV-infected individuals significantly reduces the risk of developing severe forms of TB and mortality. The goal of the work was to analyze the changes in TB/HIV co-infection over the period of 4 years.

**Methods & Materials:** A retrospective cohort study was carried out at the Zaharyan Moscow Tuberculosis Clinic from 2011 to 2014 years. A total of 1736 patients with TB/HIV co-infection were included in the analysis: 2011 (n=367), 2012 (n=428), 2013 (n=456), 2014 (n=485).

**Results:** In 2014 as well as in other years the majority of the patients with TB/HIV co-infection was male (69.5%): 2011 (77.9%), 2012 (76.4%), 2013 (78.3%). Both among men and women there was a stable high percentage of patients at age interval 30-39 years: 65%, 69.2%, 74.8% and 67%, respectively. The ratio of patients in age group 50-59 years and senior in 2011 was 1.9% versus 4.7% in 2014. The percentage of unemployed patients decreased from 71.1% in 2011 year to 58.8% in 2014. Of those who did not work only 2,1% had the university degree. The number of HIV-positive patients with newly diagnosed TB was: 2011-n=201 (54.7%), 2012-n=308 (72.5%), 2013-n=326 (71%) and 2014-n=357 (73.6%). The active TB-form (Mt+) among new TB/HIV cases was 66.7% in 2011 and 79.4% in 2014. If in 2011-2013 years most TB/HIV patients had tuberculosis of intrathoracic lymph nodes (33.3%, 45.5% and 38.9%, respectively), in 2014 the most predominant form of TB was disseminated pulmonary tuberculosis (41%). The ratio of opportunistic infections in 2011 and 2014 was as follows: viral hepatitis C/B- 32.3% and 70.5%; CMV – 2.5% and 17.3%, toxoplasmosis- 2.5% and 1.8%; Kaposi's sarcoma- 0.99% and 0.9%; Pneumocystis pneumonia -3.5% and 7.2%.

**Conclusion:** Thus TB in HIV-positive individuals is more prevalent among unemployed men under 40 years old and is characterized by severe clinical forms. The elevated number of opportunistic infections in TB/HIV-patients over 4 years along with the increased proportion of active TB-form among new TB/HIV cases (79.4%) indicates a low level of work on early detection of TB infection and prevention among HIV-infected persons.

**Purpose:** Tuberculosis (TB) is still a leading cause of death among people living with HIV. In 2015, one in 3 HIV deaths was due to TB. The timely detection and treatment of TB in HIV-infected individuals significantly reduces the risk of developing severe forms of TB and mortality. The goal of the work was to analyze the changes in TB/HIV co-infection over the period of 4 years.

**Methods & Materials:** A retrospective cohort study was carried out at the Zaharyan Moscow Tuberculosis Clinic from 2011 to 2014 years. A total of 1736 patients with TB/HIV co-infection were included in the analysis: 2011 (n=367), 2012 (n=428), 2013 (n=456), 2014 (n=485).

**Results:** In 2014 as well as in other years the majority of the patients with TB/HIV co-infection was male (69.5%): 2011 (77.9%), 2012 (76.4%), 2013 (78.3%). Both among men and women there was a stable high percentage of patients at age interval 30-39 years: 65%, 69.2%, 74.8% and 67%, respectively. The ratio of patients in age group 50-59 years and senior in 2011 was 1.9% versus 4.7% in 2014. The percentage of unemployed patients decreased from 71.1% in 2011 year to 58.8% in 2014. Of those who did not work only 2,1% had the university degree. The number of HIV-positive patients with newly diagnosed TB was: 2011-n=201 (54.7%), 2012-n=308 (72.5%), 2013-n=326 (71%) and 2014-n=357 (73.6%). The active TB-form (Mt+) among new TB/HIV cases was 66.7% in 2011 and 79.4% in 2014. If in 2011-2013 years most TB/HIV patients had tuberculosis of intrathoracic lymph nodes (33.3%, 45.5% and 38.9%, respectively), in 2014 the most predominant form of TB was disseminated pulmonary tuberculosis (41%). The ratio of opportunistic infections in 2011 and 2014 was as follows: viral hepatitis C/B- 32.3% and 70.5%; CMV – 2.5% and 17.3%, toxoplasmosis- 2.5% and 1.8%; Kaposi’s sarcoma- 0.99% and 0.9%; Pneumocystis pneumonia -3.5% and 7.2%.

**Conclusion:** Thus TB in HIV-positive individuals is more prevalent among unemployed men under 40 years old and is characterized by severe clinical forms. The elevated number of opportunistic infections in TB/HIV-patients over 4 years along with the increased proportion of active TB-form among new TB/HIV cases (79.4%) indicates a low level of work on early detection of TB infection and prevention among HIV-infected persons.

**Purpose:** The donor’s plasma and its products are used widely and the risk to be infected with dangerous viruses (hepatitis B virus, HIV, arborviruses) is not a miracle. The inactivation of viruses in plasma is highly complicated task due to the possible damages of plasma proteins. The objective of the work was to study the efficiency of millisecond technology (MST) to inactivate viruses in media and plasma.

**Methods & Materials:** Herpes simplex virus, type 1 (HSV), and poliovirus, Sabin strain, type 1ab, were used in the study. The Eagle MEM media or donor’s plasma was contaminated with virus and was treated in the unit MST “Pharma”. The principle of the method: microdroplets (50-150 microns) were obtained by spraying the liquid into the reactor through a nozzle. The microdroplets were treated with low heat-pressure (millisecond) changes of the pressure (P) and temperature (T). The samples of the virus contaminated media or plasma were taken before and after the treatment, and were tested in Vero cell culture for the infectious virus.

**Results:** The Eagle MEM media contaminated with HSV (DNA enveloped virus) with the titer up to 4.5 log TCI D50 was treated in MST device “Pharma”. The following conditions were studied: T=74.2°C – P=10 bar, T=65.0°C – P=11bar, T=60.0°C – P=11bar and T=54.2°C – P=12bar. No infectious virus was detected in all samples taken after the treatment. The
Clinical settings, but markedly less in active sore throat management programmes (14.6%, 23.6%) in active's presence.

Results: The search identified 205 eligible studies. The prevalence of GAS culture-positive pharyngitis in children <20 years in OECD countries was 23.3% (95% CI: 22.9-23.6%) in clinical settings, but markedly less in active sore throat management programmes (14.6%,

Conclusion: It was shown that the new approach to inactivate infectious viruses with millisecond technology can be used to inactivate both enveloped and non-enveloped viruses, RNA and DNA-viruses in liquid and in donor's plasma.

19.181 The risk of sustained sexual transmission of Zika virus infection in England, United Kingdom

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Purpose: Emerging infections, such as Zika virus (ZIKV), have a predilection for the unexpected. Of increasing concern, after being documented in isolated case-reports, is the ability of ZIKV to be transmitted sexually. To date, sustained chains of ZIKV within sexual networks, as observed in many sexually transmitted infections (STIs), have not been described. Here, the risk of sustained spread of ZIKV in England by sexual transmission is assessed.

Methods & Materials: Due to the dearth of information on sexual transmission of ZIKV, national experts on emerging infections, flaviviruses and STIs were consulted by questionnaire for their consensus opinion on the possibility of tertiary and sustained sexual transmission of ZIKV and its potential impact. These opinions and compiled case data were used to qualitatively assess the risk of sustained sexual transmission of ZIKV within England.

Results: People in monogamous partnerships as well as those in heterosexual or lesbian partnerships were evaluated as having an exceptionally low risk of initiating tertiary sexual transmission. In contrast, the risk of tertiary transmission within populations of men who have sex with men (MSM) was assessed as dependent on sexual behaviour. The highest risk group was the subset of MSM in dense sexual networks of multiple concurrent sexual partners, a known risk group for outbreaks of emerging sexually transmitted infections such as lymphogranuloma venereum. However, the risk of such an event occurring within this high risk network in England was assessed as very low, as it is predicated on engagement in high risk activity during semen ZIKV positivity. Importantly, the risk of congenital infections from sustained chains of sexual transmission was assessed as exceptionally low as most MSM in these networks are thought to exclusively have male partners.

Conclusion: There is a theoretical possibility of sustained chains of sexual transmission of ZIKV however; the risk of such an event was assessed as very low. Nevertheless, advice on reducing the risk of sexual transmission of ZIKV should be targeted at all sexually active individuals, regardless of sexual orientation and behaviour.

19.182 Group a Streptococcus pharyngitis and pharyngeal carriage a systematic review

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Purpose: Children living in impoverished conditions face an increased risk of acute rheumatic fever (ARF), particularly in low- and middle-income countries. ARF also persists in some deprived populations living in developed countries. Antibiotic treatment of Group A Streptococcus (GAS) pharyngitis is important for preventing these at-risk children developing ARF. In some settings, organised active sore throat management programmes operate with the aim of treating GAS pharyngitis. A proportion of patients with GAS culture-positive pharyngitis will be GAS carriers with acute viral infections, and do not need antibiotics. However, GAS carriage is usually not identified and these cases would receive antibiotics unnecessarily. This review aimed to assess the prevalence of GAS pharyngitis and carriage in different settings.

Methods & Materials: We performed a systematic literature review and generated pooled prevalence estimates for GAS culture-positive pharyngitis, serologically-confirmed (true) GAS pharyngitis, and asymptomatic pharyngeal carriage. Findings were reported by age group, recruitment method and country income level.

Results: The search identified 205 eligible studies. The prevalence of GAS culture-positive pharyngitis in children <20 years in OECD countries was 23.3% (95% CI: 22.9-23.6%) in clinical settings, but markedly less in active sore throat management programmes (14.6%,
95% CI: 14.5-14.7%). GAS culture-positive pharyngitis in children was significantly more prevalent in non-OECD countries (24.5%, 95% CI 23.0-25.1%). In children from OECD countries, the prevalence of serologically confirmed (‘true’) GAS pharyngitis was 10.9% (95% CI 9.6-12.3%) and asymptomatic GAS carriage was 11.9% (95% CI 11.9-11.9%). The sum of the prevalence of asymptomatic carriage and serologically-confirmed GAS pharyngitis approximately equals the prevalence of culture-positive pharyngitis.

**Conclusion:** These findings have important implications for clinical sore throat management and ARF prevention. The portion of a population that has true GAS pharyngitis can be predicted by subtracting the prevalence of asymptomatic GAS carriage from that of culture-positive GAS pharyngitis. In clinical settings, approximately 50% of children with GAS culture-positive pharyngitis have true GAS pharyngitis. In active sore throat management programmes, the prevalence of GAS detection is lower, and if it declines towards 12% (the asymptomatic carriage rate), there may be little benefit in continuing the programme as the majority of culture-positive cases are likely to be carriers.

19.183  Factors affecting infant feeding decisions and practices among HIV positive women attending nasara clinic, abuth 2016

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**Purpose:** This study assessed the factors affecting infant feeding decisions and practices among HIV positive women attending ABUTH

**Methods & Materials:** A descriptive cross sectional study was carried out in May 2016 at Nasara PMTCT clinic, ABUTH Zaria. Sixty five HIV positive mothers were selected by systematic random sampling. Data was collected using a semi-structured questionnaire by electronic data capture method using Epi Info mobile and analysed using SPSS version 21 software. Knowledge on infant feeding options was assessed using the Bloom’s cut off points. Univariate analysis using frequency was done to determine proportions, while bivariate (Chi-square and Fisher’s exact) and multivariate analyses (Binary logistic regression) were done to determine associations between the preferred feeding choices and the factors affecting the decisions.

**Results:** The findings revealed good knowledge (60%) of the infant feeding options among the respondents. About 42.9% of the women felt the counsellors were not convinced to certain limit which method was best, hence they not confidently guide them on the correct options. The adoption of infant feeding options as recommended by WHO was not optimal. Reasons included deterrents like financial limitations in 36.9% of respondents which was statistically significant at the bivariate (p = <0.001) and multivariate (p=0.013, odds ratio = 32.779) levels. This showed that respondents with financial constraints were 32 times less likely to practice the ideal feeding options than women who are not financially constrained. Also, sociocultural norms and beliefs in 40%, and the knowledge of risks associated with some feeding practices (38.5%) statistically significant (p=0.022 and 0.023 at bivariate and multivariate levels respectively). In the course of feeding their children, the respondents had challenges of insufficient breast milk, stigma and discrimination, among others.

**Conclusion:** High proportions of respondents know the recommended infant feeding options, though the adoption of, and adherence to the ideal methods is not practiced. Sociocultural norms, fear of stigma, discrimination, and financial constraints affect their ability to do so. It is recommended that effective counselling services should be made available to HIV positive women in the hospital by trained hospital personnel to guide such women on making correct choices.

19.184  Seroepidemiological study to assess visceral leishmaniasis in Armenia, 2015

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**Purpose:** Leishmaniasis is a vector-borne disease caused by protozoan parasites of the Leishmania genus which is transmitted by Phlebotomine sandflies. The first case of visceral leishmaniasis (VL) in Armenia was registered in 1913. From 1926-1969, 919 cases of VL...
were reported at different altitudes with the main focus being Yerevan where more than 80% of the cases were situated. A 1970 survey of dogs showed an infection rate of 7.3%. No VL cases were reported between 1969 and 1999. However, since 1999, 99 indigenous human cases of VL have been reported, mainly in Yerevan and in the Syunik, Lori and Tavush regions. The incidence rate is 0.03-0.6 per 100000. As most VL cases are asymptomatic, the objective of the study was to identify the real prevalence of VL in Armenia.

**Methods & Materials:** A systematic random sampling, pulled from the medical facilities' registry of children less than six years of age, was conducted. Dogs living within a 1500 meter radius of a human case residence were sampled. Leishmanial antibodies were tested using immune-chromatographic rapid tests rK 39. A standardized questionnaire was developed that contained 28 questions on demographic, epidemiological and clinical characteristics and on knowledge, attitude and practice questions regarding leishmaniasis and its prevention.

**Results:** Four of the 1238 children tested were positive (0.3%) including 2/91 in Syunik (2.2%) 1/100 in Tavush (1.0%) and 1/200 in Lori (0.5%). In these regions, 146 dogs were tested with eight positives (5.5%): four in Syunik (9.3%), two in Tavush (8.0%), one in Lori (5.6%), and one dog out of 50 tested in Yerevan (2%). The vast majority of parents (93.5%) had not heard of leishmaniasis. The highest levels of disease awareness were observed in Syunik (21%) and the lowest levels in Lori (2%).

**Conclusion:** Visceral leishmaniasis is a re-emerging problem in Armenia, with a high epidemic potential because of its canine reservoir. It is recommended that intersectional collaboration be strengthened including dog registration and vaccination against leishmaniasis, vector-control and host-reservoir treatment programs. Programs that raise public awareness are required.


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**Purpose:** As the regional reference laboratory for Lombardy (a region of Northern Italy) we participate to the RotaNet-Italy Study Group, in the framework of the EuroRotaNet surveillance network. In this study, group A rotaviruses (RVA) from cases of acute gastroenteritis (AGE) were investigated with the aim of evaluating the co-circulation of different strains and the emergence of uncommon or unusual rotavirus genotypes. Although most of RVA infections are supported by common genotypes G1–G4, and G9 associated with either P[8] or P[4], an unexpected spreading of uncommon genotypes could occur, suggesting gene reassortment events between strains of different origin.

**Methods & Materials:** During the first part of 2015-2016 rotavirus season, 38 positive-RVA stool samples were collected from as many children (median age: 27 months; IQR: 38.9 months) with AGE admitted to ‘ASST-CREMONA’ hospital in Cremona, a city of Lombardy region accounting for nearly 71,000 inhabitants. Molecular characterization of RVA, based on EuroRotaNet methods and algorithms, was performed by the regional reference laboratory located at the University of Milan in collaboration with the Istituto Superiore di Sanità, which coordinates the project at national level.

**Results:** A single rotavirus infection was recorded in 97.4% (38/39) of AGE and a double rotavirus infection was detected in one out 38 children (2.6%). According to G/P-type RVA characterization, the most predominant combination was the uncommon G12P[8] strain, being present in 39.5% (15/38) of children. G1P[8] was detected in 26.3% (10/38) of samples and G9P[8] and G4P[8] were identified at the same extent (6/38:15.8%); one (1/38:2.6%) RVA was G2P[4] and no G3P[8] combination was detected during the study period.

**Conclusion:** This study reports for the first time a predominant and overwhelming introduction of the uncommon genotype G12P[8] RVA in children hospitalized for AGE in a small area of Northern Italy. Continuation of RVA monitoring activities by the RotaNet-Italy can help defining a baseline of circulating RVA genotypes before mass vaccination and assess possible genotype replacement once rotavirus vaccination will be introduced.

19.186  Prevalence and risk factors of HIV Infection among prisoners in Punjab, Pakistan-2013

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**Purpose:** Sundas Foundation (An NGO; arranges blood camps for thalassemic patients) reported high prevalence of HIV among volunteer prisoner donors. It initiated intense media coverage and inquiries by the provincial legislative assembly. So the Inspectorate of Prisons
Punjab planned to conduct this study. To assess the prevalence and risk factors for HIV infection among prisoners in prison X of Punjab Province.

**Methods & Materials:** All willing Prisoners incarcerated in prison X were screened for HIV infection during 15th February – 31st August, 2013. All HIV positive cases were tested for Hepatitis-B & C and Tuberculosis co-infection using the ELISA. Their CD 4 count and routine blood tests were also done. A case-control study; selecting age matched controls with 1:2 ratio from incarcerated prisoners, was undertaken to determine association of behavioral risk factors and practices.

**Results:** Of the screened three thousand and seventeen male prisoners; having mean age of 30 years (range 10 - 85 years), 41 (1.36%) were positive for HIV. Case rate was highest among 16-20 years group and 98% cases were up to 40 years age. 88% cases had co-infection with Hepatitis-C and 8% with Tuberculosis too. CD 4 count and haemoglobin were low among 48% cases. For case-control analysis, 22 cases and 44 controls were selected. Cases were more likely to be involved in addiction (OR 11; 95% C.I. 2.8-43), injection drug use (OR 9.7; 95% C.I. 2.0-44), narcotics sale & theft (OR 7.0; 95% C.I. 2.3-24), sex with commercial sex workers (OR 34; 95% C.I. 3.0-335) and homosexuality (OR 16; 95% C.I. 1.8-144).

**Conclusion:** Prevalence of HIV among prisoners was 14 times high than general population. Multiple risk factors for HIV transmission were identified among prisoners. Screening of prisoners at the time of entry, targeted health education and risk reduction programs were recommended for prisons. Sundus Foundation decided not to collect prisoner blood donations in future.

19.187 Horizon scanning through media mining to identify medical products of human origin associated infectious risks: A pilot NOTIFY Project study


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**Purpose:** Accomplishments in medical science have led to the therapeutic use of a wide range of medical products of human origin (MPHO) that, however, have a shared exposure to risks, such as donor to recipient transmission of pathogens. The goal of the NOTIFY Project is to promote and maximize the use of vigilance and surveillance (V&S) from procurement to clinical application of MPHO at a global level.

We have performed a systematic review of informal and formal reports resulting from media mining in order to identify common and (re)emerging infections that feature possible threats to MPHO safety, initiate a core global network of public health specialists in MPHO-associated risk and keep the NOTIFY Library of adverse occurrences in transplantation, transfusion and assisted reproduction updated.

**Methods & Materials:** A 24/7 horizon scanning strategy for infectious risk associated with MPHO was designed in collaboration with the department of Global Preparedness, Surveillance and Response of WHO using the Hazard Detection and Risk Assessment System (HDRAS), that integrates a large number of distributed electronic resources. To make our search more specific, we have included the NOTIFY Library's MPHO and pathogen comprehensive taxonomy as additional screening parameters. Once the results started to appear, we were able to rate the significant items (TR topic related: RE relevant, HR highly relevant).

**Results:** A total number of 136 notifications were captured during the one month study period. Specifically, 39 notifications were MPHO-related (TR, 28.7%), 27 of which MPHO-safety related (RE) and 12 MPHO-derived infection transmissions (HR). The mean number of notifications per day was 4.6 (min 0, max 12). Outcomes have been discussed with US-CDC and E-CDC on weekly basis teleconferences.

**Conclusion:** A real time horizon scanning strategy through media mining could be a new V&S tool to support MPHO safety and contribute to the improvement of individual and public health. The initial results of this innovative approach justify further assessment over a longer period to search for possible refinements of its use and explore potentials in collaboration with national authorities in charge of MPHO safety at a global level.
19.188 Infections and antimicrobial use in Hungarian long-term care facilities in 2015: A questionnaire-based survey to evaluate initiatives and future developments
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Purpose: Due to the aging population of Europe there is a growing need for long-term care facilities (LTCF). Elderly are at higher risk of impaired immune system due to multiple underlying, chronic diseases, malnutrition, dehydration, and/or use of immunosuppressant medication. Therefore, healthcare-associated infections (HAI) and antimicrobial use can be significant among residents in these settings. This can contribute to the growing problem of antimicrobial resistance in LTCFs, including multidrug-resistant organisms. Due to the lack of national data, our purpose was to establish baseline rates and to identify priorities for improvement.

Methods & Materials: All LTCFs were invited to participate in our questionnaire-based survey between January and December 2015. The assessment covered infection prevention-related topics including: facility demographics, infection prevention practices (e.g. presence of written guidelines, hand hygiene), the most common types of infections and antimicrobial use. Descriptive statistics were used to present the data.

Results: A total of 546 LTCFs (43% of all authorized facilities in the country) participated in the survey covering 40,562 beds. The median number of available beds per facility was 74 (range: 10-720). A minority of LTCFs (2%) had assigned an infection control practitioner. Overall, there was a high availability of protocols for hand hygiene, for prevention and management of MRSA infection/colonization and for the prevention of bloodborne pathogen transmission. 70% of LTCFs reported that their residents acquired infections in the preceding year. The most common were respiratory (88%), urinary (76%) and skin/soft tissue (28%) infections. 66% of LTCFs used antimicrobials. Most frequently used antibacterials were beta-lactams, penicillins (ATC J01C) (34%), quinolones (ATC J01M) (29%), and macrolides, lincosamides and streptogramins (ATC J01F) (13%).

Conclusion: Our results indicate that HAIs and antimicrobial use constitute a relevant public health problem in LTCFs in Hungary. We have an urgent need for a national recommendation for good practice in LTCFs in order to prevent HAIs and inappropriate antimicrobial therapy leading to the risk of multidrug resistant pathogen development. In addition, limitation of antibiotic use and continuing education of prescribers on antimicrobial treatment are essential.

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Purpose: The second most common health-care associated infection is surgical site infection according to recent national and international prevalence studies, highlighting its public health importance. Although effective preventive measures exist the infection rates have not declined in the last years in Hungary regarding the National Nosocomial Surveillance System. In order to improve the national surveillance system by focusing on most frequent surgical categories with high infection rates we compared rates of surgeries performed in Hungary.

Methods & Materials: The analysis was based on the Hungarian National Health Insurance Found dataset of procedures performed in acute care hospitals in Hungary between 2011 and 2015. Results interpret the aggregated five year period. The vast majority of procedures are covered by the institution. Surgeries were grouped according to the worldwide used National Healthcare Safety Network (NHSN) operative procedure category mappings by Centers for Disease Control and Prevention (CDC) for surgical site infection surveillance. For the calculation of the numbers of operations per 100,000 inhabitants the Hungarian Central Statistics Office’s yearly population data was used.

Results: Beside the generally monitored categories, like Caesarean section, cholecystectomy, colon surgery and hip prosthesis (with 322.3, 240.7, 144.4 and 122.9 operations per 100000 per year), other, rarely under surveillance operations, frequency was high (herniorrhaphy, exploratory laparotomy, ovarian and breast surgery with 480.6, 282, 131.1 and 114.3 operations per 100000 per year, respectively). Operations with potentially higher infection rates as limb amputation, appendix surgery and hysterectomy (abdominal and vaginal combined) were also frequently performed (102.8, 100.8 and 99 operations per 100000 per year). As known, number of laminectomy, knee prosthesis, cardiac and bypass...
Conclusion: Taking into account the high numbers of certain surgical procedures even with low infection rates they can affect numerous patients. Under surveillance elevated attention could force early detection of infection or even more careful application of preventive measures. Surveillance of frequent operation categories with limited number of procedure types involved has to be considered on national or even on European level, e.g. herniorrhaphy, ovarian surgery.

19.190 Risk factors and prevalence, hepatitis B virus and hepatitis C virus among prison inmates, Chennai, India, 2015

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Purpose: Prisoners are considered to be at high risk for Hepatitis B (HBV) and Hepatitis C (HCV) virus infections due to their social behaviour. We conducted a study to determine the prevalence of hepatitis B virus and hepatitis C virus among prison inmates and identify risk factors for infection.

Methods & Materials: A Cross sectional, anonymous survey was conducted using questionnaire among the inmates of Puzhal prison, Chennai, India. We included self-completed risk factor questionnaire consisting of criminal background, education, period of stay in jail, drug addiction, marital status, sexual activity, and clinical complaints. Serum samples were tested for antibodies hepatitis C (HCV) and hepatitis B surface antigen (HBsAg).

Results: 1381 prison inmates participated in the study. The study population were between 20-65 years (median age 30±5) with 1258 of them males and remaining were females. Of the 1258 males, 926 were married. 76.6% of the males gave history of penetrative sex; while 70% of them gave history of having sex with women only and 40% of them were faithful to their partners. Some 15% were homosexuals and 5% were bisexuals. While 10% gave a history of having multiple sexual partners. About 81% reported unprotected sex with commercial sex workers (CSWs), 20% had a history of intravenous drug abuse. Nearly 45% were not aware of mode of transmission of AIDS. Among women 112 were married and 90% had penetrating sex and 27% multiple sex partners. On examination 11% had active hepatitis with or without a history of jaundice in the last 2 years. While 3.89% men and 1% women were positive for HBsAg 1.27% men and none of the women women, were found to be positive for anti-HCV antibodies. The risk factors which were significant among those found positive were intravenous drug use, frequent commercial sex worker visitor and homosexual behaviour.

Conclusion: This study reveals that Hepatitis B and C infections are prevalent among the prisoners. These findings confirm the need for interventions including health education with a view to modify their at risk behavioural practices.

19.191 Chlamydia trachomatis prevalence and serovar distribution in patients with urogenital infections

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Purpose: Chlamydia trachomatis are one of the most prevalent sexually transmitted infections. Limited data is available on typing of C. trachomatis in India. The aim of the study was to determine the prevalence of C. trachomatis in patients with urogenital infections and to characterize C. trachomatis strains by PCR-RFLP and ompA gene sequencing. Prevalence of other etiological agents of STI (viz., Ureaplasma spp, Mycoplasma hominis and M. genitalium) were also assessed.

Methods & Materials: Endocervical swabs were collected from 324 women and urethral swabs and urine samples were collected from 193 men attending the STD clinic. The samples were screened for C. trachomatis by cryptic plasmid PCR, ompA gene and nested ompA gene PCR. Genotyping was performed by PCR-RFLP and sequencing. Samples were screened for genital mycoplasmas by culture and PCR.

Results: The prevalence of C. trachomatis in men and women were 15.0% and 10.8% respectively. Serovar D, was the most prevalent followed by serovar E, F, I and G. Serovar G was detected for the first time from India. Twenty-two C. trachomatis strains were selected for
ompA gene sequencing. Neither variants nor mixed infection were picked. Both PCR-RFLP and ompA gene sequencing shows concordant results. Co-infection of C. trachomatis was significantly associated with U. urealyticum and HIV.

**Conclusion:** Patients infected with C. trachomatis have significant risk of being infected with U. urealyticum and HIV, suggesting screening of C. trachomatis alongwith other STIs. Further, genotyping studies are required to understand the geographical distribution and introduction of new serovar in the community.

19.192 Characteristics of tuberculosis (TB) and human immunodeficiency virus (HIV) coinfection and adverse effect of treatment in a cohort of hospitalized patients in Medellin, Colombia

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**Purpose:** Antiretroviral therapy has been available several years ago. We aimed to evaluate current clinic, epidemiologic and microbiologic features, resistance patterns, adverse events associated with TB treatment, and mortality in TB/HIV coinfected patients.

**Methods & Materials:** Retrospective analysis of hospitalized TB/HIV patients through medical records in a University Hospital in Medellin, Colombia.

**Results:** 178 patients were included. Diagnosis was simultaneous in 49.4%. At diagnosis of TB, median CD4 was 61/µl (27-145), and both TB and HIV diagnosis was simultaneous in 49.4%. The mainly symptoms at admission were fever, cough of any duration, weight loss, diarrhea and night sweats. Pulmonary tuberculosis (PTB) occurred in 28%, extrapulmonary tuberculosis (ETB) in 23% and mixed presentation in 49%. Ziehl Neelsen (ZN) stained smear was positive in 70% of PTB and 51% of ETB, cultures at 54% and 55%, and molecular testing (PCR) in 46% and 26% respectively. Rifampicin resistance was detected in 4.9% of these cases four had multidrug resistant tuberculosis (MDR-TB), and another had pre-extensively drug resistance (pre-XDR). Half of patients increased ALT during TB treatment, but only 10% developed hepatotoxicity criteria. There were no correlation between basal ALT values and liver toxicity. In-hospital mortality was 11.3%.

**Conclusion:** TB continues to be a leading cause to HIV diagnosis, usually as marker of advanced AIDS. In coinfected patients, diagnosis of TB is still based on conventional methods. Susceptibility tests to TB drugs is mandatory in these patients. Monitor liver function during TB treatment is always necessary.

19.193 Parasitic infections among school children in Corum, Turkey

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**Purpose:** Parasitic infections in children may affects the school success negatively. In this study, the incidence of parasites was evaluated in Corum TS Elementary School students.

**Methods & Materials:** The samples were collected from 681 students attending the first five classes. The stools were investigated by native-Lugol's iodine, Kato-katz, Kinyoun's acid-fast and trichrom staining. In addition cellophane tape method were applied. The sera were evaluated with Toxocara ELISA (Novatech, Germany).

**Results:** At least one of the method, 228 (33.5%) students were found as infected with parasites. By native-Lugol's and trichrom 20.7% were positive, and Blastocystis spp, Giardia intestinalis and Entamoeba coli were mostly seen parasites. Enterobius vermicularis were present in 123 students (18.1%). With Kato-katz additional 6 (1%) parasites have been determined: E. vermicularis, Ascaris lumbricoides and Hymenolepis diminuta that none of them could be distinguished with the previous mentioned methods. No acid-fast staining parasites could be found. IgG antibodies against Toxocara spp. were present 4.7% of students. Statistically there was no difference with gender but parasitism were higher in 4th and 5th classes. There was a strong relationship between the presence of parasites and Toxocara seropositivity (p<0.05).

**Conclusion:** The school based parasitic diseases control program is needed.
Evaluation of cystic echinococcosis suspected cases in Edirne Turkey between 2006 and 2015

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Purpose: Cystic echinococcosis (CE), caused by the larval form of Echinococcus granulosus, is one of the most important and widely distributed parasitic zoonosis in the world. This study aimed to evaluate patients who applied to laboratory with suspected CE between 2006 and 2015 retrospectively.

Methods & Materials: In this study, 3151 serum samples which were sent to the Microbiology Department of Central Laboratory of Trakya University Health Center for Medical Research and Practice (Hospital) in Edirne, Turkey between 2006 and 2015 were investigated by using commercial Indirect Hemaglutination Test (Fumuoze, France).

Results: Five hundred seventy nine (18.36%) of screened 3151 serum samples were found as seropositive. Five hundred seventy nine positive serum samples were belonged to 366 CE patients (age ranged from 4-91 years). Two hundred seventy (59.29%) of these patients were female and 149 (40.71%) of them were male. The most common organ localization was liver.

Conclusion: As a result, CE continues to be a major public health problem in this region. We suggest that new strategies be designed for E. granulosus control programs in Turkey.

Incidence of meningococcal disease in children in Astana city

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Purpose: The main aim of present study is to evaluate the clinical and epidemiological features of meningococcal disease in children under the age of 14 in Astana for 2015.

Methods & Materials: It is carried out the retrospective analysis of 109 cases with diagnosis of meningococcal disease, observed at the Municipal Children's Infectious Hospital, Astana, for 2015. For statistical analysis used program SPSS Statistics 20.

Results: Among children<14 years noted rising of the incidence of meningococcal infection in Astana in 2015. The high incidence of MI was registered in the spring (69.4%, p≤0.05), while in the winter and summer periods noted significantly lower incidence, 15.6% and 12.8% respectively. In the analysis of anamnesis, the most incidence is registered in children<5 years: from 1 month up to 1 year 18.3%, 1-2 years old 24%, 2-5 years old 34.8%, whereas in children aged 5-10 years old, the incidence rates were 16.5%, 10-14 years 6.4%, it should be noted, the incidence among male children is much higher, 68.8% of all observed patients are boys (p ≤0.05).

Determined, meningococcal meningitis without meningococcemia occurs in 1% of cases and meningococcal meningitis combined with meningococcemia occurs in 40.7%, meningococcal meningoencephalitis combined with meningococcemia occurs in 4.6%, meningococcemia in 54% of cases. Bacteriological investigation were performed in 91% of cases, and in other cases, the diagnosis was based on clinical data. Bacteriological method defined the prevalence of serotype A 78.3%, serotype B were detected in 4.9%, serotype C in 13.7% of patients.

Conclusion: Incidence of MI in 2015 was more occured in children younger than 5 years (77%), among patients predominated males 68.8%, among clinical forms most often recorded meningococcemia 53.7%, among serotype landscape often recorded serotype A 78.3%. Routine vaccination against meningococcal infection in the Republic of Kazakhstan is not applied.

The study was conducted in the Republic of Kazakhstan, Astana. We would like to express enormous gratitude to the doctor of Municipal Children’s Infectious Diseases Hospital of Astana Azhigulov Zhusupbek, to the head of bacterial laboratory - Volkova Gulnara and hospital staff.
Purpose: What was the epidemiological trend and pattern of distribution of measles infection among children less than five years in Katsina state, Northern Nigeria?

Methods & Materials: A review of the surveillance data from 2009 to 2012 was carried out. A descriptive analysis of sociodemographic and mortality data was performed including serum IgM status of children below fifteen years residing in Katsina state to determine means and proportions.

Results: There are a total of 6,820 suspected measles cases from Jan 2009 to Dec 2012 out of which 68.2% were epidemiologically linked, 27.5% were laboratory confirmed, 3.6% were indeterminate and 0.74% were discarded. The case fatality rate (CFR) was 0.73% in 2009 which is lower than that for 2010 (1.54%). The mortality data for 2011 and 2012 was however missing. Majority (78%) of the total cases occurred in 2011 with 86% from the rural areas while the lowest number of cases was recorded in 2012. Only 11.5% of cases occurred in urban areas in 2012. In 2011 and 2012, highest percentage of cases (13.5% and 18%) was from Zango and Sandamu LGAs both from southern part of the state. Also highest numbers of cases were mostly observed in March and November in all the years and the lowest in January and July. In 2009 and 2010, 86% and 69% of the cases were children below 5 years of age and no data was available on age distribution for 2011 and 2012. Cumulatively, no data was available on sex distribution of cases reported.

Conclusion: The study revealed showed a steady increase in the number of reported measles cases from 2009 to 2011 with largest cases in 2011 and peak period of transmission around March and November. The burden of measles in Katsina state also lies in the southern part affecting predominantly children aged five years and below. Routine immunization and disease surveillance and notification activities should be strengthened to reduce childhood morbidity and mortality in Northern Nigeria.

Association of genetic polymorphism of the DNA base excision repair gene (APE-1 Asp/148 Glu) and HPV type (16/18) with the risk of cervix cancer in north Indian population

Purpose: Cervical cancer is one of the most common neoplastic diseases affecting women, with a combined worldwide incidence of almost half a million new cases. Polymorphisms in DNA repair genes may contribute the genetic instability and carcinogenesis.

Methods & Materials: Cervical tissue derived from patients with cervical cancer (N = 138) and non-cancer controls (N = 180) were taken under informed consent. Genotyping for APE-I Glu 148 Asp polymorphism was performed by a PCR-CTPP as well as HPV type 16 and 118 was amplified by multiplex –PCR.

Results: It has been observed that Asp/Glu with Glu/Glu genotypes that combined we observed statistically significant with protective effect for developing of cervix cancer (OR-0.51, 95% CI 0.31-0.83, p=0.006). The combined Asp/Glu with Glu/Glu genotypes who were using oral contraceptives were shown to be statistically significant with reduced risk of cervical cancer (OR-0.22 95% CI- 0.11-0.47, p=0.002). It has been suggested that significantly correlation between HPV 16 and users of oral contraceptives in certain APE-1 genotypes with reduced risk in developing cervix cancer.

Conclusion: We observed statistical significant association with reduced risk of cervix cancer in APE-1 with different genotypes, though, on the other hand, in association between HPV type 18 and those having SCC highly increased risk of cervical cancer was observed.

Microhematospermia in acute Zika virus infection

Purpose: Zika virus (ZIKV) spreads to people primarily through the bite of infected Aedes mosquitoes. Nevertheless, prolonged presence of ZIKV and/or its components in semen of acutely infected patients has been well established, raising the possibility of sexual
transmission as an alternative mechanism of infection. Hematospermia or blood in the semen, has been documented in some of the cases where sexual transmission of ZIKV is a point at issue, but its prevalence remains unknown, as the condition is usually asymptomatic and may go unnoticed. Moreover, the occurrence of microhematospermia in patients with acute ZIKV infection has not been established.

**Methods & Materials:** Sequential samples, urine and semen were collected in three patients with uncomplicated symptomatic acute ZV infection. The medical questionnaire revealed no signs of urinary tract infection, prostatitis, urethritis, or cystitis, and urinalysis showed no abnormalities. IgM Capture ELISA tests for dengue and Chikungunya viruses (NovaTec Immunodiagnostica, Dietzenbach, Germany), were negative. Initial plasma samples and sequential paired-samples of urine and semen were assayed by means of a conventional in house end-point PCR technique at a reference laboratory (Center of Microbiology and Cellular Biology, Laboratory of Virus Biology, Venezuelan Institute of Scientific Research).

**Results:** Although direct and macroscopic examinations of the semen showed no hematospermia, tests for occult blood based on the detection of activity of pseudoperoxidase of hemoglobin and myoglobin (Combi-screen®, Analyticon Biotechnologies, Lichtenfels, Germany), were initially positive in all three cases. Of note, simultaneous urine samples were negative.

Urine was consistently negative for ZIKV by PCR. On the other hand, semen samples remained positive for several weeks. In two patients, ZIKV presence in semen outlasted the occurrence of microhematospermia (see Figure 1).

**Conclusion:** Hematospermia, either clinically evident or microscopic, appears to be common following ZIKV infection. Even though symptoms of prostatitis have been reported in men with ZIKV positive semen, it is not currently known whether disruption of the integrity of the reproductive tract occurs in this gland or elsewhere. Further studies are necessary to assess whether an infected man with hematospermia is more likely to harbor higher concentrations of ZIKV in semen and therefore, has a higher risk to sexually transmit it.


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**Purpose:** International mass events - such as the London Olympics, the World Cup in Brazil, the Expo in Milan, the Hajii - increase the risk of transmission of certain infectious diseases, either endemic or imported, leading to the emergence of outbreaks and stressing the prevention and control countermeasures. The extraordinary Jubilee 2015-16 constitutes a year-long mass event, that for intensity and duration, represents a challenge for the regional health system.

**Methods & Materials:** During the Jubilee, the Lazio Region, where Rome is located, is implementing a regional plan for surveillance and management of Infectious Emergencies. Starting from December 8, 2015 data from different integrated surveillance systems are continuously analyzed: notifications of infectious diseases (including special surveillance), syndromic surveillance, event based surveillance

Surveillance and response activities include: a) collecting and analyzing data diseases notification system; b) evaluation of syndromic surveillance data; c) International Alert monitoring; d) evaluation of alerts and activation of response and control measures.

**Results:** On June 5th, 2016, 4251 notifications have been reported with increasing frequency since the beginning of the Jubilee. For each disease, the absolute and relative frequency has been compared weekly with previous years 2013-2014 and with the trends of the previous weeks. This led to the analysis and evaluation of 70 alerts. The daily evaluation of individual notifications and its link with a Jubilee event led to the investigation of 12 events (i.e. pulmonary tuberculosis, chicken pox, measles, flu, hepatitis and rotavirus infection, listeriosis and others). Syndromic surveillance is evaluated bi-weekly and 50 evaluations have been conducted and 67 alerts have been analyzed so far, generated more frequently from “Respiratory syndrome with fever”, “Acute haemorrhagic fever with rash” and “fever with lymphadenitis”. The event based surveillance reported about 10 events per week for a total of about 200 alerts.

**Conclusion:** The implementation of the plan has made possible the close monitoring of potential infectious emergencies in the Lazio region during the reported period. Given the increase of travels and possibilità to easily participate to mass events, sharing the experience during mass gatherings is crucial to find common solutions to public health issues.
Purpose: Many studies showed that HIV-infected patients had an increased seroprevalence of HEV compared to those without HIV infection. However, no data has been reported to date in HIV-infected patients in China, where HEV is highly endemic. The aim of this study was to evaluate hepatitis E virus (HEV) seroprevalence and the situation of HEV/HIV co-infections among HIV-infected population in China.

Methods & Materials: 892 plasma samples collected from HIV-infected patients in Yunnan (YN), Henan (HN), Zhejiang (ZJ), Anhui (AH) and Xinjiang (XJ) were retrospectively tested for the presence of anti-HEV IgM/IgG and anti-HCV(by EIA), CD4⁺ count, and HIV viral load. In patients with confirmed anti-HEV IgM positivity, HEV RNA was detected by RT-nPCR.

Demographic, laboratory data and biological characteristics of HIV-infected patients were also collected and analyzed by univariate/multivariate analysis.

Results: The overall prevalence of anti-HEV in HIV-infected patients was 39.7%, which is higher than that in the general Chinese population (23.5%). The seroprevalence of anti-HEV in HIV-infected patients from YN, HN, ZJ, AH and XJ province was 58.0%, 44.2%, 41.1%, 31.9%, and 25.5%, respectively. The anti-HEV prevalence was found significantly to be associated with the issues like sex, age, ethnicity, region and HIV viral load of HIV-infected patients. No HEV RNA was detected in these samples.

Conclusion: The seroprevalence of anti-HEV in HIV-infected patients was higher compared with the general Chinese population (39.7% vs 23.5%). The risk factors analysis suggests that age, ethnicity, geographic areas and particularly HIV viral load were correlated with HEV/HEV co-infections. This is the first study to evaluate the situation of HEV/HIV co-infection in China and provides the detail information for clinical reference.

Purpose: Especially in secondary flavivirus infections specific IgM - the classical marker of acute infection – is often found at low titer, sometimes being even undetectable, while synthesis of specific IgG is rapidly stimulated. Shortly after infection, the IgG titer then levels off at moderate concentration, indistinguishable from IgG titers in convalescent infections. Similar situations have also been observed in patients with Zika virus (ZIKV) infections, recently, making it difficult to discriminate between an acute secondary and a past infection. Since ZIKV is suspected to be associated with major neurological consequences (Guillain-Barré syndrome, microcephaly in newborns), diagnosis of acute infections though is highly crucial. We analysed the course of anti-ZIKV IgA, IgM and IgG titers in two confirmed patients for whom sequential serum samples were available asking whether IgA as alternative to IgM could indicate an acute phase of infection.

Methods & Materials: Serum samples were taken from two Columbian persons at day -16, 6, 24 and 66 and at day 3, 15, 35, 52, 66, 76 and 95 with respect to onset of first symptoms, respectively. ZIKV infection was confirmed by RT-PCR at days 6 and 3, respectively. Titers of IgM and IgG against ZIKV were measured using commercial Anti-ZIKV ELISA (Euroimmun AG, Germany). Cut-off was chosen as recommended by the manufacturer. For determination of anti-ZIKV IgA, a corresponding ELISA was adapted applying an anti-human IgA conjugate. Cut-off was set at a ratio of 1.0 based on empirical data.

Results: Measurement of ant-ZIKV IgM was negative in all samples. IgG titers starting raising from the first day of blood withdrawal, reached their peak at days 24 and 15, respectively, before they decreased, clearly remaining above the cut-off. IgA titers also revealed a constant increase and decrease but only peaking above the cut-off at day 24 and 15.

Conclusion: These cases provide the first indication that determination of anti-ZIKV IgA may be helpful in the diagnosis of acute ZIKV infections and the discrimination from past infection when IgM are not detectable.
Purpose: TB patients in Nigeria and other parts of Africa often incur high costs when utilizing TB treatment and care, both within and outside of Directly Observed Therapy Short-course (DOTS) programs. For many households, TB treatment and care-related costs were considered to be catastrophic because the patient costs incurred commonly amounted to 10% or more of per capita incomes. The aim of this study was to determine the accessibility and utilization of TB DOTS services among patients attending PHC centre in Katsina state.

Methods & Materials: This study was conducted on patients with pulmonary tuberculosis receiving DOTS services in PHC centers in Katsina state. A cross-sectional descriptive study was carried out in January 2014 with a sample of 225 patients obtained by a multi-stage sampling process after obtaining ethical clearance from the Kastina state Ministry of health Ethics Review Board. A structured, interviewer-administered questionnaire was used to determine accessibility and utilization of patients. The data was entered and analyzed using SPSS Version 21.

Results: Most of the respondents were males (70.2%), married (66.7%) and Muslim (92.4%) with no formal education (49.3%) and farming constituting their major occupation. More than half of the respondents had been on treatment for less than 3 months (56.9%) and almost all the patients had easy access to treatment (98.5%) with motorcycle as the commonest means of transport (48.0%). Most of the patients had incurred cost (82.2%) due to transport (91.4%). There is statistically significant relationship between distance to the health facilities by patients and satisfaction with TB DOTS services. (P<0.001)

Conclusion: TB patients have easy access to DOTS facilities in PHCs, however majority incur cost during treatment as a result of transportation. It is recommended that more TB DOTS intake centres should be established by the State TB and leprosy control office in order to reduce the cost incurred during treatment.

Epidemiological Features of Legionellosis in a Province of South Korea, 2011-2016

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Purpose: Legionellosis is a respiratory illness caused by an aerobic bacillus acquired from environmental water system. In South Korea, legionellosis is monitored by mandatory notification from 2000, and the cases are significantly increasing since 2011. About a quarter(42/166) were the citizen of Gyeonggi province, which is the biggest province in South Korea. In this study, we analysed 42 epidemiological investigation reports in Gyeonggi province to figure out the epidemiological features of legionellosis.

Methods & Materials: In South Korea, every cases reported as legionellosis are subject to epidemiological investigation by the provincial EIS officers. From January 2011 to June 2016, 42 cases were investigated according to standardized questionnaires for demographic/clinical features, diagnostic test, underlying medical conditions, and exposure histories.

Results: 18 of 31 counties in Gyeonggi province reported cases. 32(76.2%) cases were male, and mean age was 60.6 (S.D.=15.4). 13(30.9%) cases reported in winter(mean age=55.3), 12(28.6%) in summer(mean age=63.8), and 17(40.5%) in Spring/Fall. 39(92.9%) cases were reported as 'Legionaries' disease' and 3(7.1%) were 'Pontiac fever', and 6 among 'Legionaries' disease' cases were fatal(fatality rate = 15.4%). 29(69.0%) had fever, 24(57.1%) had cough, 18(42.9%) had dyspnea, 16(38.1%) had fatigue, 4(9.5%) had confusion, and 3(7.1%) had diarrhea. 20(47.6%) cases were diagnosed by the multiplex PCR for pneumonia agents, 15(35.7%) by the urine antigen test, and 7(16.7%) by culture. 29(69.0%) had underlying medical conditions such as chronic lung diseases, COPD, asthma, immune diseases, and cancers. 20(47.6%) had risky behaviors such as alcohol drinking and cigarette smoking. The route of infection was figured out only for 9(21.4%) cases.

Conclusion: The epidemiological investigation reports would give abundant information about clinical and epidemiological features of legionellosis. In South Korea, cases reported not only in summer but also in winter, it means that regular monitoring is needed to be conducted for humidifier as well as cooling system. This study have not identified the major route of infection. To advance the hypothesis of route, we may need to adopt more structured questionnaires such as 'Hypothesis-Generating Questionnaire' developed by USA CDC.
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**Purpose:** The Severe Acute Respiratory Syndrome (SARS) outbreak was a wake-up call to healthcare facilities across the world, highlighting the impact of healthcare associated outbreaks.

We performed a review and analysis of nosocomial outbreaks post-SARS in Singapore, a modern city-state with a well-developed healthcare infrastructure and large population density, which was significantly affected by SARS.

**Methods & Materials:** A PubMed search was conducted to identify healthcare associated outbreaks in Singapore between 2003 and Jan 2016, using the terms "Disease Outbreaks"[Mesh] AND "Singapore", “Outbreaks” AND “Singapore”. We also reviewed all papers on PubMed published by the leading experts in infectious diseases in Singapore identified from the initial search.


**Results:** We identified 23 healthcare associated outbreaks over the 14 year study period. Eleven (47.8%) outbreaks were caused by viruses (most commonly norovirus), eleven (47.8%) by bacteria, and the causative organism was unidentified in one (4.3%). The majority of outbreaks (65.2%) occurred in tertiary teaching hospitals. Outbreaks were most commonly detected by alert clinicians (43.5%), then by analysis of trends of notifiable infectious diseases reported to the Ministry of Health (39.1%). Interventions performed after detection of an outbreak included enhanced infection control measures (65.2%), enhanced surveillance (17.4%), and product interception (13%).

**Conclusion:** Healthcare associated outbreaks continue to occur in busy tertiary hospitals. It is likely that the reported outbreaks are a fraction of the actual number of outbreaks. While alert clinicians remain the key to detecting outbreaks, system-based detection methods, such as data mining of electronic medical records, have the potential to improve the timely identification and control of nosocomial outbreaks. Strict attention to infection control measures remains an important cornerstone in the prevention and control of outbreaks.

19.205 Dengue virus nonstructural protein 1 induces platelet activation and promotes platelet aggregation

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**Purpose:** Dengue virus (DV) infection is currently regarded as the most important mosquito-borne viral infection. The clinical symptoms of dengue have a large spectrum from febrile illness to severe syndromes accompanied with bleeding and shock. Thrombocytopenia and vascular leak are the two common features of severe dengue. Recently, it was found that DV nonstructural protein 1 (NS1) which can be secreted into blood during DV infection, can cause vascular leak. However, whether secreted NS1 is involved in the pathogenesis of thrombocytopenia is still unclear. In this study, we investigated whether NS1 could directly bind to platelet and studied its effect on platelet activation.

**Methods & Materials:** Freshly isolated platelets were incubated with DV NS1 produced by Drosophila S2 cells. The binding of NS1 on platelets was observed by immunofluorescent confocal microscopy. The effects of NS1 binding on platelet activation were measured by src phosphorylation, P-selectin expression, cytokine secretion and platelet aggregation.

**Results:** We demonstrated that NS1 could co-localized with TLR4 on platelets by confocal microscopy. In addition, binding of NS1 to platelets induced Src-Y418 phosphorylation within 5 to 15 minutes, and this effect could be blocked by the presence of certainly anti-NS1 monoclonal antibody. P-selectin expression and macrophage migration inhibitory factor secretion were increased in platelet after NS1 stimulation. In addition, NS1-activated platelet have higher aggregation response in the presence of suboptimal dose of ADP or thrombin stimulation.

**Conclusion:** Our results suggested that DV NS1 could directly bind to platelets and induce platelet activation through src signal transduction, which may enhance platelet aggregation.
These results may explain part of the causes of thrombocytopenia and re-emphasize the critical role of DV NS1 in dengue pathogenesis.

19.206 Dengue transmission through human movement in regular and seasonal patterns on Koh Chang island in Thailand

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Purpose: Mosquitoes serve as vectors for many infectious diseases, of which a growing concern is dengue in Southeast Asia. Control of vector-borne diseases is a substantial public cost and personal mosquito control imposes a burden on many individual households. Average annual national dengue incidence in Thailand from 2000-2011 was 115 cases per 100,000 people, while on the island of Koh Chang in the Trat province of Thailand the annual incidence was on average 196 cases per 100,000 people over a similar time period. Movement from mainland Thailand and from nearby Cambodia may be driving the dengue dynamics at this local scale. As a case study of a location with high tourism and seasonal movement, this work aims to estimate the ranges of relevant parameters that could impact local disease transmission in order to understand disease dynamics influenced by human movement.

Methods & Materials: This study investigates non-seasonal and seasonal human movement through networks of human and mosquito populations in 6 residential communities in Koh Chang. Using 2-patch and 3-patch metapopulation models, we estimate the range of secondary cases, the basic reproductive ratio $R_0$, in response to two parameters: the rate of movement between Koh Chang and Cambodia and the rate of infection in Cambodia.

Results: We present results from multi-patch metapopulation models that show how movement at local and regional scales may impact $R_0$. We graph potential dengue cases resulting from ranges of parameters estimating human movement and dengue incidence to suggest what may lead to a local $R_0 \geq 1$ in Koh Chang.

Conclusion: The aim of this work is to model dynamics in disease risk and dispersal involving human movement, human disease cases, and mosquitoes in a case study of Koh Chang, Thailand. An understanding of human movement at the local and regional scale may be helpful for disease management and preparation.

19.207 Refugee health and the risk of cutaneous Leishmaniasis in Europe

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Purpose: Leishmaniasis is a vector-borne neglected disease caused by different species of intracellular Leishmania parasites transmitted by sandfly species. The clinical manifestations of the disease range from cutaneous leishmaniasis (CL), the most common form of the disease, to the potentially lethal forms of visceral leishmaniasis. CL may be transmitted as a zoonotic disease caused mostly by L. major in the Old World with small rodents serving as the animal reservoir. Anthroponotic CL on the other hand, is caused by L. tropica and transmitted through P. sergenti sandflies. In Syria, CL is a well-known disease (“Aleppo boil”), but incidence rates had decreased significantly prior to the civil war.

Methods & Materials: Risk assessments are based on World Health Organization estimates that currently, a tenth of the world population are at risk of leishmaniasis. The disease has been reported from 98 countries on four continents leading to a loss in disability-adjusted life years (DALYs) of about 2.4 million. Leishmaniasis is endemic to 14 of 22 EMRO/WHO countries, including regions where the highest number of refugees to Europe originate, including Syria, Iraq and Afghanistan, where L. tropica predominates.

Results: Although currently there is little information on the prevalence of CL in Iraq and Syria, we found that >50,000 cases have been reported in 2015 from the areas under Syrian government control, representing a dramatic increase in CL incidence in the region. Refugees may be spreading L. tropica during migration, and cases have been observed in Lebanon, Turkey, Greece and other parts of Europe. The vector, P. sergenti, is wide-spread in the Mediterranean, including Cyprus, France, Greece, Italy, Malta, Portugal and Spain. The authors present a needs-assessment for the timely detection of leishmaniasis in Europe, along with an update on the current state of diagnostics, therapy and vaccine development.

Conclusion: The possibility of transmission is high as long as the vector is present. Permissive vectors, i.e those supporting the development of most of Leishmania species, exist in many parts of Europe. Physicians taking care of refugees and migrants in Europe
Three patients from Suriname with possibly Zika virus associated Guillain-Barré syndrome

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**Purpose:** Since the Zika virus (ZIKV) outbreak in South-America, an increased incidence of microcephaly and the Guillain-Barré syndrome (GBS) is reported in several countries in South-America. Here we present three patients with GBS during the ZIKV outbreak in Suriname who were diagnosed in February and March 2016. This case series shows the diagnostic challenge to prove the clinical diagnosis of GBS in relation to ZIKV infection.

**Methods & Materials:** Plasma, serum and urine samples were collected from the patients. RT-PCR for ZIKV was performed in the urine and plasma. Presence of ZIKV antibodies in the serum was tested with a ZIKV ELISA (Euroimmun, Lübeck, Germany) and a virus neutralisation test. A multiplex serological protein microarray using recombinant proteins was used for detection of Flaviviruses and Chikungunya antibodies in the serum samples. ELISA was used to detect IgM and IgG anti-ganglioside antibodies in the serum.

**Results:** The three patients had clinical symptoms of GBS, and the diagnosis was confirmed by the findings in cerebrospinal fluid and nerve conduction studies (NCS). The clinical course of GBS in these patients was mild and specific treatment was not administered. The anti-ganglioside antibodies were negative in all patients. One patient had a positive ZIKV urine real-time PCR (RT-PCR) result. The other two patients had a negative ZIKV urine RT-PCR but a positive virus neutralisation test and presence of IgG antibodies against ZIKV in the serum. Serological testing for recent infections with *Campylobacter jejuni*, Cytomegalovirus and Epstein-Bar virus were negative.

**Conclusion:** To prove that a GBS case is triggered by ZIKV is difficult, especially after the viraemic period of ZIKV. However, taken into account the increased GBS incidence that coincided with the ZIKV outbreak in Suriname and the absence of most prevalent preceding infections of GBS in these patients, it is plausible that the GBS in these patients is triggered by ZIKV infection in the recent past.

**Analysis of dengue imported cases in South Korea, 2001-2015**

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**Purpose:** Dengue is a major public-health concern throughout tropical and sub-tropical regions of the world. Now, travelers are at significant risk of acquiring dengue and spreading it in non-endemic regions. In Korea, most cases are imported from endemic area and its cases are increasing every year. We described the characteristics of dengue fever imported cases to the national infectious disease surveillance system and analysed relation between number of dengue endemic countries traveler and major imported countries incidence pattern.

**Methods & Materials:** We analysed using dengue cases reported to the national surveillance system from 2001 to 2015. And we analysed correlation between Korean’s international traveller data from Korea Tourism Organization and monthly dengue incidence data from Ministry of Health in Philippines and Thailand.

**Results:** Of the 1,337 imported cases during 2001-2015 and a total of 828 (62%) were male and 509 (38%) of female. The median age was 30 years. Excluding overseas residents and foreigners, median travel duration is 20.8 days. Most imported cases were from Asian countries (98%) such as Philippines (36.1%), Thailand (13.2%), Indonesia (12.3%), Cambodia (7.3%), India (6.3%). Except for 35 cases reported in public health centers and clinics, all cases reported in hospitals. The 57.4% were reported from July to October, which correspond with vacation season in Korea. There was no death cases until now. A positive significant correlation exists between Korean traveler incidence and dengue incidence in Philippines and Thailand from 2006 to 2012. Country-specific Korean travelers incidence was Philippines 39.5, Indonesia 34.0, Thailand 11.7.

**Conclusion:** In Korea, imported dengue cases are increasing continuously and most common exposure country is Philippines, Thailand, Indonesia which especially korean traveler preferred destination. Our study is several limitations but this report suggested the
need for continued attention and monitoring system for tourists to visit the dengue epidemic countries.

19.210 Newly introduced RRTTRR (Reach-Recruit/Refer-Test-Treat-Retain-Resilience) strategy to increase early TB case detection and effective treatment among migrants: Thailand’s first ever experiences

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Purpose: Thailand is one of the 22 TB high burden countries for a long time and just recently enrolled in three (TB, HIV/TB and MDR TB) high burden countries according to the WHO report, 2015. Although Thailand has been on track to meet the SDG and Stop TB goals by 2015, but it continued to face challenges and issues as confirmed by an external program review which include: 1) low case notification among at-risk groups; 2) inefficient recording, reporting systems; 3) inadequate treatment outcomes; and 4) the lack of suitable care for all migrants in need. Hence, the World Vision Thailand has launched to end TB among migrants in six provinces through innovative RRTTRR (Reach-Recruit/Refer-Test-Treat-Retain-Resilience) strategy under Ministry of Health, Thailand.

Methods & Materials:
RRTTRR strategy has significant key features as follows
Reach- through outreach activities such as intensified mass verbal screening, training sessions and migrant drop in centers establishment.
Recruit/Refer - migrant health volunteers are recruited and trained for intensified case finding, sputum collection techniques and make proper referral for presumptive TB cases.
Test and Treat - use Xpert MTB/RIF for TB diagnosis and drug resistance that recently available in Thailand
Retain - provide 100 percent qualified daily DOTS watcher and routine DOTS supervisor.
Resilience- ensures to enroll migrant health insurance scheme, organize self help groups and setting up functioning inter country cross border referral mechanisms.

Results: As of May 2016, WVFT screened 31,073 migrant populations and notified 1,914 presumptive TB cases. Presumptive TB case notification rate was 6% (1914/31073) and almost 99% (1906/1914) referral and testing rate. New smear positive case detection and all TB case detection rate were 6% (110/1906) and 10% (185/1906) respectively and among them 100% (110) and 97% (179) of new smear positive and all TB cases were registered for treatment. TB/HIV co-infection rate was 9% (17/185) in migrant population. 554 migrant health volunteers were recruited and 61 drop in centers was established. 16,337 migrant populations were received TB education.

Conclusion: This evidence showed that newly innovative RRTTRR strategy significantly increase early case detection of TB and get prompt treatment in migrant population. This could be the first step to make a dynamic change to control TB among migrants effectively and efficiently.

19.211 Malaria - a new re-merging disease in Albania
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Purpose: Albania was listed as an endemic area from the beginning of the 20th century. It was eradicated in 1967 and around 1994 the movement of people for work purposes and tourism to tropical areas, malaria reappeared again, already imported. The aim is to note that malaria is re-emerging diseases in Albania.

Methods & Materials: We analyzed the etiological aspect, the cause was determined on UHC laboratory and IPH, using method the point of thin and thick blood. Epidemiological aspects defined the countries from which they came after the epidemiological investigation. It was determined the age group and gender, season and month of occurrence of malaria.

Clinical aspect - the patients were grouped according to clinical-biological data's and preclinical manifestations of different bodies taking into account the classification of cases according to the gravity and possible complications. We included 27 cases of imported malaria in our country, the age group 19-58 years old, during the period 2012 to 2016 on the infectious diseases service UHC.

Results: Based on etiological causes we found out 17 cases P Falciparum, 1 case P Vivax, 6 cases P Oval, 3 mixes (P Oval and P falciparum). Epidemiological data: 1 case from Ghana, 1 from Greece, 1 from Papua New Guinea, 1 from Cad, 2 from Nigeria, 21 from Ecuadorian Guinea. All cases were male, 19-30 years old 12 cases, 31-40 years old 9, 41-50
years old 5, 51-58 years 1. Based on seasonality. 8 cases in the spring (April-May), summer 5, autumn 8 (October-November), in winter 6. Clinical classification: simple attacks 7, with hematological, hepatic and renal alterations 16, cerebral malaria 4 cases where 1 was died. Clinical progress classification: primary attacks 19, recrudescence 3, relapses 3, reinfections 2.

**Conclusion:** We identified 4 etiological factors, P.falciparum 62.9%, P. Vivax 3.70%, P.Oval 22.3% and mix causes 11.1% (P falciparum and P Oval). Distinguished in 6 different countries. Malaria was present in all seasons. Exitus in 3.7%. Malaria already considered a re-emerging disease in Albania. The presence of transmitting vectors not excludes the possibility of return to its native form, which is in the attention of public health institute.

19.212 Ebola viral disease screening at Birmingham (United Kingdom) airport – successes and challenges

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**Purpose: Introduction:**

Ebola is a rare but serious disease caused by the Ebola virus. A large outbreak affecting Guinea, Liberia and Sierra Leone was declared over by WHO in June 2016. As part of the UK’s national Ebola response, Public Health England worked with government, National Health Service (NHS), Airport Authorities, UK Border Agency and Local Government to ensure the UK was prepared for mitigating the risk of Ebola entering the country. One of the key measures included targeted passenger screening at the UK’s main ports of entry; London Heathrow, Gatwick, London St Pancras, Manchester and Birmingham.

**Methods & Materials: Screening process:**

Screening commenced at UK’s Birmingham Airport on 31/10/2014 for all at-risk passengers who had returned from West Africa. Passengers were categorised by level of risk (relating to in-country activities), and / or describing symptoms of pyrexia (temperature of ≥37.5°C) or those clinical symptoms compatible with Ebola infection.

By August 2015, 256 at risk passengers had been screened at Birmingham Airport, some referred to NHS (symptomatic on arrival) having identified as undertaken high-risk activities.

**Results:**

Challenges:

A project of this nature had never been tackled before by UK’s local PHE team. Key challenges that were successfully overcome were: Operational delivery arrangements with partners within a dynamic commercial environment. Recruitment and training of over hundred volunteers to undertake on-site airport screening. Development of a comprehensive and continuously updated operational plan to assist screeners and management. Maintenance of effective horizontal and vertical communication. Considerable interest at the senior levels of government required the timely and accurate passage of information. Working with senior management colleagues from the local PHE Team to balance the need to resource airport screening and to maintain public health activities.

**Conclusion: Lessons Learnt:** Public Health operating in an airport environment required sensitivity to the local pressures and drivers. Success in part relied upon on previous relationships forged by agreeing an Airport Health Protection Plan. Team work was essential; both within the Steering Group, between all volunteers and with staff back at base. Partnership working was the key especially amongst Birmingham airport staff, Directors of Public Health, Local Government and UK Borders Agency.

19.213 Zika virus infection in Czech travellers

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**Purpose:** The aim of this study was to describe the clinical characteristics of Zika fever imported to the Czech Republic.

**Methods & Materials:** Prospective study evaluated symptoms, laboratory findings and outcome in patients with acute Zika fever diagnosed at the Department of Infectious, Parasitic and Tropical Diseases in Prague (Czech Republic). The infection was confirmed by RT-PCR in serum or urine and/or serology (ELISA and virus neutralization test).

**Results:** There have been diagnosed 8 cases (4M and 4 F) of Zika fever at the aforementioned clinical centre since February 2016. Age median in the study group was 42 years (IQR 38-47). A total of 7 cases have been imported from Martinique and 1 case from
the Dominican Republic. The most frequent symptoms included maculopapular rash (8 patients; 100.0%), headache (7; 87.5%), joint and muscle pain (6; 75.0%), subfebrility (5; 62.5%), fever (3; 37.5%) and conjunctivitis (3; 37.5%). Laboratory parameters were within reference values, except for leukocytopenia in 1 case and elevated serum activity of lactate dehydrogenase in 2 patients. The clinical course was mild and uncomplicated in all cases. **Conclusion:** Zika virus infection presents with mild flu-like symptoms, rash and non-specific laboratory findings. The clinical course of Zika fever is usually uncomplicated, however, intrauterine infection has been associated with a significant risk of congenital abnormalities.

19.214 FLIRT, a web application to predict the movement of infected travelers validated against the current Zika virus epidemic

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**Purpose:** FLIRT uses air traffic data and network simulations to predict where infectious disease may spread over flight networks. In this study, FLIRT is validated against the spread of the Zika virus in the U.S. Specific goals include: 1) predict where travelers infected with Zika Virus would arrive in the U.S.; and, 2) analyze and validate the application’s predictions using data collected after the prediction was made.

**Methods & Materials:** FLIRT predicts likely destinations of infected travelers through the air travel network. Using a database of flight schedules, FLIRT can display direct flight traffic and perform passenger simulations between selected airports. FLIRT was used to analyze flights departing from five selected airports in locations where sustained Zika Virus transmission occurred. FLIRT’s predictions were validated against Zika cases arriving in the U.S. during the relevant time periods. Kendall’s τ and Generalized Linear Models were computed for all permutations of FLIRT and case data to test the accuracy of FLIRT’s predictions.

**Results:** FLIRT was found to be predictive of the final destinations of infected travelers in the U.S. from areas with ongoing transmission of Zika in the Americas during the time periods from 2-1-2016 to 4-1-2016, and 1-11-2016 to 3-11-2016. MIA-FLL, JFK-EWR-LGA, and IAH were top ranked at-risk metro areas, and Florida, Texas and New York were top ranked at-risk states for the future time period analyzed (3-11-2016 - 6-11-2016). For the 1-11-2016 to 3-11-2016 time period, the region-aggregated model indicated 7.24 (95% CI 6.85 – 7.62) imported Zika cases per 100,000 passengers, and the state-aggregated model suggested 11.33 (95% CI 10.80 – 11.90) imported Zika cases per 100,000 passengers.

**Conclusion:** The results from 2-1-2016 to 4-1-2016 and 1-11-2016 to 3-11-2016 time periods support that modeling air travel can be a powerful tool in predicting where infectious diseases will spread next. As FLIRT was shown to significantly predict distribution of Zika Virus cases in the past, there should be heightened biosurveillance and educational campaigns to medical service providers and the general public in areas projected to be at risk in the future.

19.215 Tools and methods employed by community health centers in the prevention and control of Zika virus infections in Bahia, Brazil

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**Purpose:** Zika virus (ZIKV), which is mainly transmitted by Aedes aegypti and Aedes albopictus mosquitoes, has become a major health threat in Brazil and other Latin American countries. Brazil has been most impacted with ZIKV infections in addition to the existing mosquito-borne diseases of dengue and chikungunya. With the increase in zika virus infections, there have also been increasing numbers of infant brain abnormalities such as microcephaly associated to zika. This project aimed to investigate the various tools and methods employed by urban and rural health centers in North Eastern part of Brazil, to control and prevent Zika in the community. The project also examined the effectiveness of these tools as well as the challenges that are facing the health centers based on their location.

**Methods & Materials:** The methods of data collection involved participant-observation, interviews and questionnaires with the health workers over a period of 31 days. Secondary sources of articles and print outs on Zika were also used for reference purposes. The participants were chosen using snowball methodology. The major limitation of the study was language barrier, however this was mitigated by the use of translators.

**Results:** The results of this study show that house visits done by community health workers is the main tool used by health centers both in urban and rural areas to prevent the spread of zika. Another method used by community health workers is the application of Pyriproxyfen powder in areas with standing water. Although both of these methods were perceived to be
effective, most health workers insisted that the lack of education and understanding of the
disease among health workers and the public in general is the major limiting factor for making
progress in controlling infections.

**Conclusion:** Outbreak response and control demand the primary forces such as community
health workers to be well informed and educated about the disease. In addition, in most
developing countries, the rural health centers are not as equipped as urban centers in the
fight against zika and as the virus evolves genetically and habitually, rural areas are at a high
stake of future outbreaks.
20.001 Nowcasting incidence of emergent zika virus infection and its outbreaks using Google Trends data: Examples from Brazil and Colombia

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Purpose: There is increasing interest in the digital epidemiology (harnessing digital and social media data to improve disease surveillance) of infectious diseases. Data from the 2015-2016 zika outbreak in South America provides an opportunity to develop and validate models that predict contemporaneous levels of zika virus incidence using internet search query volumes from Google Trends.

Methods & Materials: Weekly incidence data for three regions in South America were obtained from national health ministry bulletins and previous studies, during the interval January 2015 - August 2016. Volume of specified health-related search queries were obtained from Google Trends for matching periods and regions. Cross-correlation and regression analyses were used to develop models to predict zika incidence from search volumes in Pernambuco. The predictive performance of the models was then internally and externally validated using data from the other two regions (Salvador in Brazil, and Colombia). Root mean squared errors and correlation analyses were used to assess the correspondence between the observed and predicted incidence data.

Results: Zika incidence in all three regions showed distinct peaks and subsequent declines to very low levels. Several of the health-related search topics correlated significantly with incidence, and multivariate nonlinear regression models based on these associations in Pernambuco had relatively very high $R^2$ values of ~0.8, and Pearson’s $r$ of ~0.9. As expected, model validation in Brazil and Colombia provided less accurate predictions, with larger prediction errors and lower but statistically significant $r$ values of -0.5 to 0.4 (p<0.05).

Conclusion: This digital epidemiology approach to outbreak modelling shows promise, but the use of big data brings a high risk of model over-fitting producing spurious correlations. The necessarily short periods of data series available for novel outbreaks fundamentally limit the complexity of models that can be appropriately created. Such models must therefore prioritise parsimony of prediction variables, and require extensive validation if digital epidemiology is to be a reliable outbreak surveillance method.


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Purpose: Influenza viruses A/B are responsible for epidemic of respiratory illnesses associated with increased rate of hospitalization while the highly pathogenic avian influenza causes huge economic loss/death. Objectives of the analysis are: To describe the seasonality of influenza, determine the circulating virus strains, determine the trend of the virus in Nigeria and provide recommendations.

Methods & Materials: The primary sources of data are the four sentinel sites (Abuja, Kano, Nnewi and Lagos). Influenza surveillance data collected from April 2008 to July 2014 was cleaned and analyzed using Epi-Info version 3.5.1. Analysis of result by age-group, sex, site, trend, and influenza types, subtypes and type of case was conducted.

Results: A total record of 11199 collected from April 2008-July 2014 documented. Influenza virus type A (FluA) was 662 (5.9%), (FluB) was 392 (3.5%) and negatives were 10185 (90.9%). The subtype of Influenza A pdm(09), A/H1N1 was 261(2.30%), A/H1 is 15(0.10%), A/H3 277(2.50%), subtype were 48(0.40%), unsubtypeable were 413 (3.70%). Female had 460 (0.46%) both FluA/B, male had 539 (0.54%) FluA/B. Type of case were ILI 9011 (80%), SARI 2096 (18%), Novel H1N1 27 (0.2%), and AI 4 (0.04%). Age-group with the highest number of positive was 1-10 with 853. State with the highest positive was Abuja 3325(29.8%) followed by Kano 3507(31.4%), Lagos 2729 (2729%) and Nnewi 1604(14.4%). The highest
peak of Influenza activity with a subtype A/H3 was in 2012, while pdm (09) AH1N1 was the highest in 2010, while A/H1 has diminished with little activity in 2009 and 2010. **Conclusion:** Thus, surveillance is effective in picking the circulating and trend of virus. The need for collaboration between the animal and human aspect of influenza surveillance to be fully integrated is highly recommended to enable track new strain of influenza virus. Sharing of data gathered between ministry of Agriculture and Health will also help in future planning.

20.003  
Screening of respiratory virus PCR panel in adults with CNS infection  
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**Purpose:** Respiratory viruses including adenovirus, respiratory syncytial virus(RSV) A, B, rhinovirus A/B, coronavirus, Influenza A, B, parainfluenza 1, 2, 3 and metapneumovirus are well known causes of upper respiratory infection in infants and young children. There are a few reports on meningitis or encephalitis associated with respiratory viruses but most of them are pediatric cases. We now demonstrate some cases of central nervous system (CNS) infection with respiratory virus in adults which are diagnosed by respiratory virus (RV) PCR panel.

**Methods & Materials:** We reviewed the registry for central nervous system infection between 2000 and 2015 at the Seoul National University Hospital, and identified patients with positive for RV PCR panel in sputum or CSF. We analyzed the clinical presentations, laboratory findings, and outcome.

**Results:** Out of total 681 patients in registry, 253 (37.1%) were viral infection. Among them, 10 patients (4%) showed positive results for RV PCR panel including Influenza A, Influenza B, rhinovirus A/B, parainfluenza virus, corona virus, RSV A and metapneumovirus. Three were positive in CSF, six were positive in sputum. Median age was 38 (range from 21-72). Four patients clinically presented as encephalitis including all three patients with CSF PCR positive results. Other six patients clinically presented as meningitis. Six patients (60%) complained URI symptoms prior to neurologic manifestations. All patients showed pleocytosis in the CSF findings, and mean WBC count was 229.2. 70% of patients showed lympho-dominant pattern. Magnetic resonance imaging (MRI) of seven (70%) patients revealed abnormality as leptomeningeal enhancement. Most of the patients (90%) had good prognosis even with encephalitis. One encephalitis patient with positive influenza A in CSF was clinically deteriorated despite of multi antiviral therapy.

**Conclusion:** We identified that CNS infection related to respiratory viruses is rare, however does exist not only in children but also in adult. Even though most patients showed good response to antiviral therapy, some patients would have higher chance of bad outcome especially with positive PCR in CSF. Screening of CSF and sputum RV PCR panel in adults with CNS infection would be helpful for proper etiological diagnosis.

20.004  
Detection of rhinovirus-associated asthma exacerbations using reverse transcriptase - polymerase chain reaction in Egyptian children  
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**Purpose:** Acute exacerbations of asthma are the leading cause of emergency department visits in pediatric patients. The development of sensitive diagnostic polymerase chain reaction (PCR) based techniques permitted demonstration of an already clinically suspected association between common viral respiratory infections and asthma exacerbations. Respiratory viruses have been identified in 80–85% of exacerbations in school-aged children, with human rhinoviruses (HRVs) being the most frequently detected. A recently identified HRV genotype, HRV-C, is circulating worldwide and is an important cause of febrile wheeze and asthmatic exacerbations in children requiring hospitalization. This study aimed to detect HRV- induced asthma exacerbations (including the new HRV-C genotype) among a group of Egyptian children

**Methods & Materials:** This cross sectional study was conducted on 31 asthmatic children in exacerbations in the period from September, 2014 till October, 2015. Patients were recruited from the emergency department and chest clinic, Children's hospital, Ain Shams University. Sputum (for children ≥7years) and nasopharyngeal aspirates (for infants and children<7years) were collected for one-step, real-time pan Rhinovirus reverse transcription polymerase chain reaction (RT-PCR) assay. One step RT-PCR was done to detect Rhinovirus C among positive cases.
**Results**: This study included 31 asthmatic children in exacerbations. They were 15 males (48.4%) and 16 females (51.6%). Their ages ranged from 7 months to 12 years with a mean and SD of (4.47 ± 3.15) years. Eight (25.8%) of the total population showed positive Rhinovirus RT-PCR test and 4 (50%) of the HRV positive patients were of the Rhinovirus C genotype (12.9% of the total population). HRV positive patients showed higher percentage of positive family history of bronchial asthma (p=0.002), higher mean values of respiratory rate (p=0.001) and temperature (p=0.001), but lower mean value of oxygen saturation (p =0.011). There were statistically significant differences regarding the exacerbation severity (p=0.024) and outcome (p=0.048).

**Conclusion**: HRVs are major triggers of asthma exacerbations among Egyptian children. The newly described HRV-C genotype accounts for a significant proportion of HRV-associated asthma exacerbations. Further studies on a larger scale are needed for HRV-C and other possibly undiscovered HRV genotypes.

**20.005**

A cross-sectional serosurvey of influenza A and B virus-specific IgG antibodies in Emirati children

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**Purpose**: Young children are at increased risk of severe influenza disease and are likely to benefit from annual vaccination. Nevertheless, vaccine administration remains infrequent. The study objectives were to measure serologic immunity against these viruses and to plan for influenza vaccination strategy for the region.

**Methods & Materials**: This cross-sectional study involved unselected cohort of children who attended the Well-Child-Care Program of Ambulatory Healthcare Services (Al-Ain, UAE) between July 2014 and September 2015. Serologic immunity (RIDASCREEN® IgG, R-Biopharm AG) for influenza A and B viruses was measured in 236 Emirati children (44% females). Medical records were reviewed for influenza immunization history.

**Results**: The mean ± SD age (y) was 4.4±1.9 (median=4.1, range=1.9-12.5). Only one child (7.8 y) had documentation of receiving the influenza vaccine once five years prior to the study sample collection; her serology was negative for influenza A virus and positive for influenza B virus. Overall, the percentage of children who were seropositive for influenza A IgG was 13.6%, equivocal 6.3%, and negative 80.1%. The corresponding values for influenza B IgG were 27.8%, equivocal 8.3%, and negative 63.9%, respectively. The percentage of children who were seropositive for either influenza A or B IgG was 35.7% and for both was only 3.6%.

**Conclusion**: The data demonstrate the majority of children are serologically naïve and, thus, are more susceptible to severe influenza disease. Therefore, annual influenza vaccine is advisable especially for young children. Vaccine policies should target the most common strain in the region.

**20.006**

Genetic characterization of isolated Influenza C viruses in the Philippines, 2006-2012

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**Purpose**: Influenza C virus (ICV) causes mild acute respiratory infection although some studies have reported severe illness causing hospitalization due to pneumonia. Only one study has been reported on ICV infection in the Philippines (Odagiri T et al 2015). In this study, we had focused on the detection of ICV from 2006 to 2012 in the Philippines and its genetic lineage identification.

**Methods & Materials**: Nasopharyngeal and/or oropharyngeal swabs were collected through national Influenza-Like Illness (ILI) surveillance from 2006 to 2012. Samples were inoculated into MDCK cells and screened for influenza A and B. Samples confirmed negative for influenza A and B were tested by RT-PCR for ICV. Partial sequencing of hemagglutinin esterase (HE) and nonstructural protein (NS) genes was conducted.

**Results**: Influenza C virus was detected in 33 of 325 samples negative for Influenza A or B viruses (IAV, IBV) from the total of 58,668 samples collected from 2006 to 2012. Thirty one out of Thirty three (31/33,94%) of cases were from children under five years old and were detected during the influenza season. Phylogenetic analysis of the HE gene using the Neighbor-joining method showed that all 33 strains formed distinct clusters within C/Sao_Paulo/378/82 strain lineage.
**Conclusion:** Influenza C virus was found to be a cause of ILI in young children. Continued surveillance for ICV will help to better understand the epidemiology, including seasonality, severity and risk factors for the disease in the tropical zone.

20.007 High-throughput bead based suspension array for the detection of acute respiratory viral pathogens among children aged <5 years in Pakistan

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**Purpose:** Viruses play an important role in causing respiratory infections in children worldwide. The burden of viruses in respiratory infections among children in Pakistan is unknown, largely due to absence of good quality diagnostic facilities. Rapid detection and identification of these pathogens, having potential to cause pandemics is important to restrict the burden of viral pathogens among children. Advanced molecular biology techniques offer great advantage as they are more sensitive and have faster identification of respiratory pathogens. Magpix system is a high throughput multiplexed microsphere-based suspension array platform capable of analyzing up to 50 unique assays in a single microplate well. The purpose of this study was to identify the burden of common respiratory viruses among children less than 5 years of age with acute respiratory infections using Magpix platform.

**Methods & Materials:** Total 935 nasopharyngeal swabs of children with acute respiratory infections were collected in viral transport medium and spiked with Bacteriophage MS2 extrinsic control to check the efficacy of nucleic acid amplification. Nucleic acid extraction was performed with a MagNaPure LC instrument by using the total-nucleic-acid kit. Amplified products were identified using a bead based suspension array for the detection of wide range of viruses and subtypes. Data were analysed and reported as median fluorescent intensity using xMAP.

**Results:** Upon testing 935 nasopharyngeal swabs 451 (48%) were positive for enterovirus/rhinovirus, 58(6.2%) for parainfluenza type III and RSV, 48 (5.1%) metapneumovirus, 39 (4%) parainfluenza type IV, 35 (3.7%) bocavirus, 31 (3.3%) adenovirus, 30 (3.2%) coronavirus OC43, 20 (2.1%) parainfluenza type I, 18 (1.9%) influenza B, 16 (1.7%) H1N1 2009, 14 (1.5%) influenza A subtype H3, 9 (0.9%) corona HUK1, 8 (0.8%) parainfluenza type II, 7 (0.7%) corona NL63 and influenza A matrix, 5 (0.5%) corona 229E and 1(0.1%) was positive for influenza A subtype H1.

**Conclusion:** Accurate and timely diagnosis of respiratory viruses in children using high throughput techniques has potential benefits, including improved treatment, decreasing the costs and reducing the empirical use of antibiotics thus preventing the emergence of antimicrobial resistance.

20.008 Influenza vaccine un-neutralized viruses associated with a specific seasonality pattern in Uganda; The HA/ HAI approach

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**Purpose:** Seasonal Influenza vaccination is important in combatting influenza virus infections and severe disease associated with the infection. Hemagglutination (HA) and Hemagglutination Inhibition (HAI) assay has been used over the years to access the influenza vaccine’s effectiveness to neutralize the circulating influenza virus strains. In this research, we analyzed the seasonality and severity of influenza viruses which were not neutralized by the World Health Organization (WHO) recommended vaccine but showed hemagglutination (HA) on red blood cells, from 2012 to 2015.

**Methods & Materials:** Clinical ILI and SARI samples positive for Influenza viruses by Polymerase Chain Reaction (PCR) were inoculated and propagated on Madin-Darby Canine Kidney (MDCK) cell line. Hemagglutination and Hemmaglutination inhibition (HA/HAI) test was carried out using guinea pig erythrocytes. All viruses that showed HA titre but were not neutralized on HAI by the WHO recommended influenza vaccine for that year were recorded in MS- Excel. Epidemiological data (seasonality and severity) corresponding to the viruses as captured on Influenza investigation forms were analyzed

**Results:** A total of 17 influenza A viruses were not neutralized on HAI by the recommended WHO influenza vaccine for the year 2012 and 2015. AH3N2 viruses showed no neutralization at 65% compared to 35% by AH1N1 2009 pandemic strains. The seasonality of circulation of the non – neutralized AH3N2 viruses corresponded to the late rainy season in Uganda from October to January for both 2012 and 2015, while AH1N1 2009 pandemic influenza viruses corresponded to the annual dry season in Uganda between
June and July 2015 with only one virus detected in January 2013. The severity of the infection was none the less similar to seasonal influenza viruses and the patients had no underlying medical condition (Asthmatic, Heart problems or TB)

**Conclusion:** Seasonal influenza A virus strains which cannot be neutralized by the recommended WHO vaccines have been demonstrated to circulate at points in time.

We recommend sequencing of these strains to ascertain genetic differences between neutralized and non-neutralized strains.

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**20.009** Short period incidence study of severe acute respiratory infection (SPRINT-SARI) initial data from a global observational study to better describe SARI epidemiology in critically ill patients


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**Purpose:** The majority of the burden of SARI-related mortality in developed countries is within intensive care units (ICUs). Increasingly, intensive care is becoming a standard element of the health care system in low and middle-income countries. However, the availability of high-quality data for critically ill patients in the early phases of a SARI outbreak is often poor. The lack of pre-populated ethics approvals, data sharing agreements, and research infrastructure makes this data often slow to help guide clinical practice for severely affected patients. This study aims to establish a rapid clinical research response capability for a future epidemics or pandemics of severe respiratory disease

**Methods & Materials:** This is a multi-centre, prospective, short period incidence observational study of patients in participating ICUs with SARI. The study period will comprise a 5 to 7-day cohort study enrolling patients, of all ages, meeting a modified SARI case-definition, who are newly admitted to the ICUs at participating sites. Through this, we have developed standardized case-report forms and a data-capture platform to better establish global readiness for evidence generation for critically ill patients with SARI.

**Results:** As of writing, we have ethics approval in 231 institutions, representing every continent and income group, with further expansion imminent. 115 sites have opened for recruitment and data collection for the first season in the Northern Hemisphere is recently completed with Southern Hemisphere collection to be completed between July and September 2016. The primary challenge in establishing this infrastructure is in obtaining ethical approvals and ensuring data quality is maintained. Preliminary results of the first season of recruitment will be presented.

**Conclusion:** Through SPRINT-SARI, we are creating a sustainable infrastructure for real-time data collection for better describing critically ill patients with SARI, in all regions of the planet. Creation of this enterprise will allow for effective risk-adjustment for SARI, as well as providing new insight into the changing epidemiology of SARI and management strategies among critically ill patients around the world. This infrastructure will iteratively improve over subsequent years to ensure data quality, accuracy of denominator projections, and applicability to diverse clinical contexts.

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**20.010** Cyclical patterns of flu incidence dynamics and their associations with variations in the diseases of the circulatory system in USA for the years 1993-2007

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**Purpose:** Multicomponent (multiannual and seasonal) variations in the dynamics of flu incidence were found earlier in different countries (e.g., cycles of 1, 2-3, 5-6 and 11 years). The first aim of this study was to test the hypothesis for the existence of such cyclical patterns in the monthly flu incidence variations in USA (1993-2007). The second aim was to extend earlier research on possible associations of such variations in the flu incidence with the dynamics of most fatal diseases of the circulatory system (e.g., acute myocardial infarction, stroke) and validate such relationships across the 4 US census regions.

**Methods & Materials:** Monthly data on the hospitalised cases (incidence) with the diagnosis of flu, acute myocardial infarction (AMI) and stroke for the years 1993-2007 (n=180 months) were kindly provided by Foster and co-authors (Epidemiol Infect 2013; 141(4): 735–44, doi:10.1017/S0950268812002890). The incidence time series contained information as stratified by US census region and age groups (e.g., below and above 65 years). Autocorrelation, periodogram regression (PRA), ARIMA, trigonometric approximation and cross-correlation analyses were applied. Statistical significance was assumed at p<0.05.
All analyses were performed by using routine (e.g., SAS, R) and specialised (6D-STAT) software packages.

**Results:** A total of 123,611 cases of influenza hospitalisation had been reported over the study interval of 180 months. A mean value per region per month ranged from 102 to 248 cases (maximum from 1800 to 4992). Cyclical patterns of flu incidence variations in all 4 census regions of USA were described, including seasonality (period T = 12 months) as well as transyears (e.g., T=17 months) and other low-frequnency (longer) cycles (T = 27, T = 38 months). Linear lagged cross-correlations (lad-periods δT=0, 3, 12 months, etc.) were established with diseases of the circulatory system (e.g., AMI) in the same regions and time intervals, especially among patients aged 65 and over (e.g., Pearson's r values from 0.17 to 0.36).

**Conclusion:** Complex cyclical behaviour of incidence variations of flu hospitalisations in USA was established over the interval 1993-2007 and these are associated with contemporaneous or subsequent dynamic patterns of such fatal circulatory diseases as AMI and stroke.

**20.011** Cross sectional survey of live bird markets, migratory and wild birds for low and highly pathogenic circulating influenza subtypes in Pakistan

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**Purpose:** In Pakistan avian influenza surveillance has been both active and passive. Here, we presented the results of a cross sectional survey effort focusing solely on the live bird markets and wild bird species from different zoos and national parks.

**Methods & Materials:** The study was conducted from Jan-Dec 2011 to identify and isolate the circulating avian influenza virus subtypes in live bird markets and wild birds from different localities in and around Islamabad. Swabs, tissues and sera were collected, screened and diagnosed by haemaglutination inhibition assay and RT-PCR.

**Results:** The highest seropositivity was recorded for H9 followed by H5 (89.4%) and H7 (72.3%). All isolates were of the low pathogenic H9N2 subtypes and no viruses were successfully isolated of subtype H5N1 or H7N7. Significantly H9N2 isolates were prevailing the most as compared to the other isolates.

**Conclusion:** it was concluded that the higher prevalence of H5N1 (89.4%) observed in the present study is an alarming threat; therefore we suggest immediate control strategies against zoonotic H5N1in live bird markets in the country.

**20.012** About the predictive positive value of the clinical diagnosis in the seasonal flu epidemic from Bucharest, Romania - 2016

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**Purpose:** Analysis of the predictive positive value of the clinical diagnosis during the seasonal flu epidemic from Bucharest municipality, 2016.

**Methods & Materials:** In an Epi Info 2000 data base were included characteristics of cases of clinical suspect flu reported to the Public Health Authority of Bucharest Municipality; in analyse were inluded cases from which in the interval of time of the weeks 3-6/2016 samples were obtained for laboratory investigation by RT-PCR of the presence of markers of acute infection with flu virus A/H1N1.

**Results:** For the 180 patients included in the study the predictive positive value (PPV) of the clinincal diagnosis of influenza was 70.6 % (63.3% – 77.1%); by type of prescribers the VPP was statisticaly similar (RR: 0,94; 95%CI: 0,74-1,20; p: 0,3830) when the diagnosis was made by family doctors (72.2%) or by hospital doctors (75.8%);

**Conclusion:** Positive predictibility of the clinical diagnosis in the flu epidemic implies favorable consequences in two ways as follows: (a) from the individual perspective the patient receives early the antiviral treatment which will shorten the clinical evolution and soften the simptomatology.; from the societal perspective the benefits will consists in decreasing the patient’s role as an active source of infection because he/she is advised to stay isolated a cople of days and to adopt the cough etiquette; suplimentary, presuming the viral nature of the condition the diagnostician doctor will not presribe antimicrobial treatment, an important antimicrobial stewardship intervention.
In the light of the advantages provided by an high PPV of the clinical diagnosis of flu it appers as perfectly right the need for sustaining the intuition of diagnostician doctor with updated, good quality information about flu epidemiology.

20.013 Prevalence of influenza A viruses in livestock and free-living waterfowl in Uganda

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Purpose: The study was aimed at establishing information on the prevalence of these viruses in swine, domestic and migratory waterfowl that seasonally visit in Uganda. Methods & Materials: Study areas included 34 live birds markets, nine landing sites and nine waterfowl sites. Samples were 1,248 cloacal and 1,328 cloaco-oropharyngeal swabs. Others included 511 nasal swabs and 929 fresh faecal samples from swine and waterfowl, respectively. Sera samples were from 2,572 birds and 417 pigs. Swabs and faecal samples were tested and subtyped for H5 and H7 using RT-PCR. Virus isolation was done in embryonated chicken eggs. Sera were analysed by ELISA and sub-types using haemagglutination inhibition test for H1, H3, H5, H6, H7 and H9 influenza A subtypes. Results: Influenza A virus prevalence by RT-PCR was 1.1% (45/4,052) while seroprevalence by ELISA was 0.8% (24/2,970). Virus prevalence was highest in domestic ducks (2.7%, 17/629) and turkeys (2.6%, 2/76), followed by free-living waterfowl (1.3%, 12/929) and swine (1.4%, 7/511). A lower proportion of chicken samples (0.4%, 7/1,865) tested positive. No influenza A virus was isolated. A seasonal prevalence of these viruses in waterfowl was 0.7% (4/561) for the dry and 2.2% (8/368) for the wet season. In poultry, prevalence was 0.2% (2/863) for the dry and 1.4% (24/1,713) for the wet season, while that of swine was 0.0% (0/159) and 2.0% (7/352) in the two seasons, respectively. Of the 45 RT-PCR positive samples, 13 (28.9%) of them were H5 but none was H7. The 19 swine sera positive for influenza antibodies by ELISA were positive for H1 antibodies by HAI assay, but the subtype(s) of ELISA positive poultry sera could not be determined. Antibodies in the poultry sera could have been those against subtypes not included in the HAI test panel. Conclusion: The study demonstrated occurrence of influenza A viruses in animals in Uganda. The results suggested that increase in volumes of migratory waterfowl in the country could be associated with increased prevalence of these viruses in free-living waterfowl and poultry.

20.014 Clinical features and risk factors predicting severe influenza: The experience of an adult tertiary care hospital in Singapore

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Purpose: Fewer reports are available on the clinical features and risk factors associated with severely ill adult patients with laboratory-confirmed influenza. We analyzed the potential clinical features and risk factors associated with severe influenza. Methods & Materials: We conducted a case-control study on severely ill influenza patients (requiring intensive care and/or died) admitted to a 1,600-bedded adult tertiary public hospital in Singapore from Jan 2011- Dec 2014. Results: During the study period, a total of 287 severely ill patients and 861 randomly selected controls were identified. The study population was predominately elderly (70% aged ≥65 years) and the male-to-female ratio was 1.1:1. 83% had Charlson score ≤3. Severely ill patients presented more often with breathlessness (p<0.001), dysuria (p=0.055), altered mental status (p<0.001), crackles (p<0.001) and consolidation changes on CXR (P=0.001), and less with fever (p<0.001), sore throat (p<0.001), productive cough (p<0.001), headache (p<0.001), myalgia (p<0.011) and nausea (p=0.002). On multivariate analysis, after adjusting for age, gender, influenza subtypes and existing co-morbidities, breathlessness (AOR 1.94, 95% CI 1.34-2.80), dysuria (AOR 5.59, 95%CI 1.96-15.90), altered mental status (AOR 10.87, 95%CI 5.44-21.73), productive cough (AOR 0.43, 95%CI 0.31-0.61), headache (AOR 0.28, 95%CI 0.08- 0.99), crackles (AOR 3.17 95%CI 2.23-4.50) and consolidation changes on CXR (AOR 2.00, 95%CI 1.36-2.94) were symptoms and signs independently associated with severely ill influenza patients. On multivariate analysis, after adjusting for age, gender, hospitalization within the past six months and admission year, factors independently associated with severely ill cases were Charlson score (AOR 1.75, 95%CI 1.57-1.94), influenza subtype A/H1N1-2009 (AOR 1.87, 95%CI 1.23-2.85), nosocomial influenza (AOR 3.43, 95%CI 1.81-6.50), and existing statin users (AOR 0.62, 95%CI 0.46-0.85). In a multivariable logistic regression model, predictors of in-hospital all-cause mortality were age (AOR 1.03, 95%CI 1.02-1.04), Charlson score (AOR 1.64, 95%CI 1.47-1.83), nosocomial...
influenza (AOR 2.86, 95%CI 1.47-5.59) and existing statin user (AOR 0.54, 95%CI 0.37-0.78) after adjusting for gender, hospitalization within the past six months, influenza subtype and admission year. **Conclusion:** Clinical presentation of adults infected with seasonal influenza did not differ between different subtypes. Chronic statin use decreased mortality risk. Further studies are needed to evaluate factors which may improve survival of hospitalized influenza patients.

20.015 Detection of respiratory viruses by multiplex RT-PCR with a GeXP analyzer

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**Purpose:** Viral infections are responsible for most respiratory infections. This study developed a multiplex and rapid identification method of respiratory virus found in children.

**Methods & Materials:** 1800 nasopharyngeal specimens were collected from pediatric patients who suspected acute respiratory infection and 87 nasopharyngeal swab were collected from healthy children for routine examination on the outpatient service at the hospital. Each of the primer pairs was designed to yield PCR products 4 to 7 bp apart, ranging from 111 to 314 bp for screening 21 kinds of respiratory viruses by multiplex RT-PCR and determine the specificity of the multiplex RT-PCR with a Genomelab Gene Expression Profiler (GeXP) analyzer. PCR partial of respiratory viruses were confirmed by sequencing with Beckman CEQ-8000. The identity of the RT-PCR product sequences was confirmed using BLAST and the NCBI database.

**Results:** A total of 67.33% (1212/1800) of samples were positive for at least one virus. The 1212 positive specimens included 475 human rhinovirus, 356 respiratory syncytial virus, 160 human adenovirus, 117 human parainfluenza 3, 96 human bocavirus, 96 Influenza A (including 39 H1N1, 21 H3N2, 9 H1N1, 6 H2N2 and 21 et al influenza A virus), 60 parainfluenza 4, 33 human coronaviruses NL63 and 229E, 27 Influenza C, 20 human metapneumovirus, 15 HCoV-HKU1/OC43, 12 Inf B, 9 HPIV-1, 9 WU polyomaviruses, 3 HPIV-2. Co-infection by at least 2 of the viral pathogens under study was observed in 13.5% cases (243/1800). Two pathogens were detected in 210 samples (11.67%) and three pathogens in 33 samples (1.83%). The commonest pathogens in co-infected samples were HBoV (78.13%), Inf C (74.07%), WUPyV (66.67%), Inf B (66.67%) and HPIV-4 (56.67%).

**Conclusion:** Viral respiratory infections are very common in children and are an enormous burden to the families and society. Epidemiologic studies of respiratory viruses are important because of prevention and treatments for virus infections are emerging. In this study, we detected 21 kinds of respiratory viruses by multiplex RT-PCR with a GeXP analyzer simultaneously. This is a rapid and simple assay that could be used in clinical diagnosis as well as for the surveillance for the spread of antibiotic resistance determinants in epidemiologic studies.

20.016 Human rhinovirus viremia in patients hospitalized with community-acquired pneumonia

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**Purpose:** Human rhinoviruses (HRVs) are common upper respiratory tract pathogens that can occasionally cause lower respiratory tract disease. Unlike other members of the Enterovirus genus, HRV viremia has only recently been documented using sensitive molecular methods. In the recently completed Etiology of Pneumonia in the Community (EPIC) study, HRVs were found to be one of the most commonly detected pathogens in the upper respiratory tract of persons hospitalized with community-acquired pneumonia (CAP), with detections in 29% and 9% of children and adults tested, respectively. However, HRVs were also detected in the upper respiratory tract of 17% of healthy pediatric controls, complicating efforts to link HRV detection with illness. The purpose of this study is to determine the prevalence of HRV viremia among EPIC enrollees to assess HRV viremia as a possible indicator of lower respiratory tract infection in CAP.

**Methods & Materials:** Sera collected within 72 hours of admission from 417 children and 154 adult CAP patients who had HRV detected from upper respiratory samples were tested by real-time RT-PCR (rRT-PCR) assay. HRV positive respiratory and sera samples were typed by RT-PCR and sequencing of partial VP4/VP2 regions.
Results: HRV viremia was detected in 56 (9.8%) of these 571 patients with the proportion of detections varying by age group: <6 months of age, 2/51 (3.9%); 6-11 months, 5/55 (9.1%); 12-23 months, 21/80 (26.3%); 24-59 months, 20/99 (20.2%); 5-9 years, 8/92 (8.7%); 10-18 years, 0/41 (0%); >18 years, 0/153 (0%). Of 56 viremic patients, 29 (52%) had corresponding convalescent serum available for testing; all were negative by rRT-PCR for HRV RNA. A second respiratory virus was co-detected in the respiratory specimen of 3 of 56 (5.4%) viremic patients compared with 82 of 321 (25.5%) patients without viremia (Chi-squared, p<0.001). Viremia was more often associated with species C viruses: HRVA, 1/278 (0.36%); HRVB, 0/39 (0%); HRVC, 55/254 (21.7%).

Conclusion: HRV viremia was identified in nearly 1 in 10 patients hospitalized with CAP who had HRV detected from upper respiratory samples and was most often associated with species C virus infection in children less than 10 years of age.

20.017 Co-circulation of multiple reassortant influenza viruses in a swine farm
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Purpose: Starting from August 2012 repeated respiratory outbreaks caused by influenza A virus (IAV) were reported for one year in a swine breeding farm in Italy. Despite the implementation of a vaccination program in sows in November 2013, the infection persisted. This study was intended to characterize the genetic diversity of the viruses circulating in the farm during the vaccination campaign.

Methods & Materials: From November 2013 to April 2014 nasal swabs were collected from a total of 249 piglets born from vaccinated sows. Extracted RNA was tested using a Real Time RT-PCR targeting the M gene and a multiplex RT-PCR for typing and sub-typing analyses. The complete genome of eight IAV-positive nasal swabs was sequenced using an Illumina MiSeq platform and phylogenetically analysed using the Maximum likelihood method implemented in PhyML.

Results: Swine IAVs of the H1N2 subtype were detected in piglets sampled between November 2013 and April 2014. Phylogenetic analyses identified the co-circulations of two different genotypes in the investigated farm between November 2013 and January 2014. Specifically, three H1N2 viruses fell within a genotype (genotype F), which has been circulating in Italy since 2003. Differently, the remaining five samples belonged to a novel genotype, never reported in Europe so far, which had likely been generated through a reassortment event between genotype F and the A(H1N1)pdm09 lineage. Within this new genotype, we identified three genetically distinct variants (V1 to V3) generated through further reassortment events of their internal genes. Analysis of the molecular sequences identified mutations associated with increased virulence and transmission efficiency in mammalian species in the PB1-F2 gene (N66S) and in the PB2 gene (701N). In addition, we observed deletions at the C-terminal of both the NS1 and PB1-F2 proteins of six viruses (F genotype and the V3).

Conclusion: The circulation of multiple reassortant genotypes in such a short time highlights the complexity of the genetic diversity of swine influenza viruses and the need for a better surveillance and control strategies. Further studies are necessary to understand the level of cross reactivity and cross protection of current vaccines against the F and novel genotypes.

20.018 Impact of host immunity in the mammalian adaptation of an H3N6 avian influenza virus
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Purpose: To date, only influenza viruses of the H1, H2 and H3 subtypes have been responsible of human pandemics. We speculate that a pre-existing immunity in the human population against the H1 and H3 subtypes may play a key role in the emergence of human-adapted viruses with pandemic potential. To test this hypothesis, we used deep sequencing to explore the within-host genetic diversity and evolution of an H3N6 avian influenza virus (AIV) in ferrets with or without a pre-existing immunity against a seasonal H3N2 influenza virus.

Methods & Materials: Two transmission chains, with three naïve and three previously immunized ferrets (convalescent) were performed in a previous study. RNA isolated from nasal washes collected 2, 3 and 4 days post inoculation (dpi) from each animal were deep
 sequenced using an Illumina MiSeq. After trimming, reads were mapped to a reference sequence using BWA-MEM. Variant calling was performed using LoFreq.

**Results:** To assess the evolution of the H3N6 AIV, we calculated the frequency of each amino acid variant at different time points. We identified three and one fixed mutations in the HA, PB1 and NS1 proteins in the viruses from the convalescent and naïve chains, respectively. Interestingly, the mutation G228S located in the receptor binding site of the HA rapidly increased in frequency in both chains. However, in the convalescent chain this mutation was rapidly selected, acquiring a frequency of 84% at 2 dpi in the first infected ferret and reaching and maintaining a 100% frequency in the second and third ferrets, while in the naïve chain this mutation required more time to become fixed and never reached a 100% frequency. In addition, estimation of the mean entropy values for each gene and of the sites likely under positive or purifying selection indicated a lower genetic diversity of the HA gene of the viruses collected from the second and third ferrets of the convalescent chain compared to the other viruses.

**Conclusion:** These findings suggest that host immunity may cause a selective bottleneck, favouring a more rapid selection of advantageous mutations during the avian influenza evolution in a new host.

**Purpose:** Vaccination is a strategic tool to control avian influenza virus infection in poultry. Improper or inadequate vaccines and vaccination schemes might favour the antigenic drift, a strategy used by influenza viruses to escape the immune system. An extensive and in-depth knowledge of the mechanisms regulating the intra-host viral evolution is of utmost importance to guarantee the application of adequate vaccine protection strategies against new emerging viruses. In this study we used a deep sequencing approach to preliminary characterize the heterogeneity and evolution of viral population in vaccinated and infected poultry under the effect of two vaccines (A and B) conferring different protection levels.

**Methods & Materials:** Two groups of ten Specific Pathogen Free (SPF) day-old chicks were vaccinated twice at a 10-day interval using two distinct influenza vaccines and challenged with an HPAI H5N1 virus 21 days from the booster dose. The challenge virus, six samples from group A and fourteen samples from group B were suitable for HA amplification and processed on Illumina MiSeq benchtop sequencer.

**Results:** Although neither of the two vaccines was able to confer full virological or clinical protection, the performances of vaccine A were higher in terms of reduction of viral shedding and survival rate. Deep sequencing analysis revealed polymorphisms distributed at nine nucleotide positions in group A viruses (frequency range from 1.12% to 6.80%) while group B showed fifty-seven positions involved in mutations with a wider frequency range (from 1.02% to 68.70%). Interestingly, seven samples belonging to group B displayed a total of ten non-synonymous polymorphisms within the receptor binding domain and/or within the antigenic sites 1 and 2, whereas no mutations in this area were observed in group A samples.

**Conclusion:** Our results suggest that a suboptimal vaccine protection might influence the diversity end evolution of a viral population, favouring the appearance of polymorphism at the very beginning of the viral infection.

**Purpose:** In this study we evaluate the performances of a commercial ELISA kit for Influenza A virus (IAV) serology by comparison with hemagglutination inhibition test (HI), gold standard test according to OIE and able to detect early and late antibody response, on swine sera collected in Northwestern Italy in 2014.

**Methods & Materials:** Briefly, 1086 sera from 43 swine herds were analysed in HI assays using H1N1, H3N2, H1N2 and H1N1pdm reference antigens (cut-off titer≥20) and with a competitive multi-species ELISA kit detecting anti-nucleoprotein IAV antibodies. ELISA accuracy and the concordance between the two assays were evaluated.

**Results:** HI positivity to at least one antigen was registered in 864 sera. In ELISA, 639 sera resulted positive, 393 negative and 54 inconclusive. The 20% (44/222) HI seronegative sera
resulted positive in ELISA, while the 26% (224/864) HI positive samples were ELISA negative and so considered as false negative. Interestingly, the 50% of them had a HI titer between 20-40; in the remaining sera, titers were distributed uniformly among 80-5120 values. ELISA performances vs HI were: sensitivity 72.65% (95%CI:69.46-75.68), specificity 63.01% (95%CI:58.44-67.41), PPV 73.08% (95%CI:69.46-76.49) and NPV 43.00% (95%CI:38.05-48.06) with K=0.3961 (95%CI:0.3405-0.4516). Considering the subtype-specific assays separately, the best ELISA performances resulted in comparison with H1N1pdm HI test with 87.89% (95%CI:83.25–91.62) sensitivity, 46.65% (95%CI:43.09-50.23) specificity, 35.21% (95%CI:31.51-39.05) PPV and 92.11% (95%CI:88.99-94.58) NPV. The best concordance was shown with H3N2 HI assay (K=0.4601, 95%CI:0.4059-0.5144).

Conclusion: One hypothesis for the higher ELISA positivity rate may be indicative of a seroconversion against another IAV strain, but the different target antibodies of the two tests might be also taken into account. The false negative sera may be due to an early immune response in some animals, not detectable by the commercial kit. In fact, as suggested by some authors, ELISA test may not identify positive animals at the early stage of infection effectively as the HI, particularly when the virus is introduced to a naive swine population. In conclusion, the ELISA performances need to be improved, but the commercial kit can be used in SIV infection serodiagnosis. However, caution may be used since the test could miss recently exposed animals.


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Purpose: Respiratory virus (RV, Influenza, RSV, parainfluenza, adenovirus, etc) cause a significant community health concern. Treating cold like symptom and managing severe lower respiratory infection are common in children and old age. Active monitoring of prevalence of specific respiratory virus is carefully follow up and monitored by hospital based laboratories and nationwide reference laboratories. We use sensitive and validated real-time PCR assay for reporting clinically important respiratory virus prevalence rate during 2015.

Methods & Materials: Seegene Medical Foundation (SMF) had nationwide local branch offices networks which collect specimen and transport in Korea. More than 4,000 clinics sent nasopharyngeal swab (NPS) and/or nasopharyngeal aspiration (NPA) everyday and the results were reported next working day. From January 1, 2015 to December 31, 2015 36,281 samples (17,062 male and 19,219 female) were tested and analyzed in SMF using Anyplex II RV16 (Seegene, Seoul, Korea). This multiplex real-time PCR (Anyplex II RV16) could detect sixteen respiratory infection causing pathogens, Adenovirus (AdV), Influenza A (FluA) and B (FluB), Parainfluenza 1, 2, 3, 4, Rhinovirus A/B/C (HRV), Respiratory syncytial virus A and B (RSV A and B), Bocavirus (HBoV), Metapneumovirus (MPV), Coronavirus 229E, NL63, OC43 (CoV), Enterovirus (HEV) in two PCR tube.

Results: During twelve months of study period, overall prevalence rates of Adenovirus (AdV), Influenza A (FluA) and B (FluB), RSV A and B, MPV and Rhinovirus were 19.1, 5.2, 2.5, 4.6, 11.6, 5.3, and 26.0 % respectively. Typical seasonal peak is demonstrated in summer with parainfluenza (May to September), winter with RSV (January and December) and Influenza A and B (from June to March).

Conclusion: Multiplex real-time PCR shows robust and consistent results in study times. It could be a cost-effective and give a rapid diagnostic tool for the detection of multiple respiratory viruses and frequently used for health care surveillance of flu season. These results have a limitation because these data are come from mostly symptomatic patients but detail medical history could not review. In clinical reference laboratory, high throughput multiplex PCR is convenient and epidemiologic data provides useful information to clinicians.

20.022 Knowledge, attitudes and practices of parents towards childhood influenza vaccination in Singapore

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Purpose: Seasonal influenza vaccination is recommended in children <5 years, but little is known about vaccination coverage and determinants of vaccination in Singaporean children. We describe the results of a survey of knowledge, attitudes and determinants of childhood influenza vaccination.

Methods & Materials: In January-March 2016, 332 parents of children aged 6 months to 5 years attending childcare centres completed an online questionnaire.
**Results:** General knowledge about influenza, perceived benefit of vaccination and willingness to vaccinate were high, with few perceived barriers to vaccination. However, only 32% of children had ever received influenza vaccine, and only 15% in the past year. In multivariable analysis, respondents were more likely to have vaccinated their child if: (i) they had a higher willingness to vaccinate (prevalence ratio (PR)=1.58, 95% CI:1.24-2.04 per unit increase in willingness score); (ii) they felt well-informed about influenza vaccine (PR=1.44, 95% CI: 1.04-1.99); (iii) their family regularly received pre-travel influenza vaccine (PR=1.64, 95% CI: 1.19-2.25), (iv) their child’s doctor recommended influenza vaccine (PR=2.47, 95% CI: 1.75-3.48), and (v) they received influenza vaccine information from a general practitioner (PR=1.47, 95% CI: 1.05-2.04). Parents who obtained influenza vaccine information from television were less likely to have vaccinated their child (PR=0.44, 95% CI: 0.23-0.85). Path analysis using structural equation modelling indicated that being recommended vaccination by a child’s doctor increased willingness to vaccinate and self-efficacy (feeling well informed about influenza vaccine). Median willingness-to-pay for a dose of influenza vaccine was SGD30 (interquartile range: SGD20-SGD50), and was higher in parents of vaccinated compared with unvaccinated children (SGD45 vs SGD30, p=0.0012). A quarter of respondents were aware that Medisave could be used to pay for influenza vaccination.

**Conclusion:** Knowledge and willingness to vaccinate are high among parents, but there is considerable potential to improve influenza vaccine uptake in children. Encouraging medical professionals to recommend vaccination of eligible children is key.

20.023 Vaccine efficacy of seasonal influenza vaccine programme among Singapore military personnel in 2012–2015

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**Purpose:** All male Singaporeans undertake two years of national service, and since 2009 active servicemen have received annual influenza vaccine. Vaccine efficacy (VE) of the seasonal trivalent influenza vaccine (TIV) was assessed among military personnel in the Singapore Armed Forces (SAF) from November 2012 to the end of 2015.

**Methods & Materials:** Consenting national servicemen who presented with febrile respiratory illness (FRI), which was defined as having fever ≥37.5°C and cough/sore throat, had their nasal swab samples and clinical data (including vaccination history) collected. All nasal swab samples were sent for laboratory confirmation using RT-PCR. Vaccination status for study participants was assessed if they had vaccination more than 14 days but less than 180 days from the disease onset date. To estimate the crude and adjusted VE, univariate and multivariate logistic models were used respectively. Variables adjusted in the multivariate logistic models were camp groups (recruit camp vs other camps) and interaction between camp groups and individual vaccine history.

**Results:** A total of 4733 observations were collected in the FRI surveillance programme conducted by SAF during the entire study period. The crude VE of TIV was moderate against influenza A(H1N1)pdm09 (VE: 57.7%, 95% confidence interval (CI): 30.6% to 74.2%) and A(H3N2) infections (VE: 52.0%, 95% CI: 39.9% to 61.6%). VE against influenza B infections was markedly lower (VE: 25.8%, 95% CI: 4.8% to 42.2%). Additional multivariate analyses showed that adjusted VE was positively significant against influenza A(H1N1)pdm09 and A(H3N2) infections in both recruit camp and other camps. However, the adjusted VE against influenza B infections was significant in the recruit camp only (VE for recruit camp: 62.8%, 95% CI: 45.4% to 86.9%; VE for other camps: -5.2%, 95% CI: -55.6% to 28.9%).

**Conclusion:** TIV provided a moderate degree of protection against influenza A(H1N1) and A(H3N2), but not against influenza B in non-recruit military camps, which may be due to vaccine mismatch on B strains during that period. Virological surveillance of this fully vaccinated population may herald strain mismatches in the general community, and in this case could suggest the use of the quadrivalent influenza vaccine rather than the trivalent one.

20.024 Molecular characterization of H6 subtype influenza viruses in southern China from 2009 to 2011

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**Purpose:** H6 avian influenza viruses (AIVs), which are prevalent in domestic and wild birds in Eurasian countries, have been isolated from pigs, a dog and a human. So, the H6 subtype AIVs might pose a potential risk to the public health.
Methods & Materials: Routine virological surveillance at live poultry markets (LPMs) or poultry farms was conducted in southern China from 2009 to 2011. This study investigated the genetic and antigenic characteristics, analyzed the receptor-binding properties and evaluated the kinetics of infectivity of the H6 AIVs in A549, MDCK and PK15 cells.

Results: A total of 14 H6N6 and 2 H6N2 isolates were obtained from 4 provinces in southern China. Genetic analysis indicated two distinct hemagglutinin (HA) lineages of the H6 strains cocirculating in southern China, and these strains facilitated active evolution and reassortment among multiple influenza virus subtypes from different avian species in nature. None of these isolates grouped with the novel Taiwan H6N1 virus responsible for human infection. Receptor-binding specificity assays showed that 5 H6 AIVs may have acquired the ability to recognize human receptors. Growth kinetics experiments showed that EV/HB-JZ/02/10(H6N2) and EV/JX/15/10(H6N6) initially reproduced faster and achieved higher titers than other viruses, suggesting that enhanced binding to α-2,6-linked sialic acids (SA) correlated with increased viral replication in mammalian cells.

Conclusion: Overall, the research suggests that the H6 viruses could evolve into a threat to humans through genetic exchange with other forms of the virus. The results also emphasize the need for continued surveillance of H6 outbreaks and extensive characterization of H6 isolates to better understand genetic changes and their implications.

20.025 Comparative evaluation of ante-mortem tuberculosis diagnostics in Asian elephants (Elephas maximus)

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Purpose: Confirmatory diagnosis of ante-mortem tuberculosis infection in wild animals is a difficult proposition. Several diagnostic techniques that utilize host cell-mediated and humoral immune responses to known Mycobacterium tuberculosis/bovis antigens have been tried for tuberculosis screening in numerous wild species. However, only a limited number of such tests in very few species are accredited by statutory agencies for field use. In Asian elephants (Elephas maximus), most reported cases of tuberculosis are caused by the human tubercle bacilli, M. tuberculosis, suggesting spillover infection resulting from humans. The United States Department of Agriculture licensed the serological test DPP Vet Assay

(Chembio Diagnostics Inc., Medford, New York) for tuberculosis screening in Asian elephants. We present a comparative evaluation of DPP Vet Assay® and six other similar yet non-accredited tuberculosis screening tests, with culture, the gold standard for tuberculosis screening in any species.

Methods & Materials: During post-mortem examinations, we obtained caseous nodules in lung parenchyma of nine captive elephants from which M. tuberculosis was isolated by culture on Lowenstein Jensen medium. Banked ante-mortem serum samples from these nine elephants and nine other known tuberculosis negative elephants were tested on DPP Vet Assay® and the six other non-accredited serological tests. Diagnostic sensitivity and specificity of DPP Vet Assay® for tuberculosis screening in elephants are reported as 100% and 95% respectively, while that of the non-accredited tests are unknown.

Results: A checkerboard pattern comparative evaluation of the positive and negative results of the eighteen elephants on the seven serological tests was done. On DPP Vet Assay®, the nine serum samples from the nine culture positive elephants tested positive and the nine negative serum gave negative results. The six non-validated serological tests gave varying numbers of positive and negative results on the known positive and negative serum samples. Differential comparison of the test results from each elephant on all the tests and each test in all the elephants were also done.

Conclusion: Our results indicate the need for ascertaining the diagnostic sensitivity and specificity as well as validation and accreditation of the diagnostic tests for tuberculosis screening in any species, including Asian elephants.

20.026 Viral load and oxidative stress in Egyptian chronic hepatitis C patients

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Purpose: Hepatitis C virus (HCV) is major cause of viral hepatitis in Egypt. This viral infection frequently does not resolve and chronic HCV carriers may then progress to serious liver diseases. The overproduction of reactive species results in oxidative stress, causing an imbalance in the host. HCV infection is associated with severe alterations of the host redox status, thus playing important roles in the development of HCV-associated liver
diseases. **Aim:** Determination of the presence of any association between chronic HCV infection and the oxidative stress, namely correlating between the HCV viral load and liver enzymes, malondialdehyde (MDA) and catalase (CAT) enzyme in chronic HCV patients.

**Methods & Materials:** Evaluations were done on a total of 150 chronic HCV Egyptian patients, having variable viral loads, in addition to a control group of 50 healthy subjects. HCV antibodies were detected by ELISA and HCV viral load was determined by Real Time PCR. Chemical detection of serum alanine transaminase (ALT), aspartate transaminase (AST), malondialdehyde (MDA) and catalase enzyme was performed.

**Results:** The study revealed that a statistically significant correlation between the liver enzymes and oxidative stress markers has been detected. Moreover, a statistically significant positive correlation existed between MDA and the HCV viral load and a statistically significant negative correlation existed between the catalase enzyme and HCV viral load.

**Conclusion:** Oxidative stress is a significant feature of chronic HCV infection. The higher the viral load, the more prominent the oxidative stress markers are.

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20.027  
**Point of care of testing in molecular diagnostics: Evaluation of GeneXpert HCV RNA for diagnosing and monitoring of HCV infection**

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**Purpose:** HCV RNA measurement is essential not only for the confirmation of diagnosis but for the monitoring of antiviral HCV therapy. Nucleic acid testing at the point of care may turn out to be a game changer for HCV diagnosis and treatment monitoring, especially in low income countries, through increased sensitivity, reduced turnaround time, and ease of performance. One such assay GeneXpert (Cepheid) has recently been released.

**Methods & Materials:** Comparative analysis between Xpert HCV RNA and Abbott HCV RNA test for diagnosing of new cases of HCV and monitoring of patients on antivirals was done. The Xpert system is a fully automated real-time PCR device, which combines steps of sample preparation, nucleic acid extraction, amplification and detection of target sequences in one cartridge. Overall, 174 CHC patients were recruited and, one time plasma samples from 154 patients and in 20 patients, plasma samples obtained at specific key decision time points of two types of therapy (baseline, 4 weeks, 12 weeks and 24 weeks) were serially re-tested on Xpert.

**Results:** Genotype 3 was the commonest, seen in 80 (66%) of the cases, genotype 1 in 34(28.3%), genotype 4 in 4(3.3%) and genotype 2 and 5 in 1(0.8%) each. HCV RNA load in the samples ranged from 0 to 6.98 log₁₀ IU/ml, with a median value of 4.69 log₁₀ IU/ml. Overall a very good correlation was seen between the two assays (R² =0.985), concordance of the results between the assays was seen in 138 samples (89.6%). High and low positive reference standards were tested ten times on Xpert to evaluate the precision and the coefficient of variation was 0.01 for HPC and 0.07 for the LPC. Monitoring of patients on two different regimes of treatment, interferon and ribavirin (20) and sofosbuvir (20) was done by both the systems at baseline, 4, 12 and 24 weeks. Perfect correlation in the course of therapy at different data point across all genotype was seen.

**Conclusion:** The study demonstrates excellent performances of the Xpert HCV assay in viral load assessment and in treatment course monitoring consistency. Assays are comparable and can be interchanged.

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20.028  
**A Diagnostic and Epidemiologic Investigation of Acute Febrile Illness (AFI) in Kilombero, Tanzania**

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**Purpose:** In low-resource settings, empiric case management of febrile illness is fairly routine as a result of limited access to laboratory diagnostics. The use of comprehensive fever syndromic surveillance, with enhanced clinical microbiology, advanced diagnostics and more robust epidemiologic investigation, could enable healthcare providers to offer a differential diagnosis of fever syndrome and more appropriate care and treatment.

**Methods & Materials:** We conducted a year-long exploratory study of fever syndrome among patients presenting to clinical settings with an axillary temperature of >37.5 C and
symptomatic onset of <5 days. Blood and naso-pharyngeal/oral-pharyngeal (NP/OP) specimens were collected and analyzed using AFI and respiratory TaqMan Array Cards (TAC) for multi-pathogen detection of 56 potential causative agents. We collected demographic and clinical data to establish epidemiologic risk for infection, using agent-specific filtered multivariate regression.

**Results:** From 15 September 2014 - 13 September 2015, 997 febrile patients were enrolled and analyzed, including: 14% (n=139) 1<5yrs, 19%(n=186) 5-14yrs, and 67% (n=672) >15yrs. AFI TAC and respiratory TAC were performed on 880 whole blood specimens and 391 NP/OP specimens, respectively. Of the 56 agents surveyed, *Plasmodium* was the most common microbe detected. AFI TAC detected seven distinct organisms in 49% of AFI blood samples, including: *Plasmodium* (47%), *Leptospira* (3%), *Bartonella* (1%), *Salmonella enterica* (1%), *Coxiella burnetii* (1%), *Rickettsia* (1%), and West Nile virus (1%). Respiratory TAC detected nucleic acid for 24 different microbial agents, including 12 viruses and 12 bacteria. The most common agents detected were: *Haemophilus influenzae* (67%), *Streptococcus pneumoniae* (55%), *Moraxella catarrhalis* (39%), *Staphylococcus aureus* (37%), *Pseudomonas aeruginosa* (36%), Human Rhinovirus (25%), Influenza A (24%), *Klebsiella pneumoniae* (14%), Enterovirus (15%) and *Group A Streptococcus* (12%). Dual and/or multiple infections were also observed. On multivariate regression, age and symptomatic presentation were significantly associated with different etiological agents.

**Conclusion:** The novelty of this study is proven in our use of highly sensitive multi-pathogen molecular diagnostics to better characterize the contributing agents of fever syndrome and establish a baseline disease prevalence in our study area.

20.029 Comparative meningitis diagnostic performance of a resource limited laboratory and a WHO accredited reference laboratory during a major outbreak of meningitis in Brong Ahafo, Ghana


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**Purpose:** Accurate diagnosis in resource limited laboratories remains a challenge. Between December 2015-April 2016, Ghana experienced a major meningitis outbreak with Brong Ahafo Region (BAR) recording 968 cases and 87 deaths. The Sunyani Regional Hospital Laboratory Microbiology Unit (SRHLMU) played a major role in the laboratory testing of suspected cases in the BAR. Tested and non-tested Cerebro-Spinal-Fluid (CSF) samples were sent from SRHLMU to a WHO accredited reference laboratory during a major outbreak of meningitis. Comparative meningitis diagnostic performance of a resource limited laboratory and a WHO accredited reference laboratory was evaluated.

**Methods & Materials:** A total of 192 CSF samples, comprising of 128 tested and 64 non-tested, were submitted to a WHO reference laboratory. The SRHLMU analysed samples using either Gram stain, Pastorex Meningitis Rapid test kit or culture depending on which laboratory testing logistics was available. The WHO laboratory used Real-time PCR method for analysis. Kappa analysis was used to determine level of inter-laboratory diagnostic agreement.

**Results:** Out of the 128 tested samples, 107(83.6%) agreed with results from WHO laboratory whilst 21(16.4%) were discordant. Of the agreed results, 38(35.5%) and 69(65.5%) were positive and negative for a pathogen respectively. Of the pathogens detected, 30(78.9%) were *Streptococcus pneumoniae*(Spn), whilst 8(21.1%) were *Neisseria meningitidis* sero-group W(Nm.w). Of the discordant results, SRHLMU reported 12 pathogens: Spn(9), Nm.w(1) and *Haemophilus influenzae* type b(2), but which were reported negative by WHO laboratory; whilst nine negative reports were reported positive for; Spn(6), Nm.w(3) respectively by WHO laboratory. There was an overall substantial inter-laboratory agreement of results between SRHLMU and WHO laboratory(Kappa value=0.65). Of the 64 non-tested samples submitted, there was no result returned for 5(7.8%). Of the remaining 59, 24(40.7%) were positive for bacterial pathogens; Spn, 20 (83.3%), Nm.w, 3(12.5%) and Nm.c, 1(4.2%) and the other 35(59.3%) were negative for any pathogen.

**Conclusion:** The SRHLMU was able to confirm bacterial meningitis outbreak in BAR and had results that compared favourably with WHO accredited laboratory. However, local laboratories need to be adequately resourced to further improve diagnostic accuracy and performance to meet demands of public health threats and emerging diseases.
Deployment of rapid and portable diagnostic test for field surveillance of Ebola virus disease in Guinea

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**Purpose:** In the past and recent outbreak of Ebola virus disease (EVD), diagnostic capacity in the local laboratories and accessibility of endemic area to reference or mobile laboratories were important factors which played a role in the spread and size of outbreak. Rapid and accurate diagnostic techniques have been required for early identification and rapid response to the disease. We have developed and evaluated a rapid detection assay for *Zaire ebolavirus* (EBOV) with a portable nucleic acid amplification and detection system. We also deployed the assay for post-mortem testing in enhanced surveillance of EVD in Guinea.

**Methods & Materials:** We developed reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay for detecting EBOV with two sets of primers which targeted untranslated region (trailer) and nucleoprotein (NP) in the viral genome. Amplification and detection was performed at 63°C for 30 min in a portable real time fluorometer (GenieIII, Optigene). The assay was evaluated using a total of 100 clinical specimens (serum, n = 44; oral swab, n = 56) collected from suspected EVD cases, and the results were compared with a reference standard test. Neither RT-LAMP nor RT-PCR test yielded a positive result in the surveillance, and no false-positive results were observed in the RT-LAMP assay.

**Results:** The RT-LAMP assay could specifically detect viral RNAs within 15 minutes without cross-reactivity to other hemorrhagic fever viruses and arboviruses. The sensitivity of the RT-LAMP test was 100% for both primer sets, while the specificity was 100% and 98.7% for the trailer and NP primer sets, respectively, compared with a reference standard test. Neither RT-LAMP nor RT-PCR test yielded a positive result in the surveillance, and no false-positive results were observed in the RT-LAMP assay.

**Conclusion:** The RT-LAMP assay was a comparably sensitive and specific diagnostic test to the reference RT-PCR test. These results highlight the usefulness of the assay as an EVD diagnostic testing for the field surveillance and the laboratory in the remote area in EVD endemic countries.

Prevalence of *Actinotignum schaalii* in patients with urinary tract infections combined with leukocyturia and inconclusive bacteriological results

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**Purpose:** *Actinotignum schaalii* (formerly *Actinobaculum schaalii*) is a Gram-positive, facultative anaerobic, slowly growing, coccoid rod bacterium. *A. schaalii* specific PCR analyses made it possible to verify this facultative pathogen more easier and faster in human samples like urine. Several cases of severe infections such as urosepsis, endocarditis, spondylodiscitis and cellulitis have been described in the literature recently. Based on these findings, there is a need for further research concerning the prevalence of *A. schaalii* causing urinary tract infection.

**Methods & Materials:** A total of 50 urine samples from patients suffering from urinary tract infection with inconclusive bacteriological results (like mixture of numerous germs, contamination or no growth) combined with leukocyturia (Addis count ≥ 500,000 leukocytes in the urine/hour) were analyzed by *A. schaalii* specific PCR. Ten urine samples from patients with low Addis count results were used as negative control.

**Results:** We were able to identify *A. schaalii* in 9 (18%; 6 female, 3 male) out of 50 (36 female, 14 male) analysed urine samples by PCR. The mean age of patients with *A. schaalii* positive urine samples was lower than from all analysed patients (40 versus 50 years). All samples with low Addis-count were tested negative.

**Conclusion:** Our results suggest that *A. schaalii* is maybe a more common uropathogen in middle-aged patients as previously thought, particularly in combination with leukocyturia and inconclusive bacteriological results from the routine microbiological testing. Further investigations to detect *A. schaalii* in combination with the leukocytes count in urine/hour in patients suffering from urinary tract infection should be considered.
Purpose: Biosurveillance is critical for the identification of emerging infectious diseases (EIDs) that pose a potential threat to global health security. Zoonoses have accounted for a large portion of EIDs, and programs such as USAID-PREDICT and the Global Health Security Agenda have begun to focus on efforts to improve EID detection. We have developed a Luminex-based multiplex assay that is able to simultaneously detect antibodies with specificity to zoonotic viruses from the *Ebolavirus*, *Marburgvirus*, and *Henipavirus* genera.

Methods & Materials: Virus species in this multiplex assay include all five species of ebolaviruses: Ebola virus (EBOV), Bundibugyo virus (BDBV), Tai forest virus (TAFV), Sudan virus (SUDV), and Reston virus (RESTV); two related marburgviruses: Marburg virus (MARV) and Ravn virus (RAVN); and three henipaviruses: Nipah virus (NiV), Hendra virus (HeV), and Cedar virus (CedPV). A mammalian cell culture system was used to produce soluble viral envelope glycoproteins (sGp) that retain their native oligomeric conformations. Following purification by affinity and size exclusion chromatography, the sGps were coupled to color-coded Bio-Plex carboxylated microspheres. Monoclonal antibodies (mAbs) and polyclonal sera from EBOV, MARV, and NiV infected non-human primates (NHP) were used to investigate specificity and cross-reactivity with the panel of sGps.

Results: EBOV, BDBV, and TAFV sGp-conjugated microspheres had the highest median fluorescence intensity (MFI) when tested with anti-EBOV NHP sera. The Bio-Plex MFI data paralleled the phylogenetic relatedness of the ebolaviruses with EBOV, BDBV, and TAFV sGp-conjugated microspheres exhibiting similarly high MFI while SUDV and RESTV sGp-conjugated microspheres had MFIs higher than sGPs from marburgviruses and henipaviruses, but lower than EBOV, BDBV, and TAFV. An additional EBOV sGp was created that lacked the mucin domain (EBOV sGp∆mucin). We compared MFI between EBOV sGp and EBOV sGp∆mucin and saw that MFI was higher with EBOV sGp-conjugated microspheres, which confirmed research that the mucin domain is an immunodominant epitope. MARV and RAVN sGp-conjugated microspheres reacted most strongly with anti-MARV NHP sera.

Conclusion: The multiplex assay is able to differentiate antibody reactivity between ebolaviruses, marburgviruses, and henipaviruses. This multiplex assay has the potential to be integrated into surveillance programs to screen domestic animals, wildlife, and humans for evidence of exposure to these EIDs.

Purpose: Zika virus (ZIKV), is a mosquito born (*Aedes* species) flavivirus, which was first isolated from a rhesus monkey in Uganda in 1947. In humans, it causes a mild illness with a range from asymptomatic to influenza like symptoms. In 2014, the virus spread eastward across the Pacific Ocean to French Polynesia, then to Easter Island and in 2015 to Central America, the Caribbean, and South America, where the Zika outbreak has reached pandemic levels. In May 2015, PAHO issued an alert regarding the first confirmed ZIKV infection in Brazil. The outbreak in Brazil led to reports of Guillain-Barre syndrome and pregnant women giving birth to babies with birth defects. On 1 February 2016, based on recommendations of the International Health Regulations Emergency Committee, WHO declared the growing Zika outbreak a Public Health Emergency of International Concern.

Methods & Materials: Fast Track Diagnostics developed an *in vitro* test based on real-time reverse transcriptase PCR for the qualitative detection of ZIKV specific nucleic acid as an aid in the evaluation of infections by ZIKV. As recommended by WHO FTD Zika virus targets the non-structural protein 5 genomic region. This test is for use with extracted nucleic acid from serum, plasma, or urine of human origin.

Results: In silico and in vitro studies confirmed 100% sensitivity and specificity of FTD Zika virus. Extended precision studies showed a high inter- and intra-assay performance, as well as a high reproducibility between different production charges. FTD Zika virus detects down to 32 copies per reaction in 100% of cases and less than copies per reaction in around 80% of cases of RNA from the Asian lineage. In a clinical study conducted in Brazil, 100% sensitivity and specificity was received for the detection of ZIKV specific RNA with FTD Zika virus and the in-house singleplex RT-PCR assay. Testing EQA samples (*INSTAND-Ringversuch*) with FTD Zika virus and a competitor kit revealed nearly identical results.
Conclusion: FTD Zika virus is a highly sensitive and specific assay for the qualitative detection of zika virus specific RNA. Our assay performed comparably to other zika virus detection methods.

20.034 Early identification and antimicrobial susceptibility testing from positive blood culture bottles using MALDI-TOF MS
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Purpose: Timely diagnosis of the etiology of infection is imperative, with mortality increasing nearly 8% for every hour of inappropriate antimicrobial therapy administered to patients with sepsis. definitive organism identification and antimicrobial susceptibility testing (AST) results of positive blood cultures are generally not available to the treating physician for approximately 24 to 72 h after culture positivity is first noted. MALDI-TOF MS has the potential to identify any organism from a positive blood culture. The work aimed to detected the earliest time with the most accurate results to perform identification and susceptibility.

Methods & Materials: Positive blood culture broths were subjected to direct Gram staining and inoculated to two blood agar plates and and incubated at 35°C. One plate was used for serial identification and susceptibility by MALDI-TOF MS (bioMérieux Vitek MS IVD system) and Vitek 2 cards every 2 hours, and the other was used for conventional biochemical identification tests after overnight incubation. The results of the 2 plates were compared. ontaminated and mixed samples were excluded. A total of positive 25 blood culture bottles were included

Results: The study included 13 gram positive isolates and 12 gram negative isolates. All isolates showed 100% concordance in identification when tested at 4 hours incubation post inoculation. All Gram negative showed 100% concordance in AST results when tested at four hours incubation except Pseudomonas sp which showed concordance at 6 hours incubation post inoculation. The AST in vitek 2 was completed after 8 & 10 hours for gram negative & positive respectively. The final results wer obtained with 99% accuracy after 12-14 hours post inoculation

Conclusion: The result of identification and AST could be obtained accurately from positive blood culture bottles in short time using MALDI-TOF MS

20.035 Fast-track Diagnostics Enteric fever assay: From pre-enrichment to multiplex real-time PCR
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Purpose: Enteric fever due to Salmonella remains a significant public health problem, predominantly in children in developing countries. Typhoid fever affects more than 20 million people per year associated to about 300,000 deaths. These high rates are mainly due to the lack of fast, reliable and inexpensive diagnostic assays. Diagnosis of typhoid remains a challenge because a low bacterial load can be responsible of an illness and also because the volume of blood that can be collected from children is limited. To overcome the problem of sensitivity, the first goal of this study was to define an appropriate pre-enrichment method of the collected sample before nucleic-acid extraction. The second objective was to develop a sensitive multiplex real-time PCR assay for detection of the common etiological agents of Enteric fever: Salmonella enterica spp., Salmonella typhi and Salmonella paratyphi A.

Methods & Materials: To determine the best pre-enrichment medium, we spiked S. paratyphi A within blood samples, added the enrichment medium, cultured for 5h at 37°C, performed nucleic acid extraction and determined the yield of specific nucleic acids by real-time PCR. We tested several commercial enrichment broths and a home-made TSB medium supplemented with Oxgall (inhibitor of Gram+ bacteria). The results show that the Selenite broth (Copan) and the TSB-Oxgall medium are the most efficient pre-enrichment methods of free bacteria in blood samples.

Results: In parallel we developed a multiplex real-time PCR assay that distinguish between infection by S. enterica spp., S. typhi and/or S. paratyphi A (+ internal control). This assay shows a limit of detection between 1E+02 and 1E+03 copies/ml and a linearity from 1E+09 to 1E+03 copies/ml for all pathogens. The specificity of the assay was validated with various negative samples which did not generate any positive signals. Furthermore, different positive materials containing bacteria, parasites, and viruses were evaluated with this assay and no other than the expected bacteria were detected.
**Conclusion:** In conclusion, this study shows the development of an efficient diagnostic pipeline that combines a quick pre-enrichment of infected blood samples with a sensitive real-time multiplex PCR assay that could be used as a reference diagnostic method for Enteric fever infectious agents.

**20.036 Comparative efficacy of diagnostic test methods for *Plasmodium* species**

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**Purpose:** Diagnostic methods are becoming a crucial component of malaria control and prevention. Improved ability to diagnose malaria may prevent many unnecessary antimalarial treatments and should also allow prompt attention to other causes of fever when malaria is ruled out. This study compared the diagnostic accuracy of histidine-rich protein (HRP-2/BSD) and (HRP-2/Aldolase/AC) based RDTs with microscopy.

**Methods & Materials:** Ethical clearance was obtained from Covenant University ethical review committee. Informed consent and assent were also sought before subjects were recruited on this study. Blood samples were collected from 200 asymptomatic and 60 symptomatic subjects in Ado-odo/Ota LGA, Ogun state. The blood samples collected were first analyzed using the Rapid Diagnostic Tests and then stained with Giemsa stain and viewed under the microscope.

**Results:** Out of the samples collected from asymptomatic subjects, 44% were males while 66% were females. Among the samples from symptomatic patients, 58.3% were females. The overall incidence of falciparum malaria among the study population by microscopy was 32.3%. Among the asymptomatic patients only, the percentage incidence from microscopy was 26.5% with 11% males and 15.5% females; by BSD (HRP-2) kits, the incidence was 17% with 6.5% males and 10.5% females; while by AC (HRP-2/Aldolase), percentage incidence was 18% for *P. falciparum*, 6% were males and 12% were females. Among the symptomatic subjects, incidence rate of *P. falciparum* malaria was 42% by ACON kits, 38.3% by BSD and 51.7% microscopic method with 58.1% males and 41.9% females. Two samples were positive for *P. vivax* when diagnosed with AC (HRP 2/ Pan) but was not detected by microscopy.

**Conclusion:** There is the need to improve on the efficacy of available Rapid Diagnostic Test methods and their sensitivity in indicating the presence of *P. falciparum* and *P. vivax* antigens in the blood.

**20.037 Effectiveness of measuring blood antibody titer and biofilm-forming ability in *Staphylococcus aureus* bacteremia**

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**Purpose:** To examine the effectiveness of measuring anti-*Staphylococcus aureus* antibody titers in the blood and biofilm-forming ability of the pathogen as supporting evidence for the diagnosis of bacteremia in patients with *S. aureus* infection.

**Methods & Materials:** Anti-*S. aureus* antibody titers in the blood were measured by ELISA using protein A-deficient *S. aureus*. The biofilm-forming ability was measured using the tissue culture plate method.

**Results:** Regarding anti-*S. aureus* antibody titers, the patient group showed significantly higher IgG and IgA antibody titers compared with the healthy control group. There was no significant difference in IgM antibody titers between the patient and healthy control groups. Furthermore, significantly higher IgG and IgA antibody titers were observed in the bloodstream infection group compared with the respiratory tract infection group. In the bloodstream infection group, the IgA antibody titers of the 27 patients for whom multiple measurements could be obtained over time were elevated prior to positive blood culture and subsequently decreased; of these patients, six with a poor prognosis died. In patients for whom the blood culture was negative, IgA antibody titers tended to be elevated or unchanged. There was no marked change in IgG antibody titers.

With regard to the biofilm-forming ability, there was no significant difference between MSSA and MRSA. However, the examination of a possible relationship with prognosis in bacteremia cases by surveying the inpatient medical records of items, including leukocyte count, CRP, the presence or absence of fever, and outcomes, revealed that patients with a poor prognosis exhibited a significantly higher biofilm-forming ability than those with a good prognosis. It is believed that an OD value of 0.8 or above indicates strong adhesiveness. Furthermore, no relationship was observed between IgG antibody titer, IgA antibody titer, and biofilm-forming ability.
**Conclusion:** Patients with bacteremia show significantly higher anti-*S. aureus* antibody titers, and it is believed that the production of large volumes of IgA antibodies can improve their condition. Conversely, low titers suggest a poor prognosis. Results also suggested that biofilm-forming ability is associated with prognosis in patients with bacteremia.

20.038 A cross sectional survey on gastrointestinal parasites in backyard poultry in Pakistan “Highly affordable model survey”

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**Purpose:** Backyard poultry is still the most important protein provider in Pakistan. A reasonable source of revenue is earned with these birds especially by poor village women’s. These birds are acceptable to rural inhabitants because it suits their socio cultural norms. In the mean while these scavenging birds get exposed to an emerging threat of gastrointestinal parasites in many ways. In backyard poultry research regarding GIT parasite is limited.

**Methods & Materials:** Bearing in mind the impact of GIT parasite infections on the production of poultry as the major constrain a model cross section survey was conducted from Jan-2015 to March 2015 in the province of Punjab, Pakistan. For comparison on agro-ecological basis the study area was divided into three regions i.e. south, north and central Punjab. An appropriate epidemiological sampling technique was used to calculate a representative sample. Randomly a total of 20006 fresh faecal samples were collected from all the districts of Punjab. Standard qualitative sedimentation technique was used for the identification of Coccidia Oocysts and helminthes eggs.

**Results:** The results showed an overall prevalence of (53.93%) GITparasites. Chi square analysis of the data showed a significantly (P<0.05) higher helminth point prevalence in south Punjab (86.69%) followed by central (50.23%) and north Punjab (13.29%). Significantly (P<0.05) higher point prevalence of coccidia species in South Punjab (38.8%) followed by central (17.84%) and North Punjab (9.99%) respectively.

**Conclusion:** It was concluded that gastrointestinal helminths and enteric protozoan’s are highly prevalent in the backyard poultry as a serious problem in the study area and the identification of predominant nematodes and coccidian species is essential for establishing a suitable control and prevention strategy.

20.039 Contribution of grain amaranth and traditional healthcare systems in intervening jointly against infectious and non-communicable diseases to control HIV

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**Purpose:** The world is experiencing a new epidemic of chronic non-communicable diseases (NCDs) even before infectious diseases such as TB and HIV have been brought under control. However, combination of major NCDs and infections can interact adversely. And because intervention against either health problem will affect the other, intervening jointly against HIV and NCDs, rather than competing for limited funds, is an important policy consideration requiring new thinking and approaches, (UNAIDS, 2012). The purpose of this paper is to demonstrate how Strategic Poverty Alleviation Systems-SPAS uses grain amaranth and traditional healthcare systems to intervene jointly against NCDs and HIV with extremely promising results.

**Methods & Materials:** SPAS assists communities to integrate Organic Grain Amaranth (a non-grass cereal and nutritional powerhouse which is also a medicinal crop) and traditional healthcare systems into interactions between People, Plants, Livestock, and environment (PeoPLE) to bear jointly against HIV and NCDs. SPAS, a not for profit community based organization’s work focuses on efforts to prevent HIV infection and to improve treatment with the ultimate goal of eradicating the virus in people living with HIV infection. Trained small scale farmer families infected or affected with HIV grow grain amaranth for food/nutrition and income and part of the surplus bought by SPAS for making grain amaranth-based nutraceuticals for community members with chronic NCDs or infectious diseases.

**Results:** Grain Amaranth Nutraceuticals have previously benefited community members with chronic communicable and non-communicable diseases irrespective of sero-status whether on ARVs or not. Those living with HIV taking these nutraceuticals have consistently maintained normal numbers of CD4+T Cells, the loss of which is a hallmark of AIDS. As for NCDs, within seven days of taking these nutraceuticals, diabetics, and hypertensives, for instance, are off all medications.
**Conclusion:** Although the PeoPLE model provides a continuum of prevention, management and treatment to compliment conventional healthcare systems for HIV, it also delivers appropriate actions and programmes to move from management situation for NCDs in people living with HIV to treatment interventions and curative therapies for improved health of PLWAs if we recognize NCDs as a high priority health problem in controlling HIV.

20.040 Risk Factors for non-communicable diseases (NCDs) among prison population in Punjab, Pakistan, 2012

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**Purpose:** The burden of non-communicable diseases (NCDs) is disproportionately carried by prisoners. Chief Justice of Pakistan during visit to Central Prison of Lahore ordered for the improvement of health status of prisoners in Punjab prisons. Death surveillance data indicated 73% deaths were due to NCDs and 85% were premature in last few years. Subsequently this study was planned to assess the prevalence of potential risk factors of NCDs among prison population in Punjab, to highlight areas for action.

**Methods & Materials:** A descriptive cross sectional study was undertaken. A total of 301 male adult prisoners were enrolled through proportionate random sampling from 29 prisons of Punjab. Anthropometric measurements taken and information on age, education, type of prisoner, recreational/work activities, and smoking gathered. Body mass index (BMI) and waist to height ratio (WHtR) calculated and WHO recommendations for Asia Pacific Region for overweight and obesity were followed. Prison diet menu reviewed. Fractions of basic dietary ingredients and per day caloric value of diet worked out.

**Results:** Mean age of participants was 40 years. Overall prevalence of obesity/overweight was 51% and Central obesity/overweight 44%. Recreational facilities were not available in any prison. Convicted prisoners (CT) have been deputed for moderate physical labor. However under trial (UT) and condemned prisoners (CP) remained sedentary almost all the time. Prevalence of overweight/obesity among CP, UT and CT was 59%, 54% and 43% respectively. Overweight/obesity depicted increasing trend with age. Being educated was significantly associated with obesity (OR 2.86; 95% CI 1.58-5.17, P < .05) and physical inactivity with central obesity (OR 1.6; 95% CI 1.03 - 2.69, P < .05). Active and passive smoking among prisoners was 44% and 78% respectively. Daily caloric intake was 2955 (recommended; 2150 for sedentary and 2350 for moderate physical work). Daily added salt, fiber and fat intake was 7gms, 5 - 6gms and 60gms respectively (recommended: added salt; < 5gms, fiber; 30gms and fat; <30gms).

**Conclusion:** Prison population has shown high prevalence of multiple preventable risk factors of NCDs. We recommended revision of prison diet menu, arrangements for recreational activities and initiation of stop smoking programs.

20.041 Interactive Identification Key for Female Mosquitoes (Diptera: Culicidae) of Euro-Mediterranean and Black Sea Regions

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**Purpose:** In the context of the recent introduction of invasive species, an urgent need for a user-friendly identification key for mosquitoes has resurfaced among entomologists and health care officials. As the MediLabSecure medical entomology network team, our experience has shown that it is essential to have a tool that can be used freely, online and offline, by both specialists and non-specialists.

**Methods & Materials:** 134 mosquito species are included in this tool. These mosquitoes are distributed in 65 countries in Europe, North Africa, the Black Sea area and Middle East. Morphological descriptors for female identification are used to build a comprehensive database. This morphological database was consequently edited using the computer software Xper², a useful tool to create interactive and easy to use identification keys.

**Results:** This interactive morphological identification key was developed using 57 morphological characters (descriptors): 11 on the head, 15 on the thorax, 9 on wings, 22 on legs and 6 on the abdomen. Geographic distribution is another very useful character where more than one country could be selected. All descriptors have detailed description, along with a picture or an illustration. Priority was to line up the descriptors, considering non-experts will use the tool so characters that are more visible than others come first. As an option there are three different models that can choose the question with the higher discriminant power that leads to a quicker identification. It allows the user to see the descriptors, states, definitions, images and all taxa on the same page.
Conclusion: Training sessions organized in the frame of the MediLabSecure Project, with participants from many of the selected countries in this task, have brought to light that a tool to train inexperienced users is a must. This identification key will be available in January 2017. This multi-entry key will allow using descriptors the user can observe. This will help when some body parts are missing on the sample since it’s possible to select a group of descriptors. Future plans are to include a key for fourth instar larva and to perform validation tests on entomologists with variable degrees of experience.

20.042 Novel rapid approach for molecular diagnosis of multidrug-resistant tuberculosis using real time PCR assay combined with high-resolution melt curve analysis

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Purpose: To develop a reliable in-house molecular method for rapid and cost-effective diagnosis of multidrug-resistant tuberculosis (MDR-TB) that is applicable to resource-limited countries with high-TB burden.

Methods & Materials: Two real time PCR reactions were set up. Reaction I comprised a newly designed LepB DR primer and the NTM primer for identification of Mycobacterium tuberculosis complex (MTC), M. bovis, and non-tuberculous mycobacteria (NTM). Reaction II used a newly designed RpoB primer to detect the rpoB mutations implicating rifampicin (RIF) resistance. Optimization and specificity of the reactions combined with high-resolution melt curve (HRM) analysis (increment = 0.04°C/s) using 22 bacterial strains were performed prior to the method evaluation using 40 mycobacterial DNA samples derived from TB-diagnosed patients.

Results: The optimal concentrations of LepB DR, NTM, and RpoB primers were 0.2, 0.2, and 0.3 μM, respectively, and the optimal annealing temperatures were 60°C for reaction I and 58°C for reaction II. No cross-amplification was observed in the reactions when testing with genomic DNAs of 13 Mycobacterium species and 9 non-Mycobacterium bacteria. Testing of 40 samples (30 MTC, 10 NTM) showed comparable results to standard culture method. The mean value of melting temperature (Tm) of MTC was 90.91±0.82°C with the Tm difference (ΔTm) averages of 0.28±0.05°C and 1.55±0.05°C compared to M. bovis and M. avium (NTM), respectively. Thus far, the rpoB mutations of 24 MTC-positive samples (12 MDR, 12 RIF-sensitive MTC) were examined and results of 23 samples were correlated with standard method. MDR showed the Tm mean value of 89.96±0.55°C and a ΔTm average of 0.38±0.34°C compared to M. tuberculosis H37Rv (wild type / Rv). The rpoB mutation was not detected in one MDR sample (ΔTm average of 0.02°C compared to Rv), in which a class -IV (A→T) point mutation was identified by DNA sequencing. Interestingly, the S522L mutation was discovered in one MDR sample along with other common mutations at codons 531, 526, and 516 of rpoB.

Conclusion: The novel targeting multiplex real time PCR in combination with HRM analysis is highly specific, rapid and cost-effective. We demonstrated its potential applications in clinical laboratories for efficient diagnosis and early treatment of MDR–TB.

20.043 Implementation of GastroBusters in Toronto: Canada’s First Online Reporting System for Foodborne Illness

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Purpose: Foodborne illness (FBI) is greatly under-reported to public health. Toronto Public Health implemented an online reporting option, GastroBusters, for the public in July 2015, in preparation for the Pan and Parapan American Games (PPAG). Mass events increase risk of FBI and also involve visitors who may not access local health services unless acutely ill. System objectives included: (1) improved ability to detect FBI clusters/outbreaks, (2) more timely data for public health interventions, (3) better estimates of FBI in Toronto, and 4) potential use as a legacy system for routine surveillance.

Methods & Materials: FluidSurveys was used to create a user friendly, secure, online data collection tool. Users had the option to provide their contact information, with clear indication that this would permit contact by a public health investigator. A 3-day food history was requested, with prompts for large events (e.g., PPAG) that were attended. Nominal reports were escalated for follow-up within two hours of receipt; exposure data from both nominal and non-nominal reports were analysed with other surveillance data sources to identify commons exposures. Data were collated in MS Access and analyzed using SASv9.3. GastroBusters was piloted during the PPAG and then evaluated after 6 months.
Results: Between August 1, 2015 and January 31, 2016, 211 food poisoning reports were submitted through GastroBusters; 79% were not reported by another surveillance data source (e.g., telephone or food premise complaint). Two-thirds (67%) provided contact information permitting public health follow-up. Eighty-five reports prompted a food premise inspection, to identify and mitigate food safety infractions. Of these, 15 (18%) identified critical infractions and three premises were closed. No widespread community outbreaks or clusters of illness were detected by any surveillance sources during the pilot period, including during the PPAG.

Conclusion: Providing an online reporting option yielded valuable and timely information on FBI in Toronto. Integration with a food safety and premise inspection program can trigger actions needed to protect the public from further illness. GastroBusters is the first online FBI reporting tool implemented in Canada, and is a valuable legacy of the PPAG that can be used to complement other surveillance data sources.

20.044 Harvesting Real Time and Historical Disease Outbreak Data from the ProMED-Mail Database: Pitfalls and Proposed Solutions

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Purpose: Internet based disease reporting systems are potential sources of vast amounts of data that can be incorporated into epidemiological models investigating disease distribution and drivers. However steps must firstly be taken in order to address the veracity, volume and variety of the available data. This poster details our attempt to harvest and transform online disease outbreak reports from Pro-MED mail into reliable and usable data detailing infectious disease outbreaks in humans and animals at a country level.

Methods & Materials: ProMED-mail issues reports regarding disease outbreaks as well as requests for information, collegial notifications (conferences, publications) and other information. Unfortunately for the epidemiologist the type of information contained within the report, as viewable through Health Map, is not immediately ascertainable. In addition the information contained within the species field is often unreliable and the location reported is occasionally that of the information source not the outbreak.

We propose an automated method to harvest infectious disease outbreak information from the ProMED-mail reports. Firstly individual reports are grouped into disease events (a series of individual reports of the same disease occurring within a specified time frame and location) thereby eliminating individual reports that do not relate to disease outbreaks. Secondly an algorithm infers the species affected by scanning the grouped reports for key words and checking the compatibility of the species identified with the disease reported.

Results: Grouping reports of the same disease at the country level with a maximum of 21 days between each report transforms the 65492 reports over 10 years into 9436 disease events containing 51023 reports. Each disease event can be reliably attributed to an outbreak.

The grouping of the reports into disease events allows a more accurate representation of the species affected. The original data did not identify a species in 14% of reports. Our algorithm identified a species in all but 1% of disease events.

Conclusion: Our methodology allows the creation of a database documenting the distribution of animal and human disease outbreaks worldwide. We aim to integrate this ‘One Health’ database with a wealth of other data resources in order to characterise and quantify disease drivers through data modelling techniques.

20.045 Pandemic propagation — pyschosocial behavior analysis with media analytics

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Purpose: Analysis of pandemic propagation is critical to control of pandemics. Researchers have developed pandemic propagation models, employing large-scale numerical simulations using human avatars to model population dynamics. Avatar simulation had proven highly successful in modeling animal behavior, such as bird flocks and fish schools, and it has proven successful in modeling human behavior. The key is sufficient understanding of the rules of action for the avatars.

Avatar simulation has been employed by Virginia Tech University and Northeastern University to model disease propagation in North America. Avatar simulation has been less successful in the developing world, particularly Africa. A key problem is lack of data with which to construct behavior rules for avatars. The psychosocial behavior of populations is insufficiently well understood, particularly under high stress environments.
Methods & Materials: Our team developed methodology for real-time extraction of emotional content of media data and for relating media metrics to psychosocial behavior indicators. It is more powerful than polling because it accesses much larger population samples, and it is real-time. It is more powerful than sentiment analysis, which is one-dimensional and insufficient for behavior prediction; rather it is many-dimensional emotions analysis based on the evolutionary psychology theories of Robert Plutchik. Plutchik has been used, for example, by the advertising community to analyze consumer behavior. Our team, in US-government sponsored research has proven the application of Plutchik to modeling of societal dynamics.

Our team employs linguists to construct emotions matrices, enabling big data processing of media data in native languages and in real time. Media include social media, broadcast media, mass print media, and local print media. Where appropriate, cellphone records are used to track population movements. Behavior indicators are developed through statistical correlation of emotions metrics with societal behavior and through analyses by linguists, cultural anthropologists, and field epidemiologists.

Results: The methodology enables real-time analyses of 1) emotional response of populations under stress; 2) response of populations to broadcast messaging; 3) population behavioral response to events and messages; 3) identifications of key influencers; 4) population micro-demographics analysis. All these data better enable population modeling in pandemic conditions.

Conclusion: Sample media analyses and results will be shown.

Purpose: Foodborne outbreaks are ubiquitous around the world. Outbreaks are commonly detected through laboratory genetic testing and investigators analyse food history in search of commonality between cases to find the source of the outbreak. Geospatial information has the potential to direct investigators to the source of an outbreak. To inform the design of surveillance countermeasures, we collected data to approximate how cases would disperse following a foodborne outbreak in a food establishment in malls located in the city centre or at transport hubs within satellite townships or in smaller food establishments within suburban regions in Singapore.

Methods & Materials: Socio-demographics, residential and workplace address and time of survey from a cross-sectional survey at randomly selected malls (10 city area, 20 satellite townships) were analysed. Distances between residence and workplace of subjects, site of survey, and pair-wise distances between addresses were used as indicators of potential geographical dispersion. We assessed if subject characteristics, survey timing and site location was associated with distance from survey site using random-intercept multilevel linear regression. We then calculated the centre of minimum distance using all residential address alone, all work address alone and both addresses together.

Results: We surveyed 5012 individuals across 30 sites in Singapore (10 city area, 20 satellite townships of which 10 were beside transport hubs and 10 at least 500m away from a transport hub). Multilevel linear regression confirmed that city centre subjects were more dispersed geographically (β=0.979; p<0.001) than for satellite township subjects far away from a transport hub; also, working subjects were significantly more dispersed (β=0.049; p=0.004), while those who ate food at the site were less dispersed (β=-0.057; p<0.001) than those who did not. Residential addresses were able to pinpoint the source of the outbreak to under 500m for sites in satellite townships, while work addresses were able to do the same for city centre sites. Using both addresses together improved overall detection in all sites to about 500m from the simulated source except one site.

Conclusion: When all cases are clearly known, residential and work addresses can help to narrow down the likely source of the outbreak.

Purpose: Tools are needed by decision-makers to enhance understanding of global disease transmission dynamics and facilitate timely risk analyses. Additionally, there is a need to include situational awareness of the presence of potential drivers of disease emergence and transmission when making early projections on transboundary risks of animal disease.
outbreak spread. We conducted a pilot study of an approach to bring diverse data on social and environmental drivers of animal disease emergence and spread together with data on animal populations and outbreaks. Information is presented geographically to visually communicate potential risks of disease emergence and potential spread.

**Methods & Materials:** Our use cases for this approach were lumpy skin disease and avian influenza. Variables collected daily from systematic searches of global biosurveillance data on unexplained increases in animal morbidity and mortality, as well as confirmed findings of diseases in livestock and poultry populations are layered with social and environmental data layers, such as climate, flood and drought, and civil unrest. Using GIS coordinates for area centroids, a time-series geospatial presentation of outbreaks and findings is developed. Data is layered on estimated population density of susceptible livestock or poultry for an initial understanding of risk potential for direct transmission and movement of the disease agent geospatially. In addition, based on knowledge of the known drivers of each disease agent, additional data are included to reflect variables associated with animal disease emergence and transboundary spread.

**Results:** Using this simple data visualization tool, the emergence and transboundary spread of lumpy skin disease and avian influenza viruses are visualized and used for situational awareness and analysis of the possible forecasted risks of local emergence of these diseases.

**Conclusion:** Data gathered to monitor and estimate local risks of emerging animal diseases are analyzed using a readily updated data visualization tool and used to visually communicate potential risks of animal disease emergence and potential spread to risk analysts. Such a tool can assist decision-makers assess local risks of disease emergences based on global drivers of animal disease transmission, as reported by open sources.

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**20.049** Employing survivors of Ebola virus disease (EVD) as a novel system to support community-based surveillance in an Ebola hotspot within Sierra Leone
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Purpose: The West African outbreak of Ebola virus disease (EVD) began in December 2013 and affected Sierra Leone, Liberia, and Guinea. Sierra Leone had the highest burden of cases amounting to 14,124 confirmed cases, 3,956 deaths, and an overall case fatality rate (CFR) of 28.0%. In October 2014, Partners In Health (PIH) arrived in Sierra Leone to assist the Ministry of Health & Sanitation (MOHS) with the Emergency Response and established the Maforki Ebola Treatment Center (ETC) in Port Loko District. As EVD patients received treatment and recovered, they were often severely stigmatized. PIH aimed to counter the stigmatization by employing EVD survivors to engage in the Emergency Response in their communities by supporting epidemiological response teams with community-based surveillance and health education.

Methods & Materials: Port Loko District (PLD) has a current population of 624,935 persons, 1,485 confirmed cases of EVD infection, and 732 EVD survivors, the second largest number of EVD survivors after Western Area District. There are 34 EVD survivors employed as Case Managers (CM) embedded throughout the 11 chiefdoms in PLD, 7 Facility Based Case Managers (FBCM) stationed at Peripheral Health Units (PHU’s), and 3 Social Support Officers (SSO) that provide supervision and reporting at the district level.

Results: Each Case Manager (CM) has an average case load of 18 survivors to monitor via weekly home visits, calls, and text messages in order to link EVD survivors to the nearest health center. Other duties involve survivor migration tracking, transporting sick survivors to health facilities, encouraging reporting of suspicious deaths/potential EVD cases, and locating patients lost to follow-up. There are several novel aspects of this system: (1) >95% of these program staff are EVD survivors that are intimately knowledgeable of EVD symptoms, (2) CMs are versatile in providing efficient healthcare linkage, and (3) staff provide valuable community-based surveillance support despite a lack of traditional healthcare training.

Conclusion: Partners In Health was in a unique position to assist the MOHS during the Emergency Response and in transitioning to recovery by maintaining staff capacity within the community to rapidly identify a suspicious event and initiate rapid response and control measures.

20.050 Using social media for better guesstimation of size of a chikungunya outbreak: A mixed methods study
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Purpose: In August 2016, authors observed an unprecedented rise of incidence of chikungunya like illness in their vicinity and other parts of the city New Delhi. However, the official govt estimates revealed only 432 confirmed cases in the city (till 31st August) and control efforts were wanting. News reports were also based on same official figures and lacked sufficient impact. Therefore, study was undertaken on social media to capture community’s perspective for a better guesstimation of size of city’s chikungunya outbreak.

Methods & Materials: Authors developed a hypothesis with conservative guesstimate of ‘true’ size (nearly 700 times larger than official figure) of chikungunya outbreak based on their observations and anecdotal evidence. Lead author posted hypothesis on his Facebook page, along with assumptions and a request to readers (general public) to indicate whether they agree or disagree with the guesstimate. Mixed methods approach was used to analyze the comments from the respondents. As an ethical responsibility, the conversation was tweeted to relevant state and national leaders with prior consent from respondents.

Results: All 93 respondents (including 60 doctors) agreed with guesstimate of atleast 3 lakh cases of chikungunya (or similar illness) in New Delhi in 2016, through various modes (sometimes multiple) of engagement on Facebook including 7 shares and several indicating that this was infact as underestimate. The following are details of responses:

<table>
<thead>
<tr>
<th>Response</th>
<th>Original post</th>
<th>Shared posts</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likes</td>
<td>62</td>
<td>18</td>
<td>80</td>
</tr>
<tr>
<td>‘Agree’ Comments</td>
<td>32</td>
<td>10</td>
<td>42</td>
</tr>
</tbody>
</table>

Others proposed reasons for govt.’s under-reporting- wrong timing on antibody testing, case management based on clinical judgment alone without lab confirmation. Many posts pointed out that the lack of coordination between various ruling parties at national and state levels was root cause of outbreak.
Conclusion: There is clear consensus among members of authors social network on Facebook that New Delhi had atleast 3 lakh cases of chikungunya (or similar illness) in 2016 (till September 18), much higher than official reported figure. Study highlights how social media can be used by authorities for better estimation of outbreak size and possibly other attributes, especially in developing countries with poor health information systems and limited resources for laboratory confirmation of diseases.

20.051 Theoretical practical and ethical implications of future-based approaches to post-disaster disease
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Purpose: Future-based, or imaginative, approaches have in the past been used for the management of security threats. These approaches seek to make situations of high uncertainty manageable. Therefore, these approaches might also be useful for post-disaster disease control. This study seeks to apply future-based approaches to the scenario of post-disaster disease control and asks if this is a feasible and ethical approach.

Methods & Materials: The study consists of a literature-review and a desk-based analysis of the use of future-based approaches to post-disaster communicable disease control. The study uses the two retrospective case studies of Hurricane Katharina and the 2002 summer flooding in Germany.

Results: The basis for the use of future-based approaches are very similar in the two cases of uncertain security risks and post-disaster disease control. Both situations are defined by either a lack of data (2002 summer flooding) or an abundance of conflicting data (Hurricane Katharina). Therefore, theoretically the use of future-based approaches can be transferred from security risk management to post-disaster disease control. In both cases, the imaginary nature of these approaches allows new ways to react to conditions of uncertainty. However, practically and ethically there are significant risks associated with the use of future-based approaches. Practically, imaginary approached demand a considerable financial commitment without sufficient evidence-base. Ethically, these approaches have the potential to infringe rights not based on probability but on possibility. Thus, the study shows that the use of future-based approaches has to be limited.

Conclusion: This study has demonstrated that the use of future-based approaches to post-disaster disease control has some value-added due to the imaginary nature of future-based approaches but relies heavily on the precautionary principle already ingrained in medical and public health practice. The ethical risks of these practices which have already been demonstrated in the field of security cannot be dismissed in public health and therefore the use of future-based approaches should be limited to scenario planning rather than disaster and outbreak response due to the unfavourable risk-benefits calculation.

20.052 Evaluating performance of natural language processing for detection of symptoms presence and duration within primary care setting in Singapore
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Purpose: Free-text data in Electronic Medical Records (EMR) are potentially useful to outbreak surveillance but needs prior processing into signs, symptoms and syndromes by natural language processing (NLP) algorithms. We aimed to programmatically read EMR and extract data on signs, symptoms, and symptom duration, and hereby describe the performance of an NLP we designed.

Methods & Materials: We extracted EMR spanning 2009 to 2014 from the National Healthcare Group Polyclinics, one of two major public sector primary healthcare providers with 9 clinics across Singapore. Randomly selected records underwent systematic manual coding for presence of signs and symptoms (based on a pre-defined list of 47 signs and symptoms), and the symptom duration. Two staff coded the data independently; third person reviewed and adjudicated on discrepancies. An NLP programmed using a keyword-based approach extracted signs and symptoms data and symptom duration on the same set of records, with outputs then compared against human coding (as the gold standard).

Results: 1338 signs and symptoms were detected from 350 medical records by human coders; of these the NLP incorrectly missed 200 (false negative) but incorrectly identified 60 instances (false positive). Thus the precision and recall of the NLP in detecting signs and symptoms were 95.0% and 85.1% respectively.
The human coders identified 253 instances with information on symptom duration, but there were 116 where NLP completely failed to detect (false negative), and 5 instances where NLP incorrectly assigned a duration when there was none (false positive), giving a precision of 96.5% and recall of 54.2%. Performance for acute (<1 week) was significantly better than for chronic (≥1 week) symptoms (recall of 57.8% vs 42.6% respectively, p=0.038). Of 137 instances where duration information was correctly detected, the NLP assigned duration was wrong in only 1 instance.

**Conclusion:** The viability of the NLP for syndromic surveillance was supported by its good performance in detecting most signs and symptoms, with moderate performance for recall of symptoms duration. Performance for symptom duration was better when restricted to more acute presentations, and may be of use in improving the temporal resolution of EMR-based surveillance.

20.053 Community event-based surveillance (CEBS) for Ebola virus disease (EVD) in Sierra Leone: Alert demographics by sex and age, February 2015-June 2016

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**Purpose:** In 2015, Community Event-Based Surveillance (CEBS) was implemented in Sierra Leone to provide an early warning system for Ebola virus disease (EVD), using a pre-established network of Community Health Workers (CHWs) to generate alerts for “trigger events” indicative of EVD. We analysed the demographic breakdown of alerts using sex and age-specific reporting trends within the CEBS system.

**Methods & Materials:** Alerts generated by the CEBS system from February 27, 2015-June 30, 2016 were described by type of trigger event (sick, death, unsafe burial, and other), sex (male and female), age category (<1 year, 1-5, 5-9, 10-19, 20-29, 30-39, 40-49, 50-59 and ≥60) and district.

**Results:** Of the 33,957 CEBS alerts that were generated during the 16-month period, 72% were death alerts and 28% were sick alerts. Of these alerts, 48% were male and 52% female; percentage of death alerts and sick alerts did not vary by sex. Alerts by ages group showed comparable trends between male and female alerts, with the largest percentage of alerts being from deaths in people over 60 (22% of male alerts and 20% of female alerts). Out of nine districts, a majority of districts generated a greater percentage of female alerts compared with male alerts within the 10-19 and 20-29 age groups, while seven districts had a higher percentage of male alerts compared with female alerts among people over 60 years of age.

**Conclusion:** Understanding community-based surveillance data is crucial for ensuring proper disease surveillance, targeting appropriate responses during outbreaks, and to improve community surveillance. The consistency across districts in sex- and age-specific demographics among both live and death alerts generated by CEBS suggests that the reporting of trigger events was not influenced by sex or age.

20.054 The zoonoses data collection in Italy: An expert system for data quality management and improvement

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**Purpose:** The need to improve data quality for a better analysis and understanding of the trend of zoonoses at country level has been increased year after year both by the EFSA and by reporting countries. This is the reason why the EFSA launched in 2014 a granting programme supporting the participating countries in updating and complementing their historical datasets in EFSA’s databases. In this context, an expert system was put in place in the Italian information system for zoonoses data collection (SINZoo) for improving data quality and for complementing the historical datasets.

**Methods & Materials:** Zoonoses data are checked in SINZoo both during and at the end of the insertion: during data entry, an automatic logic system of truth tables checks that for each zoonoses the combination of the area of interest, each possible sampling context, stage and sampling unit has been entered correctly, thus avoiding inconsistent data. Each combination available in the truth table indicates the context, the stage, the sampling Unit allowed for each zoonosis in a specific area and for a category of species.

**Results:** The missing information was retrieved following a logical pattern based on the national legislation in place on the zoonoses covered by the project. The same logical pattern
was used in SINZoo to build the truth tables specific for each zoonosis, in order to avoid mistakes during its feeding by the reporters.

After the missing/unspecified information was retrieved, the rows containing wrong combinations of zoonosis/contextstage/unit were corrected and updated following both the logical pattern of the truth table and logical rules to ensure the coherence of the data retrieved.

Overall, the goal of the project was achieved for most of the information to be retrieved: the 89% and the 83% of sampling contexts and stages respectively and the 100% of the other information were retrieved.

**Conclusion:** This project highlighted the importance of data quality during the collection and feeding of any information system. The need to retrieve historical data led to improve the rules of the national data collection foreseen by the truth tables and to define new logics and algorithms that may be used for each data reporting.

**20.055** Validation of event mobile application (EMA) for animal disease surveillance and monitoring in Uganda. milestones and challenges

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**Purpose:** EMPRES-i EMA (Event Mobile Application) is a mobile application that has been developed by FAO AGAH/GLEWS for rapid disease data collection and reporting from the field via a smartphone directly to the EMPRES-i database (http://empres-i.fao.org). The rationale for the App is that in some developing countries while access to the Internet may be difficult, telephone networks have good signal coverage over wide areas and hence rapid connection is possible while in the field. Use of smartphone reporting is a fast and effective way of transmitting disease data from the field.

**Methods & Materials:** EMA-i was customized for use and IT equipment was purchased including 15 smartphones, two computers and internet services were contracted as service providers to cover subcription. Data was collected with EMA from ten pilot different districts (Rakai, Masaka, Isingiro, Mubale, Mukono, Sironko, Kibaale, Nakasongola, Busia and Lyantonde). Reports were transmitted to the EMPRES-i platform that hosts the data for the exclusive use by NADDEC.

**Results:** Both EMA and paper reports from the ten districts were tabulated and comparisons made. Reports were obtained across the same period July 2013 to June 2016. 357 paper reports and 375 EMA reports had been submitted. 6/10 (60%) districts had more EMA than paper reports while 4/10 (40%) districts had more paper reports than EMA reports. This is expected because EMA reports are received instantly at the epidemiology unit while the paper reports come much later. There was no consistency of diseases reported between the two reporting systems. Results indicate no significantly better system in regards to amount of data each tool can collect. However, EMA reports had the advantage of timely reporting, instant feedback and information sharing among EMA users.

**Conclusion:** There is need to strengthen Early Warning, disease surveillance capabilities and improve the reporting and response to disease outbreaks in Uganda. Capabilities of EMPRES-i EMA can be fully harnessed to support communities and local governments to reduce impacts of animal disease outbreaks in livestock.

**20.056** Effectiveness of mobile phone short message service (SMS) as a post-training approach in Uganda

**R. N. Kikonyogo, L. Namara, J. W. Arinaitwe**

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**Purpose:** The Infectious Diseases Institute’s (IDI) Training and Capacity development Programme offers specialised courses in the management of infectious diseases for health workers in resource-limited settings. After each course, IDI provided onsite follow-up visits to assess the knowledge retention of the alumni. Over the years, IDI strived to innovate more cost-effective follow-up strategies; and in 2015 implemented the use of mobile phone short message service (SMS) as an approach to measure knowledge retention. SMS was a viable option due to its low cost, user-friendly interface, and high coverage in Uganda (SMS coverage is approximately 65% compared to 18% internet coverage).

**Methods & Materials:** IDI piloted SMS follow-up to a group of 49 clinicians who had attended a four-day course in Especially Dangerous Pathogens. Six weeks after the course, the 49 alumni began receiving follow-up quizzes via SMS. The SMS quizzes were in the form of multiple choice questions and were disseminated over the course of 13 weeks. Questions were based on content covered during the course. Alumni replied to the questions through a toll-free SMS platform that instantly provided feedback on whether the answer was correct or
wrong. A toll-free phone line was also used to provide reminders and receive technical queries in relation to the SMS.

**Results:** 78% of the alumni responded to the SMS quiz questions and 81% submitted the correct response. This was above the 70% pass mark that IDI approves for the award of certificates. Challenges that hindered responses included busy work schedules, poor network coverage, and lack of battery power. The high percentage of responses in general, and specifically the high percentage of correct responses, shows that SMS is effective as a post-training follow-up approach to ensure knowledge retention.

**Conclusion:** IDI has scaled up the SMS follow up to the rest of the alumni because it proved to be an effective approach.

The SMS post-training pilot was funded by United States Army Medical Research Institute for Infectious Diseases (USAMRIID)

20.057 Community-based cholera surveillance by volunteers with mobile phones: A case study from Western Area, Sierra Leone

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**Purpose:** The purpose of the project was to design and implement a digital community-based surveillance (CBS) system to understand whether non-health trained volunteers could accurately detect and report cases of suspected Cholera in their communities, using simple mobile technology.

**Methods & Materials:** In 2014-2015, 239 community-based Haitian Red Cross volunteers were trained to detect, respond to and report cases of acute watery diarrhoea occurring within their own communities by SMS. Real-time, digital data collection was set up through the online software Magpi. We used the android application SMSsync to enable SMS responses being sent to a local smartphone and data transferred to an online database through wifi or 3G. Data was extracted daily and processed in RStudio to merge SMS data with volunteer location data. The database output enabled Red Cross staff and partners to decide whether follow up, investigation or response was required for alerts. Scripts in Rstudio were furthermore used to generate automatic epidemiological reports based on SMS database and case investigation data, and shared with stakeholders to monitor trends over time.

**Results:** The CBS system had the sensitivity and specificity required to detect cholera outbreaks, as evidenced by comparing temporal variation in the rate of occurrence of cases in the CBS data with available line-list data from cholera treatment facilities. However, the data from the CBS system is noisy. Our results show positive proof of concept for disease surveillance by volunteers with mobile phones, and the ability, for the first time, to detect cholera in rural areas that are often missed in formal surveillance. Furthermore, our results illustrate that community-based surveillance works best when integrated with the formal healthcare system—coupling the high sensitivity and low specificity of community surveillance with the high specificity and low sensitivity of formal surveillance, which cannot always penetrate rural areas, or areas recently affected by environmental or humanitarian emergencies.

**Conclusion:** Community-based surveillance is an important strategy to broaden the reach of public health surveillance to rural communities in resource poor settings, especially during outbreaks. The project demonstrates the potential for scale and sustainability by utilising existing volunteer networks in response to disease outbreaks.

20.058 A Qualitative study of volunteer experiences with a mobile community event-based surveillance (CEBS) system in Sierra Leone

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**Purpose:** In 2015, Sierra Leone Red Cross Society (SLRCS) supported by IFRC implemented Community Event-Based Surveillance (CEBS) in Sierra Leone to enable early detection and response to suspected Ebola, Cholera, Measles, community death, flood and fire. The study aim was to identify benefits and challenges with the CEBS system from the volunteer’s perspective, to assist in addressing sustainability and scalability questions for further implementation.
Methods & Materials: 2202 Red Cross volunteers were trained to report through phone calls and SMS to Red Cross and existing health structures in three districts of Sierra Leone. Of these, 62 volunteers from 14 different chieftoms participated in in-depth interviews and focus group discussions, which were audio recorded. Records were transcribed and analyzed by using a thematic method, which identified key benefits and challenges.

Results: The volunteers believe CEBS has a positive impact on their communities. CEBS increases knowledge and behavior change, contribute to the prevention of Ebola and other diseases and decreases the overall mortality. Volunteers are motivated to participate by an aspiration of helping their community, encouraged by two-way communication, where communities actively participate and the health system responds to alerts. Many volunteers also participated in the hope that they would receive monetary incentives. There was initial reluctance from communities to participate in surveillance due to fear of Ebola, but acceptance increased with improved community engagement and trust in the system created by utilizing volunteers known to the community. Reporting by phone call and SMS was seen as a quick and simple way of reporting, although challenges with access to network, sim registration, cost for charging and lack of incentives was identified. A large number of false positive cases were reported, indicating that the volunteers did not have a clear understanding of case definition or purpose of CEBS, in addition to responding to high needs and expectations from the communities.

Conclusion: Community engagement and sustainability of volunteer networks are central to the sustainability and functionality of CEBS. The trust and structure of the Red Cross National Society provides an environment to support CEBS, however issues of incentives and sensitivity of the system still need improvement.

20.059 Missed opportunities for Yellow Fever Surveillance in Uganda, July 2015 - May 2016

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Purpose: Yellow Fever (YF) is a re-emerging infectious disease associated with significant mortality among patients who progress from the acute to toxic phase. There has been multiple occasional YF cases in Uganda over the past 75 years. The larger outbreak occurred across 14 districts in Northern Uganda in 2010/11 with 273 cases and 58 (21.3%) fatalities. In 2016, three smaller non-linked outbreaks were reported in the districts of Masaka (5 cases), Kalingala (1 case) and Rukungiri (1 case). These districts have concomitant high to moderate malaria transmission. Routine YF surveillance in Uganda employs weekly and monthly aggregate data reporting from health facilities and the district to the national information management system, District health information system-2 (DHIS2). Here we analyze the effectiveness of the current YF surveillance system in the detection of small YF outbreaks in Uganda.

Methods & Materials: Suspected YF case data elements for the whole country were extracted from the DHIS2 from July 2015 to May 2016. In addition, monthly indicator data for malaria rapid diagnostic test (RDT) positivity for the two sub-counties (Kebisomi in Rukungiri District; and Buwunga in Masaka District) that had confirmed YF cases were exported from DHIS2 to Excel and analyzed in Epi-Info 7.2. We computed national YF weekly and monthly reporting averages. We compared YF Weekly Epidemiological Surveillance (WES) data and malaria RDT-positivity monthly averages for acute febrile illnesses (AFI) for the two sub-counties in the same period.

Results: Nationally, 1,882 and 710 suspected cases of YF were reported monthly and WES respectively, representing a respective average monthly and weekly notification of 18 and 13 cases. Average malaria positivity rate for health facilities in Kebisomi among AFI patients ranged between 3.6 – 27.0% while in Buwunga, malaria positivity rate ranged between 40.0 – 60.0%.

Conclusion: The high number of uninvestigated suspected YF cases in Uganda and the reporting of the cases as aggregate data demonstrate a missed opportunity to detect YF outbreaks as this reporting makes retrospective suspect case follow up impossible. Additionally, case-based YF surveillance among YF suspect cases and the malaria negative acute febrile illness pool could increase the detection of YF cases.

20.060 A real-time surveillance dashboard for monitoring viral phenotype from sequence

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Purpose: Genome sequencing has become routine in outbreak surveillance. Apart from its utility in tracking evolutionary trajectories, genome sequences also contain information necessary for predicting biochemical phenotypes of outbreak pathogens, such as drug resistance and antigenic distance from vaccine strains. Having the capability to predict and visualize a pathogen's phenotype from its genome, in real-time, and at a location near the source of the outbreak, would provide epidemiologists with additional useful data that could help tailor outbreak responses and appropriately mobilize limited resources.

Methods & Materials: We provide a design for an extensible digital dashboard for infectious disease genomic surveillance. This open-source dashboard is written in the Python programming language. The application back-end is written using the Flask web framework. Automated machine learning model and parameter selection is enabled using the `numpy` and `scikit-learn` packages. Data visualization is provided by the `bokeh` package. Data are stored in SQLite databases. Automated tests using `py.test` are used to test for data and application integrity. The dashboard’s backend is modularly designed, with separate "microservices" for the web interface, data ingestion and preprocessing, machine learning, and visualization. This design enables the addition of new pathogens, phenotypes, machine learning models, and visualization types as needed.

Results: We have developed a proof-of-concept dashboard, using sequence-phenotype data from the HIV drug resistance database and the Los Alamos HIV Sequence Database. The dashboard takes in a new virus’ sequence, and returns a visualization of its predicted drug resistance profile. We show how research groups can programmatically submit standardized phenotype data to update the database and machine learning models. We demonstrate the portability of the data and models. We also provide examples on how to extend the dashboard.

Conclusion: New "gold-standard" genotype-phenotype data and integrated prediction systems are required to fully realize the utility of genomic sequencing data by connecting genotype to phenotype. We have designed this dashboard, and implemented a proof-of-concept, as part of our efforts to realize this vision.

20.061 A surveillance system of diseases of small companion animals in the Veneto Region (Italy)

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Purpose: Experts and international public health organizations stress the lack of surveillance systems of diseases of companion animals and the necessity of implementing it as a priority of the One Health perspective. This paper proposes a project addressing this public health challenge. It presents the features of a system for collection, analysis, interpretation and dissemination of data about the health status of pets in the Veneto Region, Italy.

Methods & Materials: The system provides the construction of a web-based database containing the diagnoses of transmissible and non-transmissible diseases of dogs and cats made by veterinary practitioners joining the initiative voluntarily and without profit. Each diagnosis constitutes a single record, also containing data on the identification of the individual animal and on several characteristics of epidemiological relevance. The WHO International Classification of Diseases of humans has been adapted to canine and feline diseases to standardize the diagnostic nomenclature. A software for on-line data entry and data management has been specifically created.

Results: Up to now about the 12% (n=80) of the overall veterinary practices of the Veneto Region have joined the project and about 1000 (∼750 dogs and ∼250 cats) records have been entered the database. Three basic epidemiological reports have been distributed among the network and other stakeholders (veterinary associations, people responsible for human and animal health public services, academic experts). Reports and other interactive resources are freely obtainable from the website of the project.

Conclusion: Expected outcomes are to monitor disease frequencies and their behaviour in time and space, to identify associated risk factors and to produce disease and risk maps, thus providing epidemiological knowledge supporting the everyday clinical practice. Further expected outcomes are to assess the risk of transmission to humans and to set off the possible role of pets as early sentinels of emerging health threats and as models for the study of the effects of the exposure to environmental risk factors. The perspective is to stimulate networking within the veterinary profession and between human and animal health professionals, to promote general awareness of the public health relevance of pets and to provide a useful tool to scientists and health policy makers.
Purpose: Surveillance is an integral part of every health system. Nigeria uses district vaccine data management tool (DVDMT) for surveillance data collection including routine immunization (RI). In 2012, Nigeria introduced the District Health Information System (DHIS 2) a new technology. In 2015, Enugu State commenced the use of DHIS2 alongside DVDMT to manage all health data including RI. We carried out a comparative evaluation of the two surveillance data collection tools to assess data quality, preference and effect of each in decision making.

Methods & Materials: We quantitatively and qualitatively assessed the surveillance attributes of DHIS2 and DVDMT in Enugu state 2015 using CDC guidelines 2001. We carried out desk review in the state and selected health facilities to assessed quality of the data captured by the two tools. Semi-structured questionnaire we administered to all the local immunization officers (LIOs) in the 17 local government areas (LGAs) of Enugu state to assess surveillance attributes. We also interviewed six key informants (KI), Enugu state director of public health, data manager, cold chain officer, state epidemiologist, state immunization officer and DHIS2 state implementation officer and conducted focus group discussion (FGD) with six randomly selected heads of department of health in the LGAs.

Results: In addition to the data captured by DVDMT, DHIS2 records vaccine utilization. An overall validated error of 9.5 and 3.0 for DVDMT and DHIS2 respectively was recorded. Sixteen (94%) of the LIOs preferred DHIS2 to DVDMT. Seventy six percent (76%) agreed that dhis2 is simple and more flexible than DVDMT. All KIs opined DHIS2 is better than DVDMT as it has features for data visualization and identified real-time access to data in DHIS2 as an advantage for decision making. The FGD revealed DHIS2 is better but it is cost-intensive and requires computer proficiency of current users.

Conclusion: The minimal error observed in DHIS2 is a plus. The DHIS2 is preferred to DVDMT in Enugu state as it is simpler. Real time access to data in DHIS2 allows quality and prompt decision making compared to DVDMT. We recommended the use of DHIS2 alone to Enugu state, although user capacity building and sustainability should be addressed.

Purpose: To implement and deploy the software suite namely TanRabad that supports the mission of health officers to enhance dengue surveillance and improve prevention and control strategy.

Methods & Materials: To achieve TanRabad, we started with the requirement elicitation from potential users including entomologists, epidemiologists and public health executives. We next analyzed the requirement resulting in the system specification which was clear and complete. We then implemented TanRabad that consists of 4 essential applications: TanRabad-SURVEY -- mobile application that facilitates real-time data collection from a larval survey of individual households in a village and buildings of places such as school, temple and hospital; TanRabad-WATCH -- web application that visualizes disease epidemics together with the entomological indices in the current four epidemiical weeks on map and table; TanRabad-REPORT -- web application that supports the production of regularly-used reports relevant to disease epidemic and entomological indices; and TanRabad-BI -- web application that facilitates the generation of reports related to disease epidemic and entomological indices based totally on users’ demands. Once the above applications were done, we performed their integration test to ensure that they worked well together. Last but not least, TanRabad was evaluated by an agency under the Department of Disease Control (DCD) prior to its actual deployment by potential agencies.

Results: The result has shown several TanRabad's contributions: (i) timely identification of high-risk larva sources for an effective elimination of larva sources and prevention of dengue in risk areas; (ii) visualization of integral situations to prevent epidemic re-occurrence and
control its spread; and (iii) data analysis to support the generation of relevant strategy for
dengue prevention and control.

Conclusion: TanRabad has currently been deployed by agencies under DDC. Future works
will focus on the fulfillment of TanRabad for other aspects such as outbreak investigation and
the prediction of dengue epidemic.

20.064 Sensitivity of the Ov16 serology in the elimination of onchocerciasis; a preliminary
report of 10 years of treatment with ivermectin in Ogun State, Nigeria
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Purpose: Measuring progress made in onchocerciasis treatment in endemic areas has been
challenging given the need to replace the painful skin snip method. The study investigated the
sensitivity and specificity of Ov16 Rapid Diagnostic Test (RDT) and of a Dried Blood Spot
(DBS) Ov16 Enzyme Linked Immunosorbent Assay (ELISA) for identifying exposure to
Onchocerca volvulus in Ogun State, Southwestern Nigeria after a decade of treatment with
ivermectin.

Methods & Materials: Five hundred and eighty nine (589) firstline community members who
were randomly selected from 32 communities in the 8 meso-endemic Local Government
Areas (LGA’s) provided whole blood specimen which were tested for IgG4 antibodies against
the O. volvulus antigen Ov16 using RDT and ELISA and also stored on Whatman™ Protein
Saver cards for ELISA reference testing. A gaussian mixture model and expectation
maximization was used to classify Optical Densities (OD) for positive and negative samples
from ELISA results. Data were analysed using custom scripts in R and SPSS software

Results: Of the 589 participants, 102(17.3%) and 111(18.8%) were anti-IgG4 and ELISA
positive respectively, while 79(13%) tested positive for both ELISA and RDT, with significant
difference (p<0.05). Odeda LGA recorded the highest sero-prevalence by RDT and ELISA
45.2% (33/73) 56.2% (41/73) while Abeokuta South LGA recorded the least 0.87% (1/115).
Assessing RDT to ELISA, sensitivity and specificity were calculated to be 71.82% (CI 62.44%
to 79.98%) and 95.2% (CI 92.88% to 96.93%) respectively with a 91.3% agreement.

Conclusion: The result obtained provided an information on the sero-prevalence status of
onchocerciasis. It goes further to show the efficiency of the Ov 16 RDT as a practical tool for
field use in identifying areas of endemcity. It promotes the possibility of incorporating Ov16
RDT as a new strategy in onchocerciasis mapping towards achieving elimination in Africa by
2020.

20.065 Use of genome wide gene-by-gene comparison for Salmonella enterica outbreak
investigation in Austria
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Purpose: Salmonellosis is one major cause of bacterial enteric illness in both humans and
animals. Most human cases of food-borne salmonellosis are caused by serotypes S. enterica
Enteritidis and S. e. Typhimurium. Effective epidemiological surveillance and outbreak
investigation require the accurate subtyping of strains. The aim of the project was to develop
and evaluate a new typing scheme for S. enterica based on genome wide gene-by-gene
comparison.

Methods & Materials: To cover the diversity of S. enterica a total of 37 isolates comprising
33 serovar genomes were used to define a core genome (cg) multilocus sequence typing
(MLST) scheme using the cgMLST target definer tool integrated in SeqSphere+ (Ridom,
Münster, Germany). The scheme was tested for its ability in cluster identification with 100
isolates including two outbreaks (outbreak A: N = 10 S. Enteritidis; outbreak B: N= 37 S.
Stanley). The NexteraXT Kit was used for library preparation and paired end sequencing was
performed on a MiSeq (Illumina Inc., San Diego, CA, USA) to achieve at least a 70-fold
average coverage. Raw reads were de-novo assembled using Velvet version 1.1.04. Contigs
were filtered for a minimum coverage of 5 and a minimum length of 200 bp.

Results: In total, 37 genome sequences including S. enterica Typhimurium strain L-3553 as
reference were used for cgMLST target definition and resulted in 2.396 core genome targets
out of 4,817 genes of strain L-3553. The cgMLST scheme was rechecked with 100 isolates
including isolates from two outbreaks. All 137 isolates belonging to 42 serovars had ≥ 95.0 %
good cgMLST targets. Based on the maximum number of six allelic differences within each
outbreak a threshold of eight allelic differences was determined for cluster type (CT)
definition. cgMLST correctly grouped outbreak isolates and allowed the differentiation of outbreak from non-outbreak isolates.

**Conclusion:** The defined cgMLST scheme is suitable for high resolution typing of at least 42 *Salmonella enterica* serovars responsible for > 87% infections in Austria in 2015. The typing scheme has a high discriminatory power, allows high resolution outbreak investigation and provides a typing nomenclature that can easily be shared among laboratories.

20.066 Canadian strategies for systematically assessing risks posed by emerging infectious diseases

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**Purpose:** How can the Canadian Government systematically assess and prioritize emerging infectious diseases (EID) in order to inform public health responses?

**Methods & Materials:** The Public Health Agency of Canada (PHAC), an agency of the Government of Canada, is tasked to prevent and control infectious diseases and prepare for and respond to public health emergencies, amongst other core functions described within the mandate¹. In recent years PHAC has been challenged to respond, in a timely manner, to a range of emerging infectious diseases and public health events. In order to enhance capacity the need for a systematic risk assessment platform for EIDs was identified.

The creation of a Rapid Public Health Risk Assessment Framework (RPHRAF) involved a scan of existing frameworks in order to identify common themes and structures. A literature review was conducted to broaden the evidence base. A subject-matter working group then convened to provide feedback on a draft framework and the prototype was then tested in a tabletop exercises. Finally, the RPHRAF was piloted on several real-time EID events, including: H5N1, Ebola, Measles and the Zika virus scale-up.

**Results:** The prototype framework proved to be effective and beneficial during the initial table-top and real-time tests. The framework promoted a shared understanding of risk assessments, a common vocabulary across a large government agency and response triggers for EID events. A subsequent real-time test during PHAC’s scale-up for the Zika virus revealed some weaknesses relating to vector-borne diseases and incongruence between data inputs and algorithms and risk assessments.

**Conclusion:** Drawing from the lessons learned during the testing phase of this framework, PHAC is now embarking on a revised version. The revisions will seek to make the framework more generic and capable of assessing a range of domestic and global EID events. As a part of this process PHAC will be developing systematic documentation standards and repositories, as well as event-specific risk scores.

Canada is a Federal state, comprised of provinces, territories (PT) and the federal government. An additional requirement of the revised RPHRAF will be stakeholder input from our PT and non-governmental counterparts to ensure inter-operability.

20.067 Infectious Disease Surveillance in the US Department of Defense European Region

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**Purpose:** The Counter Bio-Threats Cell (CBC) was created at the United States European Command (EUCOM) to revive planning and preparedness for infectious disease outbreaks within the US Department of Defense (DoD) European region. Global infectious disease outbreaks such as Ebola, SARS, MERS, and pandemic influenza highlight the need for integrated global strategies to combat disease emergence and mitigate risks.

**Methods & Materials:** The CBC functions in line with the US Government Global Health Security Agenda to monitor and respond to infectious disease threats according to DoD priorities. The EUCOM region includes countries with highly developed public health infrastructures to those suffering from ongoing conflict with limited public health and economic capacity to handle infectious disease outbreaks. US DoD active duty, civilians, contractors, and their families live and work within these host nation populations, sharing risk for infectious disease transmission. The DoD has developed a Phase 0-V infectious disease management plan, from Phase 0 to prepare for the possibility of disease emergence to Phase V for transition and recovery from a pandemic event.

**Results:** The CBC functions to synchronize infectious disease preparedness throughout EUCOM and integrates with US Government-wide approaches, beginning with Phase 0. Host nation partnerships are key to managing infectious disease risks, so CBC Phase 0 activities include refining the EUCOM infectious disease surveillance strategy, developing relationships
with host nations, and assisting and planning collaborative exercises with host nations to address challenges.

**Conclusion:** By developing an infectious disease surveillance and response plan, the CBC can function to increase surveillance effectiveness for the benefit of US and host nation populations in the event of an infectious disease outbreak.

20.068  **Google dengue trends: An indicator of epidemic behavior.** The Venezuelan case

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**Purpose:** Dengue Fever is a neglected and increasing public health threat. Developing countries are facing surveillance system problems like delay and loss of data. Lately, the access and the availability of health-related information on the Internet have changed how people seek on the web. In 2004 Google developed Google Dengue Trends (GDT) based on the number of search terms related with the disease in a determined time and place. The goal of this review is to evaluate the accuracy of GDT in comparison with traditional surveillance systems in Venezuela.

**Methods & Materials:** Monthly and yearly Pearson’s correlation between GDT and official reported data from 2004 until 2014 was obtained. We defined epidemic weeks as those where the number of cases was higher than the expected according the Ministry of Health (MH). The data was clustered in epidemic and non-epidemic weeks and correlations between GDT and official data was obtained. Significance criteria were 0.01. SPSS 22.0 was used as statistical package.

**Results:** The overall Pearson correlation between GDT and official data was r=0.85 while between official data and expected cases according the MH was r=0.35. After clustering data in epidemic and non-epidemic weeks, correlations were r=0.86 and r=0.65 respectively. Important interannual variation of the epidemic was observed.

**Conclusion:** The model shows a high accuracy in comparison with the expected cases according to the MH, particularly when the incidence of the disease is higher. Google Dengue Trends is free, available and can forecast the shape of an outbreak in “real time”, which it’s almost impossible for the epidemiological regular data that takes weeks/months to be available. This early warning tool can be used as an indicator for other communicable diseases in order to apply effective and timely public health measures especially in the setting of weak surveillance systems.

20.069  **Knowledge and practices regarding rabies in urban and rural communities in Thailand and Cambodia**

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**Purpose:** Rabies is endemic in South-East Asian countries which the major reservoir is domestic dog. We assessed the knowledge and practices regarding rabies of local people in urban and rural communities of Thailand and Cambodia.

**Methods & Materials:** Participatory epidemiology (PE) was used to identify the knowledge and practices of local people in four urban, five rural communities in Thailand and five rural communities in Cambodia as a part of the field working of ASEAN veterinary student volunteer Project 2016. In each community, five to ten of local people were participated; questions regarding knowledge on rabies, transmission and reservoir were asked. Additionally, the questions about their practices related to rabies such as what they usually do after dog bite, how they do to prevent rabies in domestic dog and their concern about rabies in both human and domestic dog were included.

**Results:** People in all settings have ever heard about rabies. They knew that rabies transmitted by dog bite, however, less information according to other mammal reservoirs. People in Thailand stated that vaccination in dog is the major prevention strategy, while people in Cambodia did not state about vaccination in dog. In both Thailand and Cambodia, local belief related bite wound and rabies was illustrated.

**Conclusion:** Lack of knowledge on rabies was identified. The information regarding wound cleaning and post-exposure prophylaxis is in needed to be transferred to local people.
ICARES: Two years experience with a promising early warning tool for infectious disease clusters


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Purpose: Clusters of infectious diseases are frequently detected late and tempo spatial information to analyse a cluster is often unavailable in the acute phase of an outbreak. Contemporaneous information about possible associated conditions is usually scarce as evidenced by the current Zika virus outbreak. ICARES (Integrated Crisis Alert and Response System) is a secure web-based system designed to facilitate the automated early detections of clusters of infectious diseases as they evolve in real-time.

Methods & Materials: ICARES has been developed and used in the Netherlands over the last two years on three pilot syndromes: respiratory tract infection, hepatitis and encephalitis/meningitis. ICARES harvests data automatically from local General Practices, General Practice Out-of-Hours services and three hospitals. A special interface has been built to capture anonymised numbers of routinely entered syndrome codes in real time. Actual numbers compared to historic data combined with information about patients' age cohort, gender and postal code area are used to detect and analyse clusters of cases. This is mapped onto a dashboard for the local unit of infectious diseases control.

Results: ICARES detected 10 possible clusters of infectious diseases. Analysis by local public health authorities revealed that two were real clusters. One detected encephalitis cluster was part of an enterovirus encephalitis outbreak. The second cluster related to respiratory tract infections and was part of the 2014-2015 influenza season. Other alerts were judged as false positive.

Conclusion: ICARES is able to detect clusters of infectious diseases in real-time without any administrative burden for clinicians. Furthermore, even during the annual flu season, incidence rates per postal code, which exceed the seasonal rates, are detected. This is in line with retrospective observations that 2014-2015 flu season was the longest since 1970 with the highest peak incidence in years. Future directives would be to decrease the number of false positive alarms without losing possible clusters. Conditions associated with specific infections can readily be detected by adding their diagnostic codes to ICARES yielding information about birth defects (e.g. during a Zika virus outbreak) or haemolytic uremic syndrome during a STEC outbreak.

Performance of community volunteers in human and animal diseases surveillance by using mobile application

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Purpose: PODD disease surveillance system was developed for monitoring animal diseases, human diseases and environmental issues by using mobile application called "PODD". It was developed and implemented since early 2015 in 74 communities in Chiang Mai, Thailand. Total of 296 community volunteers, 4 people per community, were recruited into the system to be volunteer reporter for their communities. They were trained every 3 months about animal and human diseases and how to report those abnormal events through PODD mobile application. This study aimed to determine the performance of those community volunteers in reporting animal diseases, human diseases and environmental issues by using mobile application in PODD disease surveillance system.

Methods & Materials: The regularity of reporting, participation in training, leadership, enthusiasm and possibility of being role model for other volunteer were evaluation criteria to determine their disease reporting performance. According to the criteria, community volunteers were divided into four groups: A was reporter who reported more than 75% of request, attended the training more than 50% and showed excellent performance, B was reporter who reported between 50-75% of request, attended the training 30-50% and showed excellent performance, C was reporter who reported between 25-50% of request, attended trainings less than 30%, but did not show good performance and D was reporter who reported less than 25% of request, attended the training only 1 time and need to be improved their performance.
Results: The results indicated that 20.42% of volunteer reporters showed great performance with Group A. Most of them were grouped in Group B and C (34.60% and 37.72%, respectively). These groups attended PODD training and activities and showed the regularly report. The system needed to maintain these performances and motivate them to proactive for moving to Group A. Group D (7.27%) was focused to improve their performance. This group could be the characters of undesirable people, who would be recruited to be disease surveillance volunteer.

Conclusion: In conclusion, disease surveillance system with digital technology, that needed the participation from volunteers, could evaluate performance of the volunteer and empower them to make better surveillance system in the future.

20.072 Characterization of a Culex theileri flavivirus variant in field-collected mosquitoes from Turkey
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Purpose: The Flaviviridae family includes several strains that only replicate in mosquitoes or mosquito-derived cell lines, generally called as insect-specific flaviviruses, along with the well-known mosquito-borne important human pathogens. Recent evidence indicates wider-than-expected prevalence and dispersion insect-specific flaviviruses in mosquito populations. Here, we describe the near-complete genomic characterization of flaviviruses isolated during a field surveillance campaign in Turkey.

Methods & Materials: Four virus strains, isolated using C6/36 cells from mosquito pool extracts with positive results in screening via generic flavivirus PCR were evaluated. The pools originated from collection sites in Aegean and Thrace regions, and comprised individuals previously identified as Culex theileri via morphology and DNA barcoding. Culture supernatants of the 3rd passage were subjected to nucleic acid purification, cDNA synthesis, library preparation and sequencing via Illumina HiSeq 1500 sequencing platform. Assembly of the contigs, alignment with the complete or near-complete insect flavivirus genomes deposited in public databases and further analyses were performed with relevant software.

Results: Near-complete genomes of 10299-10420 nucleotides, that cover the complete coding region of the flavivirus polyprotein were assembled. Neighbor-joining analyses placed all isolates within a distinct group, which further forms a cluster with two culex theileri flavivirus isolates from Portugal. No association of nucleotide/aa variations with collection sites was observed. The putative polyprotein comprised 3357 amino acids and followed the universal flavivirus polyprotein organization of structural proteins C, preM and E, and nonstructural proteins NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5. The putative protease cleavage sites were noted as identical to the Culex theileri flaviviruses. Pairwise amino acids comparisons demonstrated 0.99-1.33% diversity rates among intramural and phylogenetically-related strains.

Conclusion: The findings indicate the circulation of a novel culex theileri flavivirus variant, tentatively called as the “Culex Theileri Flavivirus Turkey (CTFT)”, in various regions of Turkey. This report constitutes the first detailed characterization of insect-specific flaviviruses in Asia Minor.

20.073 A metagenomic survey reveals rhabdo and negevirus sequences in mosquito pools from Turkey
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Purpose: Mosquitoes are among the most frequently-observed arthropod vectors, with the potential to transmit several viral diseases of significant human health impact. In addition to the viruses associated with vertebrate infections, several viruses belonging to diverse viral families have been identified in mosquito populations, suggesting the frequent circulation of these agents. In this study, a metagenomic sequencing approach was utilized to investigate the abundance and diversity of viruses that replicate in mosquitoes, collected during a field surveillance campaign in Turkey.

Methods & Materials: Four mosquito pools that comprise 13-45 individuals of Culex pipiens sensu lato (n=2) and Aedes caspius (n=2) mosquitoes, collected in Mediterranean and Thrace regions during 2015 were evaluated. The pools were processed via standard protocols employed for virus isolation and subjected to sequencing on an Illumina HiSeq 1500 platform. Sequences were analyzed with a workflow for the identification of highly divergent viruses that include trimming for quality reads, substraction of irrelevant sequences, alignment, taxonomic binning and visualization steps, and further assembled into contigs and near-complete genomes.

Results: In an Ae. caspius pool, previously positive for flavivirus screening, sequences belonging to an Ochlerotatus caspius flavivirus were identified. Negevvirus sequences were detected in two Culex pipiens pools, originating from different regions. In the Culex pipiens pool from Thrace region, complete genome of a rhabdovirus, closely-related to Merida virus, a putative novel rhabdovirus recently-identified in Cx. quinquefasciatus mosquitoes and in the Yucatan Peninsula of Mexico, was characterized. The assembled sequence comprised over 11800 nucleotides and demonstrated 83% sequence similarity to Merida virus in pairwise comparison.

Conclusion: Viral metagenomic analysis can overcome the limitation of standard methods in virus screening and proves to be an effective approach for investigating the potential viral diversity associated with field-collected mosquitoes. The employed approach have resulted in characterization of negevvirus and Culex rhabdoviruses for the first time in mosquitoes from Turkey.

Purpose: Bats are increasingly recognized as hosts and sources of viruses with important evolutionary relevance or zoonotic capability even from Europe. Based on a recent study of virome analyses of bats in China, circular single-stranded DNA viruses along with members of the family Parvoviridae constitute the main groups of DNA viruses within bat faecal virome. In the last couple of years several novel circular replication-associated protein encoding single-stranded (CRESS) DNA viruses were described in bats from Brazil, China, USA and Tonga, Oceania. Over decades, metagenomic studies of bat samples along with surveillance studies using degenerate, group specific primers have expanded the number of newly described, often unclassified, viruses within the family Circoviridae. Other CRESS DNA viruses were also described from bat fecal samples, such as Gemycircularviruses of the family Mycopodnaviridae. The aim of our study was to reveal the diversity of CRESS DNA viruses in Central-Eastern European bat fecal samples collected in Ukraine, Hungary, Romania, Serbia and Georgia.

Methods & Materials: Faecal samples were collected from multiple localities in Georgia, Hungary, Romania, Serbia and Ukraine by trained chiropterologists during bat ringing and bat rehabilitation activities. In addition 42 guano samples, collected for previous studies in Hungary were randomly selected and subjected to random primed reverse transcription PCR and semiconductor sequencing by using the Ion Torrent PGM platform. Samples from Georgia, Romania, Serbia and Ukraine were further tested with nested-PCR, targeting the rep gene of Circoviridae family. The identified viral sequences were used for inverse PCR (iPCR) primer design, using Geneious software v9.1. iPCR amplicons which corresponded to the size of 1.5-3kb were purified, than PCR products were sequenced by genome walking method with Big Dye Terminator Cycle Sequencing Ready Reaction on ABI-PRISM 3100 Genetic Analyzer sequencing platform.

Results: Several novel viruses were described, with a potentially novel viral species within the family Circoviridae. Neminivirus from the family Geminiviridae was also described at the first time from Europe. CRESS DNA viruses of the family Mycopodnaviridae were also detected.
Conclusion: The results of this study provide the first dataset on CRESS DNA viruses, circulating among European bat species and discuss their phylogenetic relationship with other previously described viruses.

20.075 Assessing risk of transmission of dengue and yellow fever viruses in major Kenyan Cities by estimation of Stegomyia Indices

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Purpose: The emergence of dengue (DEN) and yellow fever (YF) in east Africa is of growing public health concern and linked to unplanned urbanization and highly adaptable Aedes (Stegomyia) vectors. Entomological surveys remain the main stay in investigating the risk of transmission of these diseases, yet are lacking despite recent outbreaks especially dengue at the Kenyan coast. This study sought to establish entomological indices as a means of risk assessment of DEN and YF in three major urban areas of Kenya and to characterize the positive container types for targeted vector control.

Methods & Materials: We surveyed mosquito immature (larvae/pupae) during the long-rains, short-rains and dry seasons in DEN-endemic (Kilifi County) and DEN-free (Kisumu and Nairobi Counties) areas of Kenya from September 2014 to June 2016. In randomly selected houses, immature were surveyed both indoors and outdoors, reared to adults and morphologically identified to species level. The House index (HI) and Breteau index (BI) were estimated based on houses and container types positive for Aedes immature per site and season.

Results: Of the 900 houses surveyed at all the sites, 88 were positive indoors for Aedes (Stegomyia) immature. 16% of the containers surveyed in all the sites were positive, with 75% located outdoors. The overall HI was 17%, 11% and 0.3% and the corresponding BI was 89, 93 and 14 for Kilifi, Kisumu and Nairobi respectively. HI and BI were highest in the long rainy season than the short rainy and dry seasons. The most productive container types in all three sites were jerricans, plastic drums, metal drums and tyres. Aedes aegypti and Aedes simpsoni were the only stegomyia species identified.

Conclusion: Our findings suggest that Kilifi and Kisumu are at high-risk of DEN and YF transmission while Nairobi is at low-risk, based on the threshold levels of HI (>5%) and/or BI (>20) defined by the Pan American Health Organization. Metal drums were mostly positive in the dry season in the high-risk areas and could be targeted for vector control. However, risk of transmission is highly dependent on the competence of the local vector species to these viruses, which will be determined in ongoing studies.

20.076 ZIKA VIRUS: Improve diagnostic strategies

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Purpose: Zika virus (ZKV) is a mosquito-borne virus transmitted by Aedes genus. It is a RNA flavivirus genetically close to dengue, West Nile, yellow fever and Japanese encephalitis virus. A causal link between ZKV and microcephaly was observed in newborn babies in Brazil. Several Guillain-Barré Syndromes’ associated with an increase in cases of ZKV infection were described in French Polynesia in 2014 and Brazil in 2015-16.

Methods & Materials: By using litterature analysis we established existing methods of diagnostic and treatments. Diagnosis of ZKV disease in humans is mainly based on RNA detection, which is present only in a brief period of viraemia, in serum or plasma samples. Specific antibody detection is difficult due to strong serological cross-reactivity with other flaviviruses diseases (dengue, yellowfever). The virus has been detected in other body fluids such as semen, saliva, and urine. WHO (March 2016) identify these three urgent R&D strategies which must be intensely developed: a vaccine that will be safe for pregnant women and for fetus, a better vector control and, more urgently, a reliable diagnostic.

Results: Diagnosis of ZKV disease is mainly based on RNA detection, but serological cross-reactivity with other flaviviruses diseases (dengue, yellowfever) existing. It is essential to have reliable and more sensitive and specific serological tests. In other body fluids RNA detection is longer because of higher level of RNA. The urine sample is positive for ZKV more than 10 days compared to serum sample about 6 days. In the same way, laboratory confirmation of ZKV is established by presence of RNA or
antigen in serum or other samples; or presence of IgM antibody against ZKV. Current diagnostics tests are difficult to implement in laboratory routine.

**Conclusion:** Next Olympic games in epidemic country of Zika leads us to ask ourselves about efficient diagnostic, treatment and vaccine.

It is essential to have reliable and more sensitive and specific serological tests. The control of Aedes mosquitoes expansion are also been a priority as well as the development of vaccines for pregnant women at first. The discovery of new DNA vaccine by scientists (Larocca et al. 2016) its a promise for future available vaccine.

20.077 The Zika outbreak: An overview of suspected Zika cases presenting at a tertiary hospital in Singapore

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**Purpose:** Zika virus (ZIKV) is a zoonotic disease transmitted by the Aedes mosquito. Singapore reported its first case of locally transmitted ZIKV on 27 August 2016 and the cases surged to 384 by mid-September. This abstract presents a summary of Zika testing at a tertiary hospital during the first three weeks of the Zika outbreak in Singapore.

**Methods & Materials:** An observational study of patients presenting to NUH, a 1,250 bed academic hospital in Singapore, was conducted. All patients tested from 29 August to 18 September 2016 were included. The initial testing strategy from 29 August to 6 September 2016 was aimed at identifying positive cases and isolating them in order to contain the outbreak. Free testing was provided based on Singapore Ministry of Health (SG-MOH) case definitions with links to known geographical clusters. From 7 September onwards, the SG-MOH definition for suspect cases was not limited to specific geographic locales, and clinicians were given greater leeway when ordering ZIKV tests especially for pregnant women. The costs of tests were henceforth charged to patients but at a subsidized rate for Singapore residents. We reviewed the impact of these changes on test positivity rates. Positive ZIKV Real time Polymerase Chain Reaction (RT-PCR) of the blood and/or urine was used to define a confirmed case.

**Results:** A total of 370 patients were tested during the study period, overall 38 (10%) (25 blood, 34 urine and 21 blood and urine) were ZIKV positive. Patients were more likely to be positive during the initial containment period (Odds Ratio 16.1, 95% CI 3.1-68.1, p <0.0001) when 36/211 (17%) were positive compared with 2/159 (1%) when tests were charged and freely available to all including those who did not meet any case definition.

**Conclusion:** A system of free testing using geographic and clinical criteria had a much higher yield than a looser testing criterion which incorporated user fees. Countries trying to get an accurate epidemiologic understanding of their emerging Zika outbreaks should consider using a wider net of free testing to identify cases for targeted vector control to avoid catering primarily to the “worried well”.

20.078 Ebolavirus pseudotypes as antigen surrogates for serological studies

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**Purpose:** The recent outbreak of Ebola virus disease in West Africa focussed the global scientific community’s efforts on the need to develop efficacious vaccines, antivirals and improved diagnostic assays which are applicable in the resource-limited countries where outbreaks occur. To allow serological assessment of these vaccines and antivirals to be undertaken in low containment laboratories, we generated a panel of replication-defective ebolavirus pseudotypes. We sought to optimise their method of production and assess whether the use of a chimeric or truncated glycoprotein (GP) could increase pseudotyping efficiency. Further, we identified highly permissive cell lines and noted their effect on neutralisation profiles using a range of sera and monoclonal antibody samples.

**Methods & Materials:** Primers were designed to generate truncated GP and chimeras formed using overlapping extension PCR, switching the GP cytoplasmic domain with that of a virus which pseudotypes efficiently. Generation of ebolavirus pseudotype followed a three plasmid transfection protocol using a luciferase reporter gene. A panel of cell lines were
infected with these pseudotyped viruses and neutralisation assays undertaken by incubation with doubling dilutions of mAb or sera before the addition of permissive cells.

**Results:** Highest pseudotype titres were achieved using lentiviral cores and transfecting HEK 293T/17 cells. The use of a chimeric or truncated GP failed to increase infectious titre. Evaluation of five target cell lines identified HEK 293T/17 cells as being most permissive to infection, yet neutralisation assay data proved clearest when using CHO-K1 cells.

**Conclusion:** Comprising the main antigenic target, the ebolavirus envelope GP, these pseudotypes alleviate the need for wild-type virus in serological studies and can be handled in a low containment laboratory setting. As such they have been utilised in a range of projects, including testing the potency of an ebolavirus vaccine and the development of novel antibody therapeutics.

20.079 Septic arthritis caused by *Streptococcus suis* – A case report

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**Purpose:** Human *Streptococcus suis* infections are zoonotic infections related to swine animals. It is an emergent public health concern in Asia, where it is associated with food-borne transmission and manifestations of meningitis, endocarditis and sepsis in humans. Arthritis is less common.

**Methods & Materials:** We report a case of a 22-year-old portuguese previously healthy man presented to the hospital with fever, myalgias, swelling and pain of the right wrist with two days duration. Laboratory exams revealed leukocytosis (total leucocytes count 15,34x10⁹/L, 70% neutrophil) and a C-reactive protein of 176,2 mg/L. Wrist's ultrasound showed effusion and synovitis of the radio-carpal joint. Arthrotomy was performed for debridement and synovial fluid analysis. Blood cultures were collected. Patient was admitted with the diagnosis of septic arthritis and ceftriaxone was started empirically. *Streptococcus suis* was isolated on both synovial fluid and blood. According to bacteria susceptibility antibiotherapy was adjusted to penicillin. After 14 days of iv treatment he was discharged home recovered.

The patient worked as a butcher who had handled raw pork. He recalled an abrasion injury on his right first finger 2 days before illness onset.

**Results:** In this case, we had unspecific clinical findings but epidemiological context that raised the possibility of a zoonotic disease. The final diagnosis was based on the results of appropriate microbiological tests timely collected.

**Conclusion:** In Europe, as in this case, *Streptococcus suis* infections are mostly occupational exposures.

20.080 A fatal malaria caused by *Plasmodium knowlesi* infection in a healthy man, Betong, Yala, Thailand April, 2016

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**Purpose:** A zoonotic malaria caused by *Plasmodium knowlesi* is an emerging malaria in South East Asia. The disease was newly recognized and can cause severe illness. The reservoir is an old world macaques in a rain forest of Malaysia-Thailand border. *Anopheles* mosquito is known as a vector. We reported a first fatal case of *P. knowlesi* in a heath man who worked in the jungle of Thailand-Malaysia border.

**Methods & Materials:** We interviewed the case's relatives about the clinical presentation and exposure history including reviewed medical records in the hospital. Blood smear was done and subsequently performed molecular detection by RT-PCR for *P. Knowlesi*. Active case finding among the case's colleagues who recently went to the jungle within a month was also done and then laboratory testing was performed by blood smear and RT-PCR.

**Results:** A 46 year-old healthy man developed symptom in 10 days after spending 2 weeks in the jungle at Thailand border to Malaysia. He was admitted at Betong hospital, Yala province in comatose and then intubation was done. He was renal and hepatic failures and pulmonary hemorrhage. He died within four days after admission. Peripheral blood smear for malaria parasites indicated hyperparasitaemia and malaria antigen test revealed non *P. falciparum*. Then *P. knowlesi* infection was suspected and treated. A single species of *P. knowlesi* was confirmed by PCR. Ricketsia, Dengue and Leptospira of FTD Tropical Fever Core PCR were all negative. There was no additional case or laboratory confirmed among 31 colleagues who recently went to the jungle in one moth ago. In Thailand, 45 cases of *P. knowlesi* were reported and Thailand-Malaysia border are the highest incidence.
Conclusion: Malaria caused by *P. knowlesi* can cause severe and dead in healthy human. Currently, there is an epidemic and potentially life threatening in Malaysia. Early diagnosis and treatment with antimalarial chemotherapy is necessary to prevent multiple organ failures which leading to death. A history of jungle visiting in the epidemic area particularly Thailand-Malaysia border should be pay attention for a *P. knowlesi* infection. Preventing *Anopheles* mosquito bite in the jungle is advised for people who stay overnight in the jungle.

20.081 Prevalence of *Bartonella henselae* infection and its diagnosis in diverse clinical conditions in a tertiary care hospital in North India


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Purpose: *Bartonella henselae* is an emerging zoonotic pathogen which is ubiquitous among mammals. It establishes intraerythrocytic infection which enables its transmission through blood sucking arthropods. It causes myriad of infections which closely resemble febrile illness and chronic diseases like tuberculosis and haematological malignancies. There is a dearth of studies from India regarding its prevalence. The present study was undertaken to diagnose *B. henselae* infection and report its prevalence in diverse clinical conditions.

Methods & Materials: 130 patients including those with fever and lymphadenopathy, infective endocarditis and neuroretinitis were enrolled in the study. Whole blood, serum, and lymph node aspirate and valvular vegetations if available were obtained. Samples were plated on chocolate agar and brain heart infusion agar containing 5% fresh rabbit blood and were incubated at 35°C for at least 4 weeks in 5% CO2 with high humidity. Indirect fluorescence assay (IFA) was done for the detection of IgM antibodies in the serum using a commercial kit. Whole blood was used to perform Polymerase chain reaction (PCR) for the citrate synthase gene (*glt A*).

Results: IFA was positive in 11 (8.46 %) patients and PCR was positive in 3 (2.3%) patients. Culture was negative in all the cases. Out of the eleven IFA positive cases, seven presented with fever and lymphadenopathy. Five of these were diagnosed as having Pyrexia of Unknown Origin (PUO), one with Cat Scratch Disease (CSD) and one with Granulomatous Disease (CD). Among the four IFA positive ophthalmology cases, one was a case of Parainuda Oculoglandular syndrome and the rest showed symptoms of neuroretinitis. A higher incidence of Bartonella infection was seen in patients with fever and lymphadenopathy i.e. 9/39 (23.07%) eight of whom were children.

Conclusion: The present study shows that the threat of Bartonella infection is very much a reality in India. It is also an important treatable cause of fever and lymphadenopathy in children. Serology and PCR are useful tests for its diagnosis. Clinicians should consider Bartonella infection in the differential diagnosis of febrile illnesses and chronic diseases.

20.082 Breaking the Ebola virus disease chain of transmission; the role of Montserrado County sectorial surveillance system Liberia

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Purpose: World Health Organisation (WHO) declared Ebola virus disease (EVD) a Public Health Event of International Concern (PHEIC) in August 2014. This followed the largest West Africa EVD epidemic both in magnitude and geographic spread with morbidity and mortality exceeding all previous outbreaks combined. Montserrado county sectorial EVD surveillance system initiative in 2015 was aimed to achieve a more effective and rapid response to EVD control in Liberia through decentralisation of the response structure. The objectives of the evaluation were to assess the attributes of the system, evaluate the distribution and spread of EVD and communicate findings to key stakeholders for appropriate actions.

Methods & Materials: The 2001 CDC updated guidelines for evaluating public health surveillance system, was used for the evaluation. A suspected case of EVD was defined as “any person with an illness characterized by a history of acute fever and three or more acute clinical symptoms or signs of hemorrhage or death of a person with such a history or any unexplained death”. Sector 3 surveillance data from January to March, 2015 was reviewed and analysed, and stakeholders were also interviewed.

Results: A total of 108 suspected cases were captured by the system. The median age of suspected cases was 37 years, range (0 – 91) with more cases recorded among males 58
entry, transcription and replication. While some RVFV proteins are known to interfere with apoptosis and antiviral signalling, a significant knowledge gap exists concerning the host cellular factors required for the virus cycle. Using a forward genetic screen, we aim to identify those host genes and pathways essential for RVFV replication.

Conclusion: The surveillance data generated serve to guide decision making at the sector and national levels regarding planning, implementation and coordination of EVD control strategies. Sustainability of Montserrado EVD surveillance continues to pose a challenge since it is donor driven. Holistic ownership of the system by the Liberian ministry of health and social welfare remains the eminent solution.

20.083 Middle East Respiratory Syndrome Coronavirus (MERS-CoV): A systematic literature review
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Purpose: Since the detection of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) among humans in 2012, questions remain unanswered regarding the virus’s origins; clinical, epidemiological, and virological characteristics; and potential therapeutics. A systematic literature review was conducted to synthesize current knowledge and identify critical knowledge gaps.

Methods & Materials: We conducted a systematic review on MERS-CoV using PRISMA guidelines and identified 312 relevant, peer-reviewed publications from Embase, Google Scholar, and PubMed. Of these, 206 were selected for inclusion based on their contributions to four pre-defined themes (virology, epidemiology/clinical characteristics, origins/reservoirs, and therapeutics/prevention).

Results: Virological research identified the functional human receptor (CD26/DPP4) and shed light on MERS-CoV’s broad species tropism. A variety of molecular and serological assays have been developed for surveillance and research. The epidemiologic profile is incomplete, although initial data suggest values for the basic reproductive rate, proportion of primary cases, case fatality rate, and demographic and geographic distributions. There have been sustained outbreaks in Saudi Arabia and South Korea, and potential risks for infection include camel contact, nosocomial exposures, and close contact to active cases, but not Hajj pilgrimage. The primary mechanism of transmission in health care settings appears to be environmental contamination of medical devices and surfaces from respiratory secretions.

MERS and MERS-like CoVs have been detected in bat species in numerous countries, and MERS-CoV is genetically similar to bat coronavirus HKU-4, which contains cell surface-expressed CD26/DPP4. Dromedary camels have demonstrated MERS-CoV seropositivity throughout the Middle East and Africa and there is preliminary evidence of camel-to-human MERS-CoV transmission events. Various potential therapeutic agents have been identified from high-throughput screening and other methods, but none have been clinically evaluated in human trials. At least one candidate vaccine has progressed to Phase I human trials.

Conclusion: Although there has been substantial MERS-CoV research since 2012, significant knowledge gaps persist. Uncertainties remain about the zoonotic origin, clinical characteristics, risk factors for infection, asymptomatic transmission, effective therapeutics, and vaccine candidates. These areas merit urgent attention by the global community to better understand, detect, and control MERS-CoV using a unified One Health approach.

20.084 Mammalian haploid genetic screen to identify host factors essential for Rift Valley fever virus
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Purpose: Rift Valley fever virus (RVFV, family Bunyaviridae, genus Phlebovirus) is currently referenced by the WHO as one of the 8 emerging pathogens likely to cause serious outbreaks in the near future and for which few medical countermeasures exist. Transmitted by mosquitoes, RVFV causes large and devastating epidemics in Africa and Arabia, leading to hemorrhages and massive abortions in livestock ruminants, and hepatitis and hemorrhages in 10-20% of human hospitalized patients.

While some RVFV proteins are known to interfere with apoptosis and antiviral signalling, a knowledge gap exists concerning the host cellular factors required for the virus cycle. Using a forward genetic screen, we aim to identify those host genes and pathways essential for RVFV entry, transcription and replication.
**Methods & Materials:** Haploid organisms allow the study of gene knockouts, since recessive mutations will show a clear phenotype due to the absence of a second gene copy. Combined with the pluripotency of embryonic stem cells, the effect of the knockout on viral cycle can be assessed in any disease-relevant cell type. We used the recently developed mammalian haploid embryonic stem cells (mESCs) (Elling, 2011, Cell stem cell), which were mutagenized with a revertible genetrap vector, for infection with the RVFV MP-12 vaccine strain.

**Results:** While wild-type cells massively died upon infection, a higher number of cells survived within the mutagenized cells. Surviving cells were submitted to next generation sequencing, and statistical analysis gave a list of 5 genes potentially involved in RVFV MP-12 cycle, but not essential for normal cell life. Those genes will be validated by infection of specific knockout mESCs clones (and their reverted version) by different strains of RVFV.

**Conclusion:** Such an approach allows the identification with a great confidence of important host cofactors to RVFV, increasing our knowledge on the viral cycle, and giving access to several potential targets for antiviral design.

20.085  
Sero-prevalence of Crimean Congo Haemorrhagic fever (CCHF) in small ruminants of Pakistan

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**Purpose:** Crimean Congo Haemorrhagic Fever (CCHF) is a major zoonosis prevalent in Pakistan caused by CCHF virus a Nairovirus of family Bunyaviridae. CCHFV is reservoired in tick vectors which spend a significant part of their haematophagous life cycle on domesticated animals, during which CCHFV can be transmitted. CCHFV can also be transmitted to humans during the slaughter of viremic animals and directly from bite of infected ticks. Both are important transmission routes of virus to humans and a case fatality rate (CFR) of up to 40% is common. Nosocomial transmission between humans is less common, but associated with higher CFRs. All types of transmission have been reported in Pakistan but very limited information is available about its epidemiology in animals which may act as silent carrier. Therefore, it is very important to develop a sensitive and effective surveillance system for CCHF carrier animals. This would lead to better understand its epidemiology in animals for devising appropriate control strategies.

This study was conducted with the aim of estimating baseline levels of CCHFV exposure in small ruminants in Pakistan using Enzyme-linked immunosorbent assay.

**Methods & Materials:** One hundred heat inactivated sera from sheep (n = 57) and goat (n = 43) were shipped to Public Heath England and tested by ELISA at 4 dilutions (1/100; 1/400; 1/1600; 1/6400). Anti-CCHFV nucleoprotein (CCHF-NP) positive control rabbit serum and CCHF-NP antigen and negative control antigen were provided by National Institute of Infectious Immunoflorescence Diseases, Japan.

**Results:** Overall sero-prevalence of CCHF in small ruminants was 9%. Highest CCHF sero-prevalence was observed in sheep and goats of Loralali (22.2%) followed by Quetta (8.3%) and Abbottabad (0%). Sero-prevalence of CCHF in goat was 10.53% (6/57) and 6.98% (3/43) in sheep. Sera dilution 1:400 and 1:100 showed best results using Receiver Operating Characteristic analysis having cut off OD value > 1.538 with 100% sensitivity and 92% specificity & cut off OD value > 1.207 with 100% sensitivity and specificity for goats and sheep, respectively.

**Conclusion:** This study reported the presence of antibodies against CCHF in sheep and goats of Pakistan indicating their role in CCHF transmission.

20.086  
Using the Incidence Decay and Exponential Adjustment (IDEA) model to understand transmission dynamics of MERS-CoV in a camel herd

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**Purpose:** Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic disease that emerged in 2012. To date, there have been over 1700 human cases reported. Camels appear to be the reservoir host, with infection sporadically spilling over to humans. However, little is known about the transmission dynamics of MERS-CoV in the reservoir population. We
used the Incidence Decay and Exponential Adjustment (IDEA) model to examine the spread of MERS-CoV within a single, closed camel herd in Egypt.

**Methods & Materials:** A longitudinal study of a closed camel herd conducted from December 2014–February 2016 documented an outbreak of MERS-CoV over 8 site visits between January–May 2015. By the end of the outbreak, 96% (79/82) of all camels had been infected. Nasal swabs were tested and confirmed using the UpE and ORF1a rRT-PCR assays. We applied the 2-parameter IDEA model to cumulative incidence data to estimate $R_0$ and a control parameter ($d$) for the within-herd transmission using a generation time of 12 days (range: 10–15 days). The best fit $R_0$ and $d$ parameters were estimated using maximum likelihood. We attempted to project the expected time course of the outbreak by iteratively fitting successive generations of data using a limited number of epidemic generations.

**Results:** The best-fit estimate for $R_0$ was 2.62 (95% CI: 2.20–3.20) and for $d$ was 0.09 (95% CI: 0.06–0.13). Parameter estimates stabilized at 5 generations, however, final outbreak size projections were underestimated until the full dataset was used (range: 38–90). Model performance was weaker when higher values of $R_0$ were estimated, consistent with previous applications of the IDEA model.

**Conclusion:** Using a simple model and cumulative incidence data, our results provide a preliminary estimate of $R_0$ for camel-to-camel transmission of MERS-CoV. Our findings suggest that infection within a susceptible camel herd spreads rapidly ($R_0 > 2.5$) with very little decay of the transmission dynamics ($d < 0.1$). These findings are consistent with a pathogen that causes mild or asymptomatic infection in the reservoir host population with little to no active intervention to prevent spread. This paper highlights the need for further epidemiological studies, which in turn will support evidence-based decisions regarding interventions aimed at reducing zoonotic transmission.

20.087 Setting research priorities to control zoonoses in smallholder dairy farms of periurban India

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**Purpose:** The systematic neglect of the rapidly emerging periurban fringes has led to an evidence vacuum. In order to address the problems of this emerging ecosystem with directed policies, it is essential to identify critical gaps in the existing knowledge and identify priority research to address those gaps. In this study we aim to identify the critical gaps, and identify a strategic research agenda to fulfill those gaps.

**Methods & Materials:** The modified CHNRI methodology, which was previously deployed to identify research priorities for zoonoses in India, was used for this priority setting process. The method has three steps: defining the context of the research and identifying experts; generating research options through a systematic interview; independent ranking and scoring of options using weights from pre-defined criteria. A wide range of experts were interviewed to generate research options, followed by the prioritization of the same by another group of expert independent scorers.

**Results:** Brucellosis was identified as a priority disease. Primary consumers of farm products and farmers were identified as highly susceptible groups. Solid wastes and their improper disposal was identified to be a major farm-related commodity of concern. The experts identified 316 research options to address the identified knowledge gaps. Most were related to basic epidemiological research (121/316, 38%) and research to develop newer interventions (94/316, 30%). The experts put the most stress on research that leads to deliverable, affordable and sustainable interventions, with the highest potential for reduction in the disease burden.

**Conclusion:** The experts acknowledged the lack of evidence-driven policy to address zoonotic vulnerabilities arising at the human-animal-environment interface in periurban smallholder dairy farms. The overt stress on developing newer interventions, added to the ignorance of the current epidemiological situation, reflects the chaos that has taken over the systematic understanding of the peri urban milieu. This reflects the need to undertake advocacy to sensitize the major stakeholders – funding agencies, researchers, policy makers and program managers – to the need of the hour so that the research-reality disconnect can be reduced. The results from this study may help bridge this gap.


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**Methods & Materials:** A longitudinal study of a closed camel herd conducted from December 2014–February 2016 documented an outbreak of MERS-CoV over 8 site visits between January–May 2015. By the end of the outbreak, 96% (79/82) of all camels had been infected. Nasal swabs were tested and confirmed using the UpE and ORF1a rRT-PCR assays. We applied the 2-parameter IDEA model to cumulative incidence data to estimate $R_0$ and a control parameter ($d$) for the within-herd transmission using a generation time of 12 days (range: 10–15 days). The best fit $R_0$ and $d$ parameters were estimated using maximum likelihood. We attempted to project the expected time course of the outbreak by iteratively fitting successive generations of data using a limited number of epidemic generations.

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**Conclusion:** Using a simple model and cumulative incidence data, our results provide a preliminary estimate of $R_0$ for camel-to-camel transmission of MERS-CoV. Our findings suggest that infection within a susceptible camel herd spreads rapidly ($R_0 > 2.5$) with very little decay of the transmission dynamics ($d < 0.1$). These findings are consistent with a pathogen that causes mild or asymptomatic infection in the reservoir host population with little to no active intervention to prevent spread. This paper highlights the need for further epidemiological studies, which in turn will support evidence-based decisions regarding interventions aimed at reducing zoonotic transmission.
Purpose: To investigate the sequence analysis of Manadonese G4P[6] rotavirus strain associated with diarrhea.

Methods & Materials: From April 2013 to July 2014, 441 fecal samples were collected from pediatric inpatients in RSUD Dr. R. D. Kandou Hospital, Manado. The samples were screened for group A rotavirus and 10 other target viruses by multiplex PCR. Positive samples for rotavirus were further analyzed for genotyping and sequence analysis. Phylogenetic tree were constructed by neighbour-joining method.

Results: Group A rotavirus was found as the predominant viral agent (49.9%), with G1P[8] being the most common (69.5%) strain. One case of G4P[6] was found among the positive samples and sequence analysis of VP7 gene showed a close relation with porcine rotavirus strains, while VP4 genes showed a closer relation with human rotavirus strains.

Conclusion: The case of G4P[6] rotavirus strain in this study suggested an evidence of human-animal reassortment and showed a possibility of interspecies transmission between porcine and human.

20.089 Absence of MERS-CoV in domestic camels, Republic of Korea, 2015
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Purpose: In the Republic of Korea (ROK), the first human case of Middle East Respiratory Syndrome coronavirus (MERS-CoV) infection was reported on 20 May 2015. It occurred by a traveler who has been traveled to Middle East, since then outbreaks associated with health care facilities were rapidly spreading. Unlike previously reported MERS-CoV occurred countries, explosive outbreaks of MERS-CoV caused public health threat and socio-economic loss in ROK. In these circumstances, increased concern about the camels known as an animal reservoir of MERS-CoV triggered the necessity of MERS-CoV surveillance in domestic camels. Thus, we investigated MERS-CoV infection in all domestic camels in June, 2015 in ROK.

Methods & Materials: We collected nasal swab specimens from 46 camels both dromedaries (Camels dromedarius) and Bactrian (Camels bactrianus) in 10 provinces in ROK. Real-time reverse transcription PCR (RT-PCR) was performed as recommended by the World Health Organization (WHO).

Results: All samples were confirmed as negative for MERS-CoV.

Conclusion: The MERS Co-V outbreaks in ROK are characterized by super-spreading events involving several healthcare facilities, poor infection control and so on. This situation caused panic and strike excessive fear into people thus we took a preventive action against camels. Therefore, we investigated MERS-CoV in domestic camels. Our results indicate the absence of MERS-CoV among domestic camels in ROK. In addition, it suggests that there is no epidemiological relation between camels and outbreak of MERS in ROK.

20.090 Ocular manifestation of Mediterranean spotted fever in Bulgaria
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Purpose: Mediterranean spotted fever has become an emerging infection in Bulgaria since 1993. The pathophysiological hallmark of its causative agent, R. conori, is the invasion of vascular endothelial cells causing endothelial injury and tissue necrosis. Ocular involvement is common, but frequently asymptomatic, self-limiting and easily overlooked.

Methods & Materials: Case presentation: A 30-year-old man was referred to our hospital in early August 2013 because of skin rash, fever, myalgia, arthralgia for 15 days and painless blurred vision and floaters in his left eye that occurred 2 days before the admission. He was treated by his general practitioner with Amoxicillin but without effect. At the admission the patient was febrile with diffuse maculo-papular skin rash, involving palms and soles, but no tache noire was seen, nontender hepatomegaly, the remainder of his physical examination was normal. He gave no history of tick bite, but owned a dog. The patient was unemployed and drank alcohol socially.

Results: Complete blood count and routine biochemistry tests were within reference range apart from mild elevation of alaninaminotranferase (75 E/l), his serology for syphilis was
negative. Consultant ophthalmologist revealed ciliary flush, keratic precipitates on the corneal endothelium, visible cells and flare in anterior chamber without synechia, consistent with diagnosis of uveitis in the left eye. The patient was started on doxycycline, ciprofloxacin and intravenous fluids for 10 days and additional therapeutic agents for ocular condition – mydriatic drops, topical steroid and nonsteroidal medications.

His general condition gradually improved as was the ocular involvement. The patient was discharged after 7 days with recommendation to continue treatment for ocular condition and to see an ophthalmologist as an outpatient. Later serology for R. conorii (ELISA) came back positive for IgM and IgG. Unfortunately, the patient had never returned for a follow-up visit and no information was available about recovery of his vision.

**Conclusion:** Our case serves as a reminder how broad the clinical presentation of MSF might be and R. conorii should be included in the work-up of a patient with uveitis who lives in endemic area.

20.091 IL-28B polymorphisms differently influence IFN system induction, antiviral activity and inflammatory response by african and asian ZIKV strains

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**Purpose:** Since ZIKV Africans and Asian lineages have been associated with different clinical manifestation, the purpose of this study was to explore whether the two strains have different sensitivity to IFN type I, type II and the combination of both. Moreover, we investigated their ability to induce IFN system and antiviral activity in PBMC from healthy donors and whether IL-28B rs12979860 polymorphisms could contribute to explain the different host susceptibility to ZIKV infection.

**Methods & Materials:** Vero E6 cells were treated with IFN-α/IFN-g and infected either with ZIKV MR766 or ZIKV isolate Brazil/2016/INMI1 (ZIKV INMI1) to establish IFN sensitivity. Quantification of IFN-α mRNA, MxA mRNA, IFN-g mRNA was performed in PBMC from healthy donors with different IL28 rs12979860 polymorphism.

**Results:** ZIKV MR766 was able to induce IFN-α mRNA after 24h of infection in IL-28B rs12979860 CC, but not in IL-28B rs12979860 TT while an effective IFN-g mRNA induction was observed only in IL-28B rs12979860 TT. Consistently, the african strain was able to induce MxA mRNA in both IL-28BCC and TT healthy donors, with a peak at 48h p.i. ZIKV INMI1 showed the same trend of induction of IFN-α mRNA with a delayed kinetic (48h p.i) in IL-28B rs12979860 CC. On the contrary, in IL-28B rs12979860 TT the induction of IFN-α mRNA was more rapid and sustained over time after ZIKV INMI1. Both IL-28B rs12979860 CC and IL-28B rs12979860 TT showed a rapid and constant production of IFN-g mRNA after ZIKV INMI1 infection. Interestingly, ZIKV INMI was not able to induce prompt antiviral activity. Looking to the cytokine profile, IL-28B rs12979860 TT polymorphism was associated to a polyclonal inflammatory response.

**Conclusion:** Our data indicate that IFN-α and IFN-g are able to inhibit the replication of both ZIKV strains, being ZIKV INMI1 more sensitive to the antiviral activity of IFNs. Moreover, a different ability of the two ZIKV strains to induce IFN-α mRNA, IFN-g mRNA and MxA mRNA was observed, being ZIKV INMI1 not able to induce a prompt antiviral activity. The differences among the two ZIKV strains seem to be strictly related with IL-28B rs12979860 polymorphisms

20.092 Serological survey of hantavirus infection among rodents in Hungary

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**Purpose:** Hantaviruses are found worldwide and are known to cause serious human diseases. Natural hosts are rodents, soricomorphs and bats. Three types of hantaviruses are circulating in Hungary, Dobrava, Puumala and Tula which are carried by mice and voles. Dobrava and Puumala viruses cause human disease. In Hungarian forests the dominant species of rodents are yellow necked mouse (Apodemus flavicollis) striped field mouse (Apodemus agrarius) wood mouse (Apodemus sylvaticus) and bank vole (Myodes glareolus) which are natural reservoirs. The aim of the study was to survey the prevalence of
hantaviruses among rodent populations and examine the potential correlation between population densities and seroprevalence.

**Methods & Materials:** The rodents were trapped at 13 sampling plots on a 149 ha area in the Mecsek Mountains, Hungary from March to October between 2011 and 2014 using a capture-mark-recapture method. Rodent sera were tested for antibodies against the two most significant hantavirus species in Europe, Dobrava-Belgrade (DOBV) and Puumala (PUUV) viruses by ELISA.

**Results:** Among the 3720 tested sera samples 350 were positive for hantaviruses (DOBV, PUUV). In 2011 the seroprevalence was 14.5% (178/1229), but in 2012 despite of the higher number of rodents it was just 7.4% (122/1638), in 2013 and in 2014 it was about 6%. The seroprevalence was the highest for the Apodemus species in the first two years, then in 2013 and 2014 for the Myodes glareolus. In every year among the hantavirus positive rodents the number of males were higher.

**Conclusion:** Since the seroprevalence in 2012 decreased to half despite of the higher sample size, it may indicate that the number of infected individuals and community size are not directly proportional. Since in the subsequent years we registered great decrease in population size, but the infection rate stagnated throughout, it may indicate a time delay between community size changing and infection rate alteration. In four years the number of Apodemus species which were originally abundant in the area decreased and the population density of Myodes glareolus became higher. Therefore at the last year of the survey of 2014, the Puumala hantavirus seroprevalence was much higher compared to Dobrava seroprevalence.

**Purpose:** Leptospirosis is an important zoonotic disease in the world, particularly in tropical and subtropical regions. A cross-sectional survey was conducted from 2011 to 2012 to assess the prevalence of leptospiral antibodies in domestic animals from Senegal and to identify the predominant serogroups.

**Methods & Materials:** Three hundred and twenty blood samples were collected from 88 equids (68 horses and 20 donkeys), 151 domestic ruminants (52 goats, 43 sheep and 56 zebus), and 81 dogs, and were tested for twenty-four serovars of pathogenic *Leptospira* species by the microscopic agglutination test.

**Results:** From the total number of 320 samples, 186 (58%) were found to be positive towards one or several serovars of pathogenic leptospires (cut off: 1:100). According to the species, the positive sera were obtained from 83% of dogs, 96% of horses, 80% of donkeys, 35% of goats, 7% of sheep, and 30% of zebras. The serogroup Icterohaemorrhagiae is the most frequent in donkeys (43%) and sheep (50%). The most frequent serogroups in horses are Icterohaemorrhagiae (36%) and Australis (35%). Serogroup Australis is the most frequent in zebu (27%). In dogs, the most frequent serogroups are Icterohaemorrhagiae (33%), Canicola (20%), and Australis (17%).

**Conclusion:** This study shows the broad dispersion and the high prevalence of the different serogroups of pathogenic *Leptospira* species tested, particularly among dogs and equids. The disease is endemic in domestic animals and concerns all the species. This high biodiversity has already been reported in tropical countries and is supposed to be linked to a wide range of reservoir mammals. Additional studies are required to identify the reservoirs for the different serogroups in this part of Africa.

**Vaccinia virus** in feces of wild rodents from São Paulo State, Brazil

**Methods & Materials:** The rodents were trapped at 13 sampling plots on a 149 ha area in the Mecsek Mountains, Hungary from March to October between 2011 and 2014 using a capture-mark-recapture method. Rodent sera were tested for antibodies against the two most significant hantavirus species in Europe, Dobrava-Belgrade (DOBV) and Puumala (PUUV) viruses by ELISA.

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Purpose: The origin of Vaccinia virus (VACV) outbreaks in Brazil remains unknown, but since the isolation of VACV in Mus musculus mice during a zoonotic outbreak that peri-domestic rodents have been suggested to be a link between cows and wild animals. Considering that experimentally infected mice eliminate viral particles in their feces, we investigated the presence of VACV in the feces of wild rodents captured in the forest areas surrounding milking farms in the central west region of São Paulo State, Brazil.

Methods & Materials: Samples were collected in 47 farms randomly selected, including 10 in Torre de Pedra (23°14’58.76”S48°11’39.49”W) in which outbreaks were registered in 2007 and 2010, 15 in Bofete (23°05’54.51”S48°11’26.61”W) and 22 in Anhembali (23°05’54.51”S48°11’26.61”W), both without reports of outbreaks. Wild rodents were captured from May to September of 2011 in Pitfall and Sherman traps. The rodents were anesthetized in plastic autoclavable bags containing gauze soaked in ethyl ether. Blood samples, organs, and feces were collected. Viral DNA was extracted from feces using the RTP® DNA/RNA Virus Mini Kit (Stratec Molecular, Berlin, Germany). A nested PCR was used for amplification of the vaccinia growth factor (vgf) gene, and positive samples were submitted to gene sequencing.

Results: Fecal samples were collected from 115 animals, of the following species captured: Akodon montensis, Calomys tener, Jumolmys pictipes, Necromys lasiurus, Nectomyx squamipes, Oligoryzomys flavescens, Oligoryzomys nigripes, and Sooretamys angouya. Six (5.2%) were positive including 14% of Sooretamys angouya, 8% of Oligoryzomys flavescens, and 4% of Oligoryzomys nigripes., however, only one of the positive samples was sequenced because the low DNA concentration of the other five were not sufficient for sequencing. The sequencing and phylogenetic tree revealed that our strain clustered with the Brazilian VACVs.

Conclusion: For the first time, we reports detection of VACV DNA in feces of naturally infected Oligoryzomys flavescens, Oligoryzomys nigripes and Sooretamys angouya. Indeed, the transmission of VACV via feces of experimentally infected mice to cows has not yet been described, and no cases of rodent-to-cow or rodent-to-human VACV transmission have been reported during the Brazilian outbreaks. Additional studies are needed to further elucidate this epidemiologic situation.


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Purpose: Although reported in Kenya, Uganda for the first time investigated and responded to a Rift Valley Fever (RVF) outbreak in 2016. Uganda last reported yellow fever outbreak in 2010, but experienced a new outbreak in 2016 in three districts. We present the epidemiological description of the cases, identified risk factors and control efforts for RVF and Yellow Fever outbreaks.

Methods & Materials: In March 2016, two cases presenting with fever, general body weakness, severe haemorrhagic tendencies were reported by Kabale and Mbarara Regional Referral Hospitals as suspected viral haemorrhagic fever cases. In March, Masaka Regional Referral Hospital (300 km from Kabale district), and in April, Rukungiri and Kalangala districts reported cases with fever and haemorrhagic presentation. Through the coordinating National Task Force on epidemics in Ministry of Health, a multidisciplinary team with epidemiologists, veterinarians, environmental health experts and statisticians investigated these cases. Blood sample collection and referral, line listing, active surveillance were done.

Results: For the RVF outbreak, the index case was a 42 year old male, a butcher by occupation whereas the second case was a 16 year old school boy. Although up to 24 cases were line listed, samples sent to Uganda Virus Research Institute confirmed two cases as positive for RVF virus by reverse transcriptase Polymerase Chain Reaction (RT-PCR). Out of 1051 samples collected from animals in the sub-county of the index case’s residence, two goat samples were RT-PCR RVF positive; and IgG seropositive cattle (27%), goats (6.5%) and sheep (5.7%), indicating the possible source of infection. Risk factors included close animal contact, working in forested areas with the mosquito vector and being male.

For Yellow Fever, 32 cases were line listed but five in Masaka and one each in Kalangala and Rukungiri districts were confirmed positive by RT-PCR. Preventive vaccination of population above 6 months achieved more than 90% coverage in each district. No new cases were recorded after immunisation.
Conclusion: Uganda has developed capacity to investigate, test and confirm RVF and Yellow Fever virus diseases. Detailed active surveillance has been set up to investigate any additional cases in humans and animals to reduce chances of international spread.

20.096 Characterisation of the increasing numbers of autochthonous hepatitis E infections in England and Wales 2010-2015

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Purpose: Hepatitis E virus (HEV) was previously known for causing acute infections in travellers returning from hyper-endemic countries. However, autochthonous, food–borne transmission of HEV genotype 3 (GT3) has been increasingly recognized as an emerging problem in industrialized countries. Although asymptomatic in most cases, HEV GT3 can cause mild and self-limiting to severe acute hepatitis and, most worryingly, chronic hepatitis in immunocompromised patients. Over the last decade, cases of acute HEV infection in humans have been increasing across Europe. Since 2010, a year on year increase has been reported in England and Wales. Previously the only phylotype circulating in England and Wales was HEV GT3 group 1, however, the substantial increase in HEV cases since 2010 has been associated with the emergence of a novel group of the virus (HEV GT3 group 2). Enhanced surveillance of cases of acute HEV infections in England and Wales was established in 2005 to investigate and characterise non-travel associated cases of HEV and to identify potential risk factors.

Methods & Materials: Newly diagnosed cases of HEV are reported to the Second Generation Surveillance System (SGSS, a voluntary electronic reporting database of clinically significant pathogens by NHS hospital laboratory departments). In addition, Health Protection Teams across England and Wales also report new cases of HEV and complete an enhanced surveillance questionnaire on each case. For case ascertainment, these findings are compared with cases reported to SGSS.

Results: The number of HEV cases tripled from 2010 to 2015. As previously reported, the majority (>70%) of cases of HEV GT3 infections are male and their median age is above 60 years. HEV GT3 group 2 virus appears to be responsible for a large part of the increase in indigenous cases, whilst a trend towards more severe and prolonged illness has been observed in cases of HEV GT3 clade 1 as demonstrated through higher rates of hospital admissions and prolonged illness.

Conclusion: The increasing rates of HEV across England and Wales remain a concern, particularly as the natural history of this disease continues to be unclear. The emergence of a new phylotype largely responsible for the increase in cases warrants further research.

20.097 Congenital Zika Syndrome: Establishing obstetric and paediatric surveillance in the UK and ROI

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Purpose: The recent Zika virus outbreak was declared a Public Health Emergency of International Concern by WHO in February 2016. There is scientific consensus that Zika virus is a cause of microcephaly and other congenital anomalies. Cases of maternal-fetal transmission have been confirmed and congenital abnormalities associated with maternal Zika virus infection have been described. In 2015, 1.6 million UK residents travelled to South and Central America and the Caribbean, 28% of these were women of child bearing age. The risks to and impact on UK travellers to affected countries is not yet clear. Surveillance studies described below aim to establish the number of cases and the risk of congenital abnormalities associated with travel to countries with active Zika virus transmission. Findings may help to describe the typical features and natural history of this condition, including presentations in relation to time of infection during different stages of pregnancy as well as long-term outcomes.

Methods & Materials: The UK Obstetric Surveillance system (UKOSS) conducts surveillance for pregnancy outcomes in mothers with a relevant travel history. Detailed reports are collected on adverse pregnancy outcomes including miscarriages, stillbirths and terminations. The British Paediatric Surveillance Unit (BPSU) records all infants ≤ 6 months of age with microcephaly or neurological abnormalities born in the UK and ROI to mothers who have travelled to affected countries. Developmental follow-up is scheduled at 2 years of age. Case
ascertainment is facilitated by conducting both surveillance systems in parallel and findings will also be compared to those of the National Congenital Anomaly and Rare Disease Registration Service (NCARDRS) which collects data on congenital abnormalities including microcephaly.

**Results:** Surveillance for congenital Zika syndrome has been rapidly established in the UK and Republic of Ireland (ROI). UKOSS surveillance commenced on 1st March 2016, surveillance by BPSU started on 1st April 2016. To date, no live born case has been identified and the number of adverse pregnancy outcomes such as miscarriages reported has not exceeded expected levels.

**Conclusion:** Ongoing surveillance of this developing situation is required to monitor the risks and impact on the UK population to guide future recommendations and planning for services.

20.098 Screening for Viral Proteins of Some Emerging Viruses in African Straw-coloured Fruit bats (*Eidolon helvum*) Captured in Zaria, Nigeria

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**Purpose:** This study was carried out to screen for the presence antibodies to Nipah virus soluble G protein (NiV sG), Nipah virus N protein (NiV N), Nipah virus soluble F protein (NiV sF), Hendra virus soluble G protein (HeV sG), Zaire Ebola virus G protein (EboV G), Cedar virus soluble G protein (CedV sG) and Menangle virus N protein (MenV N) in *Eidolon helvum* captured in Zaria, Nigeria.

**Methods & Materials:** A total of forty-five bats were trapped, and captured from two residential bat roosts in Zaria. Bats were slaughtered for consumption with whole blood spotted on Whatman FTA cards and transported to CSIRO, Australia for screening by Luminex binding assay.

**Results:** The result revealed low Median Fluorescent Intensity values for most samples but high values were obtained for HeVsG (2.2%) and MenV N (6.7%): this is suggestive of the presence of antibodies to these viruses.

**Conclusion:** There are no evidences of NiV sG, NiV N, NiV sF, HeVsG, EboV G, CedV sG and MenV N proteins in bats captured in Zaria. Further investigation may recognize novel viruses of public health importance.

20.099 Targeting Ebola Virus Disease Glycoprotein processing via Phenyl-sulphonyl-derived Host Furin inhibitors

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**Purpose:** Against the backdrop of high frequency non-synonymous substitutions in Ebola virus glycoprotein in a recent publication, the suitability of current treatment regimen (glycoprotein-based vaccine) for future outbreaks seem bleak. Here, we took advantage of the dependence of Lassa virus on host Furin for secretory and surface glycoprotein protein (SGP, GP1 & GP2) processing to design mechanism-based inhibitors built on phenyl-sulphonyl scaffold. Attention was focused on building compounds which form pi-pi interaction with active site histidine-194 and readily accepts electron from nucleophilic serine-364.

**Methods & Materials:** Using host Furin active site pharmacophore, a library of the compounds was designed based on phenyl-sulphonyl scaffold using Fragment-based de novo Med-Chem Transformation (FdMT) method for docking and scoring using autodock vina. High affinity compounds were custom-syntheised and tested for in vitro human furin Inhibition (Biotek-FL600) and Ebola virus glycoprotein precursor processing using western blotting technique.

**Results:** immunoblot results showed that the selected compounds blocked recombinant Ebola virus glycoprotein lacking the transmembrane region (rEBVO-GPd7M) processing following 24-hr incubation period

**Conclusion:** Phenyl-sulphonyl-derived compounds respresent novel stategies to developing pharmacologics against Lassa virus disease.
Purpose: On July 5th, 2016 Phang Nga Provincial Health Office, Thailand Ministry of Public Health received a notification of cluster skin lesions and severe itching in a family. We started outbreak investigation with aim to confirm diagnosis and outbreak, to identify the source of outbreak and to implement control measures.

Methods & Materials: We interviewed cases about clinical presentation and physical examination of all suspected cases including exposure history. Skin lesions were carefully explored and identify a parasite by direct microscopy. Complete blood count was done in all cases. Active case finding was carried out in the village with definition of any skin lesion and itching. Environmental survey was done in and surrounding the case’s household.

Results: Total six cases were met case definition with age between 11 and 32 years old. All cases are living in a same family. Their onset was during June 22nd – July 3rd, 2016. There is no additional case in a village. The most common skin lesion was erythematous papule and severe itching. Body (83%) and neck (67%) was the most affected area. Leukocytosis was found in 2 cases. One mite was removal from one case and subsequently identified as Tyrophagus longior mite. Copra itch is confirmed the diagnosis. This house previously did a cottage industry of handling copra and the dried kernel of the coconut 6 months ago. This family was moving in 3 months later. Three unused copra beds were also found in the bed rooms.

Conclusion: Copra itch is the most likely cause of the outbreak of skin disease. The previous was reported since 1953 in the Netherlands. The clinical presentation was also matched with this disease. The copra beds were suspected source of outbreak. We removed and burn them. Gamma Benzene Hexachlorine (0.1%) applied the skin lesions and Doxycycline was prescribed to prevent secondary skin infection. Deltamethrin was spaying indoor and outdoor to control the vector.

Purpose: Trichinellosis is a neglected zoonotic disease caused by infection with Trichinella roundworms and is endemic in Thailand. This parasite is most often spread through the consumption of raw or undercooked pork, which is a common ingredient of traditional foods in northern Thailand. The ethnic minorities living in the northern Thai highlands rely on swine production for their livelihoods and cultural practices. Trichinellosis is commonly reported in these populations and has a negative impact on health, livelihood, and socioeconomic development. This study was carried out to investigate regional risk factors for trichinellosis to better inform public health interventions.

Methods & Materials: Fifty-three households within Huai Chan Si and Huai Ma Fueang villages, which had recent trichinellosis outbreaks, were randomly selected for interviewing. The households were interviewed using a questionnaire developed to collect data on trichinellosis risk factors in four subsystems: animal husbandry, environment, food chain, and economics. This multidisciplinary study involved students and faculty members from the Faculty of Veterinary Medicine and Faculty of Economics at Chiang Mai University, who were trained on how to conduct the questionnaire and on One Health concepts.

Results: The survey results suggested that animal husbandry, the environment, and household economic circumstances are entirely interdependent in terms of trichinellosis risk. Our findings suggested that eating raw pork was part of important rituals and likely not subject to behavioral modification; however, most villagers were unaware of the association of raw pork consumption and trichinellosis. A range of pig production methods were assessed, associated with different levels of trichinellosis risk, indicating that those of lower risk required higher costs in terms of capital and time to implement. Villagers generally considered investment in improved husbandry a lower priority compared to direct livelihood improvements.
Conclusion: A complex assortment of interdependent factors must be considered as an interdependent social-ecological system when devising disease interventions for trichinellosis. These and other findings could be used as basis for expanding the research to include a component of participatory prevention and control aimed at reducing *Trichinella* transmission in northern Thai highland villages.

20.102 Epidemiological and clinical characteristics of hantavirus infections detected in three geographically close natural foci during the 2014 Croatian outbreak

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**Purpose:** The aim of this study was to analyze epidemiological and clinical characteristics of hantavirus infections detected in Croatia during the 2014 outbreak.

**Methods & Materials:** From January to December 2014, a total of 122 patients from three geographically close natural foci in the central mountainous Croatian region (Ogulin, Slunj and Plitvice Lakes surroundings) with clinical symptoms suggestive of hantavirus infection were tested for the presence of IgM/IgG antibodies to hantaviruses. Serologic tests were performed using a commercial IFA for the simultaneous detection of Puumala (PUUV), Dobrava (DOBV), Hantaan (HTNV), Seoul (SEOV) and Saaremaa (SAAV) virus (Euroimmun, Lübeck Germany). Cross-reactive samples were additionally tested using western blot (Euroimmun, Lübeck Germany). For hospitalized patients clinical symptoms and laboratory parameters were analyzed.

**Results:** Acute hantavirus infection (IgM/IgG antibodies) was confirmed in 57/46.7% patients, while 10/8.2% patients were IgG seropositive. PUUV infection was detected in 88.1% and DOBV infection in 11.9% patients. Among acute cases, 75.4% were hospitalized. Males were infected more commonly than females (75.4%). The mean patient's age was 37.9 (15-69) years. Contact with rodents was noted in 59.3% patients. The outbreak started in winter months with majority patients (80.7%) reported from May to July. According to disease severity, mild clinical form was noted in 66.7%, moderate in 18.5% and severe in 14.8% patients. The most common clinical symptoms on admission were high fever (96.3%), chills/shivering (62.9%) and lumbal pain (48.1%). In 11.1% patients, respiratory symptoms with interstitial inflammatory infiltrates on X-ray were reported. Acute renal failure was noted in 74.1% patients but only 3.7% required hemodialysis. Laboratory abnormalities included albuminuria (96.3%), thrombocytopenia (81.5%), elevated urea and/or creatinine levels (70.4%) and elevated transaminases (25.9%). The mean duration of hospitalization was 9.6 (4-19) days. Only one PUUV infected patient developed multiorgan failure and died.

**Conclusion:** Our results indicate that central mountainous Croatian regions are still endemic areas for hantavirus infections with circulation of both PUUV and DOBV. Compared to previous Croatian outbreaks, respiratory symptoms that are frequently detected in PUUV infection were reported less frequent.

20.103 Identification of spatiotemporal patterns of African swine fever behavior in wild boar in the European Union

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**Purpose:** African swine fever (ASF), one of the most important diseases to affect pigs, is currently spreading widely through eastern EU especially in wild boars. The objective of this paper was to identify regions and periods of time in which notifications of ASF in wild boar occurred in the UE from 2 years (2014-2015) in order to describe the dynamic of the disease in this epidemic helping to identify risk areas and the behavior of ASF in UE.

**Methods & Materials:** A spatiotemporal analysis of cases (2014-2015) in wild boars notified in EU has been carried out in order to identify: 1) the maximum distance of association of ASF cases that allows determine the spatial window for the space-time analysis 2) the space-time clusters of ASF cases in EU in wild boar. A combination of space–time analysis was used here: K Ripley analysis and the permutation model of the time-space scan statistic of Kulldorf et al (2005) respectively.
Data used for the analysis included the location (latitude, longitude) and the date of start of the 1451 ASF notifications reported by EU countries to the world animal organization for animal health (OIE) (WAHID, 2016) from January 2014 through December 2015.

**Results:** The K Ripley analysis indicated a yearly maximum distance of spatial association between ASF wild boar cases of 111 km in EU, which was adopted as average maximum distance for the subsequent analysis. Nine significant (P < 0.005) time–space clusters of ASF cases were detected, which were located in Lithuania (2), Poland (1), Latvia (3) and Estonia (3). Clusters included a total of 652 cases. Six of the clusters occurred between September and December, the other three clusters occurred from May to August. Mean of clusters radii was 38.77km (SD=41.21), and mean of cases include in each cluster was 72 (SD=77). Most of them are located in eastern zones close to the endemic areas borders.

**Conclusion:** Results presented here identify risk areas and the behavior of ASF in EU, this information will help to focus preventive and control measures of ASF in the EU.

20.104 Effectiveness of alternative control measures for foot-and-mouth disease in swine farms in the USA estimated using simulation modeling

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**Purpose:** Conventional strategies for controlling foot-and-mouth disease (FMD) epidemics are based on the implementation of three control zones, referred to as infected, buffer, and surveillance zones, which are imposed around infected premises. In those areas, surveillance activities and movement restrictions are selectively applied. The U.S. has not suffered an FMD epidemic since 1929. Here, the impacts of alternative sizes of the three control areas on the duration and size of hypothetical FMD epidemics affecting the U.S. swine industry were estimated.

**Methods & Materials:** FMD epidemics were simulated in three distinct U.S. regions encompassing 10,468 swine farms. A stochastic spatial model (InterSpread+) was used to simulate four scenarios representing baseline (S1) and three alternative variations (S2: -20%; S3: +20%; S4: +50%) of control area radii. For each scenario, 1800 simulations were run. Baseline conditions (S1) reflected current USDA guidelines for infected (3km), buffer (10km), and surveillance (20km) zones. The model was parameterized using peer-reviewed literature, expert opinion, and USDA and industry data. The predicted mean number of infected farms (PNIF) and the mean duration of the epidemic (MDE) were compared between scenarios using a Wilcoxon test.

**Results:** For the baseline scenario, the PNIF and MDE were 183 premises (IC 95% 177.67-188.33) and 152.13 days (149.31-154.95), respectively. S2 significantly (p<0.05) increased the PNIF (248%) and MDE (43%), whereas S3 and S4 significantly (p<0.05) reduced the PNIF (50% and 76%) and MDE (39% and 80%). Most of the PNIF (67%, 74%, 65% and 53% for S1-4, respectively) were located outside of the control zone

**Conclusion:** Results provide quantitative knowledge on the influence that variation of control zone radii may have on the predicted size and duration of an FMD epidemic in the U.S. swine industry. Model outputs will help to inform decisions in the face of a hypothetical FMD epidemic in the country.

20.105 Development and parameterisation of a between-flock model to assess the utility of a risk-based surveillance scenario for the early detection of low pathogenic avian influenza

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**Purpose:** Mutation of avian influenza (AI) to its highly pathogenic form can cause significant illness and death amongst poultry flocks. Early detection of low pathogenic AI (LPAI) could therefore avoid high levels of morbidity and mortality, prevent economic losses and trade restrictions, and significantly reduce the onward spread of disease. Current surveillance requires owners to report signs of infection, however, symptoms of LPAI are typically mild, and suspicion of disease may not occur until weeks after the infection is introduced. This study aims to assess the performance of a risk-based surveillance scenario, whereby AI is automatically suspected if thresholds for mortality and/or a drop in egg production are exceeded.

**Methods & Materials:** A spatial network model to describe the spread of LPAI across UK poultry holdings has been developed. The model uses a deterministic compartmental model to spread disease within an infected holding, and incorporates expert opinion on the frequency of visits between potential drivers of disease to simulate spread between holdings. To parameterise the within-flock model we applied a novel approach, Approximate Bayesian
Computation (ABC), which bypasses the need for an expression for the likelihood function by utilising the within-flock model.

**Results**: We illustrate use of the ABC approach by estimating the key parameters that describe the within-flock spread of LPAI using data from two separate AI outbreaks. We also show the effectiveness of the approach in characterizing uncertainty by using it to determine the probability distributions describing our confidence in each of the parameters of interest. Using these parameter estimates to inform our between-holding model, we compare the performance of current surveillance with and without a complementary risk-based surveillance scenario for two strains of LPAI.

**Conclusion**: The results of this study indicate that the inclusion of risk-based surveillance could improve the early detection of LPAI. This would provide an objective means for suspicion of AI and reduce the risk of a mutation of LPAI to its highly pathogenic form, thereby avoiding devastatingly high levels of morbidity and mortality, the implementation of statutory disease control measures and considerable economic losses.

**20.106 Google trend tool as a predictor of Chikungunya and Zika epidemic in a environment with little epidemiological data, a Venezuelan case**

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**Purpose**: The aim of this paper is to analyze Google trend as a tool in a environment where no epidemic or epidemiological data is available in real time.

**Methods & Materials**: Google trend tool was fitted to Venezuela case regarding to Chikungunya and Zika outbreaks, this tool extrapolates the search results based on keywords, "Chikungunya" and "Zika", Data and curves obtained from the Google trend tool was compared against the data and curve of “febrile cases” definition (regular monitoring strategy) from Venezuelan Ministry Of Health. “Febrile cases” according to ministry of health are those cases without other specific or clinical clear diagnosis as Dengue, measles, pneumonia, diarrhea, etc.; our main assumption is that the extra number of “febrile cases” are related to the epidemic. Using graphic epidemic curves in consecutive epidemiological weeks from May 2014 to December 2014 for Chikungunya and January to April 2016 to Zika. Starting date of the outbreaks, duration of the plateau phase and date to ending of cases were evaluated in both events. Pearson’s correlation coefficient was performed in both cases. Significance criteria were 0.05. STATA 11.0 was used as statistical package.

**Results**: A high correlation between the curves obtained by Google trend and epidemic curves Ministry of Health (febrile cases) was observed 0.89 (0.05) Pearson correlation coefficient. Starting time, duration and decline of epidemic curve are well correlated. Magnitude in terms of number of cases needs to be fitted to strong data (as confirmed cases, no available data so far). National events as Holidays (Christmas) or Political events (elections) could affect the curves related to behavioral changes search patterns, also Internet penetration in rural areas.

**Conclusion**: The Google trend tool can be useful where epidemiological information is not available on a regular basis, has a high ability to forecast the timing of the epidemic, the relative magnitude and duration of the outbreaks, it’s free and available to all who can use the information, also can predict the shape of the outbreak in “real time” fashion which its almost impossible for regular epidemiological data that takes week/months to be available.

**20.107 Empirical estimation of the incubation period for hand-foot-and-mouth disease from school outbreaks**


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**Purpose**: Outbreaks of hand-foot-and-mouth disease (HFMD) occurred frequently in Asia and occasionally in Europe and the US. The incubation period, defined as the time elapsed from infection to symptom onset, is important to guide disease control and prevention. The incubation period of HFMD is commonly described as 3–7 days but was supported by limited quantitative evidence. In this study we estimated the incubation period of HFMD from school outbreaks in Hong Kong.

**Methods & Materials**: In 2015-2016, we recruited 24 kindergartens, 3 primary and 8 secondary schools which reported to have HFMD cases. We collected information on the dates of symptom onset and absence for the diagnosed HFMD cases, and potential epidemiological link to other HFMD cases in household.
We extracted classes with ≥2 HFMD cases for the analysis. The distribution of incubation period was estimated allowing for interval censoring. We assumed the HFMD cases were infectious after symptom onset. For each HFMD case, the possible exposure period for other students in the same class was defined from the day of symptom onset to the school day before taking sick leave. The time differences between the exposure period to all potential infectors and the symptom onset day of the subsequent HFMD cases were used to estimate the incubation period. We fitted log-normal, gamma and Weibull distributions by maximum likelihood method and selected the best-fitted distribution by Akaike information criterion. We estimated the median, 5% and 95% percentiles and computed the relevant 95% confidence interval by bootstrap method.

**Results:** Among the 35 schools, 105 cases from 29 classes in 19 schools were included for analysis. Weibull distribution was the best-fitted model with an estimated median incubation period of 4.5 (95% CI: 4.1-5.1) days. The estimated 5% and 95% percentiles were 0.6 and 14.3 days respectively.

**Conclusion:** The median incubation period of HFMD is consistent to the range of 3–7 days commonly cited by different health agencies. However, the incubation period can be more variable and not uncommon to be longer than a week. The finding may have implications on the recommendation of disease control in a school setting.

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Outbreak of Suspected Pertussis in Kaltungo, Gombe State, Northern Nigeria, 2015: The Role of Sub-optimum Routine Immunization Coverage

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**Purpose:** Although pertussis is a vaccine preventable disease, outbreaks still occur in vaccinated populations. Despite the availability of vaccines, pertussis outbreaks still occur in developing countries. In December 2015 we investigated pertussis outbreak in Kaltungo to identify determinants of infection and institute control measures

**Methods & Materials:** We enrolled 155 cases and 310 controls in an unmatched case-control study. Residents of Kaltungo with paroxysmal or a whooping cough lasting 2 weeks with or without vomiting were cases, while randomly selected neighbours without paroxysmal or a whooping cough were controls. Using structured questionnaire, we collected data on socio-demographic, clinical and risk factors and analyzed using Epi-info7. We collected twelve Nasopharyngeal swabs and submitted to a reference laboratory using Polymerase chain reaction

**Results:** We enrolled 155 cases and 310 controls in an unmatched case-control study. Residents of Kaltungo with paroxysmal or a whooping cough lasting 2 weeks with or without vomiting were cases, while randomly selected neighbours without paroxysmal or a whooping cough were controls. Using structured questionnaire, we collected data on socio-demographic, clinical and risk factors and analyzed using Epi-info7. We collected twelve Nasopharyngeal swabs and submitted to a reference laboratory using Polymerase chain reaction

Bivariate analysis revealed contact with a case (Odds Ratio (OR)=8.8; 95% Confidence Interval (CI)=5.4-14.3), parental refusal (OR=20.2; CI=10.2-29.8), mothers with informal education (OR=5.4; CI=3.4-8.6) and being a Muslim (OR=3.9, CI=2.5-6.0) were more likely to have pertussis infection, while receiving pentax (OR=0.3, CI=0.2-0.4) was protective. Multivariate analysis revealed parental refusal (adjusted OR=27.8; CI=8.8-87.7), contact with a case (AOR=7.9, CI=4.3-14.7, P=0.000), belonging to the Muslim faith (AOR=2.0; CI=1.1-3.5) and having mothers with informal education only (AOR=4.7, CI=2.6-8.4) as independent predictors of pertussis infection.

**Conclusion:** Sub-optimal vaccination due to parental refusal and informal education were major determinants of pertussis infection. We conducted awareness campaigns of key immunization messages targeted at the informal education sector. We mobilized resources for case management, contact vaccination and health education in public gatherings, worship places, and schools.
Evaluation of the performance of randomly amplified polymorphic DNA (RAPD) method for microbiological typing in an invasive Serratia marcescens NICU outbreak


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Purpose: Serratia marcescens is a common pathogen in the hospital environment and colonizes patients. It is a serious cause of nosocomial outbreaks of invasive infections. There is limited experience using polymerase chain reaction (PCR)-based randomly amplified polymorphic DNA (RAPD) method for typing of S. marcescens isolates for timely investigation of an outbreak. We sought to evaluate the performance of PCR-RAPD method using different primer sets compared to a reference method in the context of a neonatal intensive care unit (NICU) outbreak of invasive S. marcescens.

Methods & Materials: Stored isolates of S. marcescens and cluster-related patient isolates over 2 months, were sub-cultured and genomic DNA was extracted. Blinded PCR amplification of each isolate DNA was performed using 4 primer sets, 2 of which were previously published primers for fingerprinting S. marcescens and 2 RAPD primers which were tested for the first time (272 P. aeruginosa and 270 B. cepacia primer sets). The PCR products were run on agarose gels, and the band patterns were compared visually and with the aid of computer analysis. Then the results were compared to those obtained from the traditional typing methods of pulse field gel electrophoresis (PFGE).

Results: Overall, 4 blood isolates, 4 non-invasive samples, a control S. marcescens and a control Pseudomonas aeruginosa were included. All the invasive outbreak isolates (4 blood cultures from 3 different patients) were found to harbor identical S. marcescens strains using PFGE and RAPD typing methods, and they clustered separately from non-invasive isolates. Of the 4 primer sets used, the 272 P. aeruginosa primer gave the highest discrimination and the most number of bands allowing for accurate clustering of for the S. marcescens isolates. Results for RAPD were available within 24 hours from testing initiation and were replicable.

Conclusion: In a small invasive S. marcescens outbreak in the NICU, typing by PCR-RAPD using the 272 P. aeruginosa primer set performed well compared to the reference method. A particular attraction of this method is that it is economic and provides rapid results to inform infection control measures in real time. The use of PCR-RAPD could be a reasonable option for outbreak investigations of S. marcescens.

Quantifying the risk of nosocomial infection within Ebola Holding Units: A retrospective cohort study of negative patients discharged from five Ebola holding units in Western Area, Sierra Leone

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Purpose: A central pillar in the response to the 2014 Ebola Virus Disease (EVD) epidemic in Sierra Leone was the role of Ebola Holding Units (EHUs). These units isolated patients meeting a suspect case definition, tested them for EVD, initiated appropriate early treatment, and discharged negative patients to onward inpatient care or home. Positive patients were referred to Ebola Treatment Centres. We aimed to estimate the risk of nosocomial transmission within these EHUs.

Methods & Materials: We followed up a cohort of 543 patients discharged with a negative EVD-test from five EHUs in the Western Area, Sierra Leone, and examined all line-listed subsequent EVD-tests from any facility in the Western Area to see if the patient was re-tested within 30 days, matching by name, age and address. We defined possible readmissions as...
having the same name and age but uncertain address, and confirmed readmissions where name, age and address matched.

**Results:** We found a positive readmission rate of 3.3% (18 cases), which included 1.5% confirmed readmissions (8 cases) and 1.8% possible readmissions (10 cases). This is lower than rates previously reported. We cannot ascertain if EVD was acquired within the EHUs or from re-exposure in the community. No demographic or clinical variables were identified as risk factors for positive readmission, likely due to our small sample size.

**Conclusion:** These findings support the EHU model as a safe method for isolation of suspect EVD patients and their role in limiting the spread of EVD.

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**20.111 Outbreak of measles in children of a remote district of Afar region, Eastern Ethiopia**

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**Purpose:** Measles is an acute viral illness caused by a virus in the family paramyxovirus, genus Morbillivirus. On February 1, 2016 an outbreak of measles was reported by a remote district in the eastern part of Ethiopia. The investigation team was formulated to assess the distribution and associated risk factors of Measles outbreak in the district, so as to implement control measures.

**Methods & Materials:** We applied a descriptive analysis of the collected line list of cases followed by age matched case control study with a case to control ratio of 1:2, in order to identify the possible risk factors of the outbreak.

**Results:** We identified 21 cases of Measles from which the first five cases were laboratory confirmed using the conventional PCR method and the remaining are epidemiologically linked making the overall attack rate of 44.7 per 100,000 populations. Most of the cases were female (66.6%) and the maximum and the minimum age was 1 and 14 year respectively with a mean age of 4.7 year (S.d=3.7). Most of the caregivers (85.7%) were illiterate and housewife with an average house hold size of 9 person per household. 81% of the cases were not vaccinated for measles and the average measles vaccine does taken by the remaining vaccinated cases were one does. On multivariate analysis using the logistic regression model vaccination status of case AOR=0.16 (0.02-0.96) and contact history with person with similar symptoms AOR= 46.2 (6.6-532.2) were statically associated with probability of acquiring the disease. The outbreak was controlled by implementing mass vaccination and health education activities and no death was reported.

**Conclusion:** We have confirmed the incidence of measles outbreak in Adilala Kebele of Telalake Woreda of Afar region, the major risk factors being accumulation of susceptible unimmunized children and contact with infected person. We recommend to strengthen health education and the routine and catch up immunization programs activities by the district health office to prevent similar outbreaks.

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**20.112 Measles outbreak investigation in Darolabu district of oromia region from Ethiopia 2014**

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**Purpose:** From 31 December 2013 to 17 February 2014 a series of measles outbreaks identified in rural Kebeles of the Darolabu District from West Hararghe Zone in Oromia, Ethiopia. These outbreaks raised questions regarding the effectiveness of immunization programs and other risk factors associated to the disease. The purpose of this investigation was to assess the most important factors leading to these outbreaks.

**Methods & Materials:** During the study period, the investigator analyzed surveillance data and performed a case-control study. A case was any person in Darolabu District who developed fever and maculopapular generalized rash and cough, coryza or conjunctivitis (red eyes). A control was a person resident of Darolabu District without such diagnosis during the same period. To confirm cases, five samples taken and IgM test performed. We interviewed study participants by using structured questionnaire and data was analysed by using epipinfo version 7.1.

**Results:** A total number of reported cases during the study period was 96, male 57 and Female 39. In total 74 (77%) were below 14 years, from which 54 (73%) was children below 5 years old. The overall attack rate was 1.5 per 1000 population with variation among kebeles. Variables found to have a significantly positive association with Measles cases in the
Factors associated with acquisition of glycopeptide-resistant enterococci during a single-strain outbreak

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Purpose: The spread of glycopeptide-resistant enterococci (GRE) is a worldwide public health problem. In France, GRE remains an emerging micro-organism certainly due to recommendations published from the first outbreaks. Many researches studied the risk factors for colonization with GRE but few studies tried to highlight the risk factors for GRE acquisition via cross-transmission. This implies the study of a single-strain outbreak. The aim of our study was to investigate the factors associated with GRE acquisition during a hospital outbreak.

Methods & Materials: We designed a single-center case-control study (1:4) from a vanB E. faecium outbreak (genetically identical strains) which occurred from September 2013 to January 2014 in University Hospital of Strasbourg. Analyzes were performed using Bayesian methods. Univariate logistic regressions with informative priors from anterior studies (Servais, 2009; MacIntyre, 2001; McEvoy, 2006; Karki, 2012; Hoshuyama, 2007) were realized. A multivariate model was build including variables with a probability of odd-ratio exceeding 1 (Pr) greater than 85% or less than 15%. Sensitivity analysis with non-informative priors was performed.

Results: Sixty-five patients were included (13 cases and 52 controls). After univariate analyzes, 10 variables were included in the multivariable model (with Pr ≥ 85% or Pr ≤15%): diabetes, immunosuppression, age, antibiotics within the 3 months and during hospitalization, dependency, antacids, bedpan/adult nappy/stoma, number of hospitalization days in the year and isolation measures. After multivariate analysis, only antibiotics during hospitalization (OR=3.4 IC95% [1.3-9.5], Pr=99.2%), number of hospitalization days in the year (OR=1.01 IC95% [1.00-1.03], Pr=95.4%), diabetes (OR=2.0 IC95% [0.8-5.1], Pr=92.1%), immunosuppression (OR=0.1 IC95% [0.0-1.3], Pr=4.1%) and isolation measures (OR=0.1 IC95% [0.0-0.6], Pr=0.6%) were associated with GRE acquisition.

Conclusion: We highlighted that history of hospitalizations, taking antibiotics and isolation measures had an effect on the GRE acquisition during this hospital outbreak. The use of Bayesian statistics was useful for our study because of small population size and available prior information. Cross-transmission factors were difficult to collect, therefore it would be interesting to make prospective studies and search other cross-transmission factors such as room, medical devices or nursing staff sharing.

Outbreak Investigation of Measles in Swati Mohalla Landhi, April 2015

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Purpose: On 29th March 2015, five cases of measles were reported from National Institute of Child Health in Karachi. An outbreak investigation was initiated through FELTP and local health department to identify the risk factors and propose control measures.

Methods & Materials: The investigation was carried out from 29th March to 31st April 2015. A case was defined as “any child <15 years of age, resident of Swati mohalla, presenting with fever, generalized maculopapular rash with one or more symptoms: Coryza, cough or conjunctivitis with onset of symptoms from 29th March to 31st April 2015. A case-control study was conducted. Active case finding was carried out and age and sex-matched controls were chosen from the same community. Blood samples from four cases were sent for laboratory analysis.

Results: A total of 450 children <15 years of age, from 259 households in the neighborhood were surveyed. A total of 22 cases and 44 controls were identified. Mean age was 30.8 months +23.8 (range 7-120 months) with an equal male-to-female ratio. Complications of measles were found in seven (31.8%) cases; 5 with pneumonia & two with diarrhea. Among
cases, seven (31.8%) were fully immunized, 13 (59.09%) were unvaccinated and two (9%) were partially vaccinated. Vaccine efficacy was calculated to be 53.3%. Out of 22 cases, 13 were found to be unvaccinated and at risk to develop measles (OR 11.89, 95% CI 2.72-56.60, p-value 0.001). All four blood samples were positive for measles.

**Conclusion:** Poor vaccination coverage was the most likely cause of this outbreak. Mop up vaccination activity was carried out in all affected and adjoining areas. Vaccine coverage should be improved to prevent future outbreaks.

20.116 Ciguatera outbreaks in Germany caused by imported tropical fish

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**Purpose:** Ciguatera is the most common fish poisoning worldwide caused by ciguatoxic tropical reef fish. This foodborne disease is characterized by a combination of gastrointestinal and neurologic symptoms. The occurrence of cold allodynia is pathognomonic for ciguatera. Severe symptoms may occur during the acute disease phase. Neurologic symptoms may persist several months.

Due to climatic changes, ciguatoxin producing dinoflagellates now occur in subtropical coastal areas. In the Canary Islands, for example, ciguatera has been endemic for about 10 years. Dinoflagellates have also recently been found in the Mediterranean Sea. Due to the rising volume of imported tropical fish, the number of ciguatera cases is also growing in temperate climate zones.

In November 2012, the first ciguatera outbreak occurred in Germany. Further outbreaks occurred in Germany during the following years. In Germany, there is no notification system for ciguatera.

**Methods & Materials:** The cases of the outbreaks should have been retrieved from different data sources and were registered pivotally at the BfR. The cases of the first outbreak 2012 have been investigated by a clinical 3-year follow-up.

**Results:** In 2012, more than 20 persons were affected. Two cases showed severe symptoms. Neurologic symptoms of 2 other patients persisted nearly 3 years. At least, 20 further cases could be avoided by the product recall of the importer. In 2013 - 2015, outbreaks with 1, 6 and 16 cases respectively occurred in Germany. All cases were caused by tropical fish caught in India during the same stormy season.

**Conclusion:** The ascertainment of cases and the outbreak investigations proved to be difficult due to inconsistent case reports to poisons centers, local health and veterinary authorities. In Germany, many physicians are unaware of the disease pattern of ciguatera and risks caused by tropical fish. Physicians of the poisoning centres played an active role in the elucidation of the outbreak. The occurrence of further outbreaks during the consecutive years emphasizes the increasing significance of ciguatera in Germany. The BfR is an active member of the EFSA-project for risk assessment of ciguatera in Europe. Cases of ciguatera occurring in Germany may be reported to 32@bfr.bund.de

20.117 Fourth Dengue Fever Outbreak in Ethiopia, July 2015: Investigation of Risk Factors

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**Purpose:** Dengue Fever (DF) is a rapidly spreading vector-borne viral disease. Globally, estimated 50-200 million cases and 20,000 deaths occur annually. In Ethiopia, DF is an emerging health problem since 2013 when the first outbreak reported in Dire Dawa city. Subsequently, outbreaks occurred in Afar and Somali regions in 2014. Because of increment report of acute febrile illnesses and one confirmed DF case from Goday town, we investigated the outbreak to identify the risk factors and strengthen control measures.

**Methods & Materials:** We conducted a descriptive study followed by a case-control study in Goday town, Ethiopia from 8-22 July 2015. Cases were defined, according to the WHO guideline, as any person living in Goday town who presented with fever and any three of headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, abdominal pain and laboratory confirmation by PCR/RDT; the controls for the study were individuals with none of the case-defining signs and symptoms and living in the same town. We recruited 50 cases and 100 controls in the study. Data were collected at household level using structured questionnaires and analyzed using SPSS 20 software.

**Results:** We identified 223 cases with no death of which 116 (52%) were male. The mean age of cases was 25.8 years. Ten cases were positive for DF by PCR at national laboratory. Lack of formal education (AOR=3.1 [95% CI: 1.30-7.49]), living with ill person (AOR=2.8 [95%
Purpose: Lassa Fever (LF), endemic in Liberia, is difficult to distinguish from other viral haemorrhagic fevers such as EVD and other diseases that cause fever. Definitive diagnosis requires laboratory confirmation not widely available in relevant settings. Here we report the significant challenges encountered when mounting an effective response to a Lassa fever outbreak in a post-EVD resource limited setting, Liberia 2016

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Methods & Materials: A rapid response team was deployed to Bong County in February 2016 in response to a cluster of three LF cases. Case investigation, contact tracing and community education and awareness raising were undertaken. Specimens were tested for EVD in-country and the referral system to the regional laboratory in Sierra Leone for LF testing was re-established. Previously notified LF cases were reviewed to determine the size of the outbreak and epidemiological links.

Results: In 2016 to date 54 suspected LF cases have been reported through IDSR across four counties. All cases tested negative for EVD. 39 samples were sent for LF testing, 7 (18%) were confirmed (RT-PCR or ELISA Antigen), 8 (21%) were in convalescent stage (ELISA Antibody) and 24 (61%) were not a case. A lack of response interventions to early cases were identified.

Conclusion: The delay in response to this outbreak can be related to a number of challenges in the post-EVD setting. There is a need to strengthen the IDSR system, develop preparedness plans, train rapid response teams including specific response activities for epidemic prone diseases and build laboratory capacity to test in country. Prioritising these actions will aid timely response to future outbreaks.

Purpose: There is a lack of empiric evidence and global standard for selection and use of personal protective equipment (PPE) for highly infectious diseases. Using human factors methodologies, we identified contamination issues during donning and doffing of common PPE in order to inform PPE procurement criteria and design.

Methods & Materials: The study took place between October and December 2014 in four academic health sciences centers (2 adult, 1 pediatric and 1 trauma center) in Toronto, Canada. Participants (n = 82) were representative of potential users of PPE within Western hospitals and included the roles of primary health care provider, assistant for donning and doffing, and (in some centers) trained observer. Trials included completion of a task-based scenario by the primary, followed by administration of a questionnaire to all participants. A mixed methods approach was used. Usability testing assessed the appropriateness, potential for errors and ease of use of various combinations of PPE. A qualitative constructivist approach was used to analyze participant feedback.

Results: Seven combinations of PPE were tested in the 4 environments. PPE tested included: surgical gowns; one-piece coveralls (varied across centers); 2 types of apron; shoe covers; rubber boots; surgical gloves; nitrile gloves with extended cuff; N95 respirators; face shield; bouffant; safety goggles and glasses; surgical hoods with ties; shroud hoods; and, powered air-purifying respirator (PAPR) systems. Not one of the tested combinations provided a complete solution for PPE. Twenty-three PPE design flaws were identified, including multiple problems with doffing and disposal of PAPRs, one-piece coveralls and foot
protection. Environmental factors, such as anteroom layout, and the design of protocols and instructional material were also found to impact safety. The study identified the need to design PPE as a complete system, rather than mixing and matching components.

**Conclusion:** Healthcare organizations are encouraged to use human factors methods to identify risk and failure points with the usage of their selected PPE, and to modify on the basis of iterative evaluations with representative end users. Manufacturers of PPE should consider usability when designing the next generation of PPE.

20.120 Outbreak investigation of Dengue fever in cases reporting to a tertiary care hospital in District Rawalpindi, Pakistan-September 2015

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**Purpose:** During August 2015, an unusual increase (7 cases / day) indengue fever cases was reported from a tertiary care facility in District Rawalpindi, designated as the main referral center for management of dengue cases by Health Department. An outbreak investigation was conducted to evaluate the risk factors and recommend preventive measures.

**Methods & Materials:** A suspected case was any resident of District Rawalpindi presenting with an acute febrile illness with or without retro-orbital pain, headache, rash, myalgia, arthralgia, hemorrhagic manifestations, during 29 Aug-30 Oct 2015. A confirmed case was identified as any person testing positive for dengue IgM on ELISA. Age and sex-matched population-based controls were selected. DEN 1-4 serotype identification was carried out using RT-PCR.

**Results:** A total of 659 suspected cases were identified out of which 373 were confirmed. Mean age was 36 years (range 10-69 years) with a male to female ratio of 3:1. Age group of 21-30 years was most severely affected (n=151 AR 0.04%). Two deaths were reported (CFR 0.53%). Serotype identification for 322 cases was carried out. DEN-2 (n=126; 39%) was the most prevalent serotype followed by DEN-3 (n=96; 30%), DEN-4 (n=58; 18%) and DEN-1 (n=42; 13%). Out of 373 cases, 153 (41%) stored water in open containers (aOR 2.35, CI 1.71-3.24, P value 0.000). Use of mosquito repellents (aOR 0.128, CI .07-0.21, P value 0.000) was found to be protective.

**Conclusion:** Mosquitos breeding in stored water in open containers inside the house was the most probable cause of the outbreak. Vector control measures and health education activities were initiated regarding use of repellents and removal of indoor uncovered stored water.

20.121 Differential serological and molecular arbovirus testing of Yellow Fever suspected blood specimens in Uganda 2016

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**Purpose:** To investigate and confirm arbovirus natural infections using serological and molecular methods and rule out cross reactions due to virus relatedness

**Methods & Materials:** Between 1st April to 26th July 2010, 131 blood samples were sent and tested at the arbovirus laboratory at UVRI for the presence of immunoglobulin M (IgM) antibodies against West Nile Virus (WNV), Dengue virus (DENV), Chikungunya virus (CHIKV), and Zika virus (ZIKAV) using IgM capture ELISA. The serum samples were also subjected to RT PCR in order to detect pathogen RNA for the listed viruses including O’nyong nyong (ONN).

**Results:** A total of 8 samples were seropositive for YF. 2 were ZIKA positive, and 7 were CHIK positive. Seropositivity for more than one agent was detected in 2 samples. IgM antibody cross reaction was observed between YF and ZIKA. In addition, 8 samples were found to be positive for YF viral RNA; 2 for DEN viral RNA using real-time RT PCR. All these harbored single infections by RT PCR. No viral RNA was detected for WN, CHIK, ZIKA, SFV and ONN.

**Conclusion:** Positive YF IgM and PCR results confirmed a natural infection and outbreak of yellow fever in Uganda. However, a differential Plaque Reduction Neutralization Test (PRNT) will confirm an infection between alpha- (CHIK and ONN) and flaviviruses and rule out cross reaction between these viruses.

20.122 Ongoing vaccine-derived poliovirus type 1 (cVDPV1) outbreak - Lao PDR 2015

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**Purpose:** Bolikhamxay is one of provinces in Lao PDR that reported low coverage of Oral Polio Vaccine (OPV). On 6 October 2015, the Laos Government and WHO were notified of a case of circulating vaccine derived poliovirus type 1 (cVDPV1) from the National Institute for Infectious Diseases (NIID) in Japan. This study aims to describe on going cVDPV1 outbreak in Lao PDR.

**Methods & Materials:** We enhanced Acute Flaccid Paralysis (AFP) active case finding in affected and all provinces in Lao PDR, collected 2 and 3-5 stool samples from the AFP case and close contact for each AFP case, to identify VDPV strain by CODEHOP RT-PCR at NIID, Japan. Immediate interventions such as OPV campaigns and social mobilization have been implemented to prevent disease transmission.

**Results:** A total of 11 out of 87 AFP cases have been confirmed for cVDPV1 in Bolikhmaxay (3 cases), Xaysomboun (4 cases) and Vientiane province (4 cases) since 8 October 2015 till 7 July 2016. Two of the positive cases have died with Case Fatality Rate (CFR) was 2.29%. Twenty five out of 384 stool samples of close contacts were also positive for cVDPV1 in those 3 provinces. Six rounds of OPV campaign and social mobilization were implemented nationwide to reduce the case morbidity and mortality in the country.

**Conclusion:** This cVDPV1 outbreak is the biggest outbreak after Lao PDR is free from polio since 2000. This reminds Ministry of Health Lao PDR continuing strength routine polio vaccination coverage, and routine AFP surveillance should be enhanced for early detection and rapid response.

20.123 Outbreak of Crimean Congo Hemorrhagic Fever in a butcher family at Havaillian Abbottabad Pakistan, September 2014

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**Purpose:** Congo-Crimean Hemorrhagic Fever (CCHF) transmitted by tick-bite or exposure to blood of infested animals or infected person. Since identification of the first case in Pakistan in 1976, sporadic cases have continued to occur. Animal movements before Eid-ul-Adha increasing propagation of ticks & disease transmission risk. A case was reported on Sept-9, 2014 and investigation started on Sept-11, 2014. objectives of the investigation were: To identify risk factors and control the outbreak To prevent disease transmission during Eid-ul-Adha

**Methods & Materials:** Using descriptive study design, case definition used for identification of active cases/contacts was “a person from Hawaiian/hospital staff who remained in contact with a confirmed case or livestock and febrile for 14 days or less with hemorrhagic manifestations”. Samples were tested using RT-PCR. Patients record, previous health and livestock data reviewed, hospitals staff and relatives were interviewed using pretested questionnaire.

**Results:** All four fatal cases from Abbottabad district; father and three sons, were butchers, with common symptoms; fever, hemorrhage, lower limbs paralysis and altered consciousness. Timeline of events showed common source infection. All identified contacts, 18 family members and 19 hospital staff, remained asymptomatic except four who developed fever; however their samples turned negative. Previous data revealed that disease is endemic in the district and record of livestock departments confirmed the presence of ticks in animals.

**Conclusion:** Animal handlers are at risk of acquiring infection due to poor knowledge. Strengthening surveillance, launching awareness campaign and sensitization of health staff is recommended.

In response of the study recommendation, preventive measures were taken by relevant stakeholders to control the outbreak and media campaign was launched to minimize the panic in the area due to deaths of four adults in a family and to reduce the chances of transmission due to gross animal movements before "Eidul Azha".
Purpose: Ebola viral disease (EVD) outbreak 2014 in West Africa declared as Public Health Emergency of International Concern (PHEIC) according to IHR (2005). Sudan as member state committed to plan and respond to PHEIC. Sudan was in a great risk to be affected by EVD due to the previous history of EVD outbreaks in 1976, 1979 and 2004, long open borders with Limited resource and poor infrastructure. In addition to presence of united nation mission in Darfur (UNAMID) coming from all over Africa. Research Question: What was the level of preparedness and response in Sudan to EVD 2015 within the context of IHR 2005?

Methods & Materials: This descriptive cross-sectional assessment was conducted using; WHO checklist to evaluate Ebola preparedness in different sectors, field visits, on desk review to Ebola committee’s and IHR Focal Point reports, and in-depth interview with stakeholders.

Results: According to the IHR Domains analysis; existing of coordination mechanism at all level and active participation of National Focal Point (NFP) was (92%) Surveillance core capacity (53%), surveillance system was sensitized and capacity building for surveillance officers were achieved however Event and community based surveillance were still lacking behind Response and preparedness core capacity (40%); NFP lead the implementation of the Ebola Preparedness Plan. National response plan in place, command center was identified; border states were classified as high risk states accordingly, re-habilitation of isolation centers were priority, although the Protocols, guidelines and case managements were available, however there is inadequate staff capacity Majority of IHR requirements for ground crossing (POEs) were not fulfilled, although the preparedness at Khartoum International Airport scored (60%) Risk communication core capacity (42%); Distribution of fact sheets, educational messages and awareness raising sessions of different group conducted.

Conclusion: Risk of importation of EVD cases is moderate. Strong political commitment exists to support and enhance preparedness for EVD. The majority of IHR requirements for the points of entry are not fulfilled, urgent coordination mechanize with neighboring countries needs to be activated. Appropriate training for staff in case management and IPC is needed. Risk communication plan should be implemented. Sudan need to Enhance and implement Event base and community based surveillance.

Purpose: Natural disasters have increased by 94% in the last three decades in the West African sub-region. Epidemics represented 40% of these disasters. The sub-region accounts for 20% of world epidemic alerts, because the surveillance system in a number of African countries. Nigeria specifically is riddled with difficulties in providing timely reports of the first cases of epidemic-prone diseases, leading to delayed identification and response. The aim of this study was to Assess the core activities and supportive functions for disease surveillance system in Katsina state, Nigeria.

Methods & Materials: We conducted a retrospective, cross-sectional study among 13 Local Government Areas and 65 health care facilities in Katsina State to assess the core activities and supportive functions for disease surveillance system in Katsina state, Nigeria.

Results: Of the total number of LGAs and health facilities surveyed, shortages of data tools were noticed in 91% and 79% respectively. Only 40% of the LGAs carry out data analysis while same was not conducted at health facility level at all. Surveillance Resources were found to be adequate at state level but grossly inadequate at both LGA and health facility levels. There was no epidemic preparedness and response plan, budget line and emergency stocks of drugs and supply at LGAs. Timeliness and completeness of reporting improved across the state between years 2010 – 2013.
Conclusion: The disease surveillance system in Katsina State is weak as evidenced by inadequate resources for its core and supportive functions at LGA and health facility levels. It is recommended that capacity building on data analysis and adequate resources should be made available to make disease surveillance system functional at all levels in Katsina State.


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Purpose: The unprecedented and devastating epidemic of Ebola virus disease (EVD) in West Africa has ignited increasing global concerns. In relation to this outbreak, health facilities especially hospitals are the front liner to detect diagnose and manage EVD suspects. Ethiopia has an airline connections as well a seat for African Union, there has to be strong readiness to defend the existence of EVD. We conducted an assessed to identify the hospitals preparedness towards EVD in order to improve the capacity of early EVD detecting and reporting.

Methods & Materials: We conducted a cross-sectional health facility based survey from January 1-3, 2015. A structured questionnaire was used to interview all (33) public and private hospitals found in Addis Ababa city administration. Frequency and Percentage analysis was made using Epi Info 7.

Results: We obtained a total of 33 Hospitals, of these 13(39.4%) were public and 17(60.6%) were Privates. Eleven (84.6%) have done staff awareness on EVD in public hospitals, and 15 (75%) private hospitals. Of the total assessed hospitals 31(94%) and 25(78.5%) hospitals have functional incinerator and triage units respectively. 24(72.7%) have Ebola case definition, 9(27.3%) have national Ebola interim guideline, 16(48.5%) have Ebola cases screening procedure, 17(51.5%) have isolation rooms, 13(39.4%) and 3(9.1%), isolation rooms were separated from other wards and labeled with safety signage respectively. 15(45.5%), and 6 (18.2%) isolation rooms have bed and specific toilet respectively. 11(33.3%) and 19(57.6%) isolation rooms have separated hand washing and referral systems respectively. 22 (66.7%) have delegated focal person for referring EVD cases to the existing treatment unit and 8(24.2%) were having EVD preparedness and response committee.

Conclusion: We confirm greater than half of assessed hospitals have a good preparedness on emergency triage unit and isolation room for EVD. However, we observed poor infection prevention and control practice, coordination and referral system in some assessed hospital. We recommend the hospital coordination, referral system to the hospital, infection prevention and control to EVD should be improved through continuous gap filling training and supportive supervision.

20.127 Factors associated with cerebrospinal meningitis outbreak in Kebbi State, Nigeria, February 2015

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Purpose: Cerebrospinal Meningitis (CSM) is an epidemic prone disease and remains a major public health challenge in the African meningitis belt. In February 2015, Kebbi State Ministry of Health reported outbreaks in four local government areas (LGAs). We investigated to establish, confirm the epidemic, and assist the state to strengthen surveillance for CSM in the affected LGAs and institute appropriate control measures.

Methods & Materials: We carried out advocacy visit, data collection, active case search, public enlightenment, cerebrospinal fluid (CSF) sample collection and analysis. Using World Health Organisations’ criteria, we defined both suspected and confirmed cases of CSM in time, place and person. Descriptive study was conducted to identify the risk factors of the infection. Univariate and bivariate analyses were performed using Epi-Info 7 Statistical package and Health Mapper software version 4.3. Odds ratio at 95% confidence interval (CI) was used to determine the association between variables. Level of significance was set at p-value ≤0.05.
Results: From Epidemiology week 1 to 7, the state recorded a total of 92 cases with 13 deaths (Case Fatality Rate: 14.13%) in 4 LGAs of the state (Cumulative Attack Rate: 28.4%). Majority (95% of cases) were in age group 6-15 years. Males were more affected (61.3%). While 81.1% of cases shared rooms with >3 persons, 48% slept in rooms that had <2 windows. Only 12.1% of cases had received CSM vaccination in the last 2 years. Aleiro LGA was mostly affected (64%). Of the 29 CSF samples collected from suspected cases, 12 (41%) were confirmed positive for Neisseria meningitides (Serogroup C). Age < 16 years was found to be a significant predictor of contracting CSM (OR: 13; 95%CI: 1.7-104) p< 0.001). The affected LGAs were yet to commence mass vaccination campaign due to unavailability of vaccine for Nm type C.

Conclusion: The outbreak of CSM in Kebbi State was confirmed and the associated risk factors were age <16 years, overcrowding and poor vaccination coverage. We recommended intensified surveillance, continued health education on compliance with vaccination and housing standards, adequate logistics, and mass vaccination campaign.

20.128 Cholera outbreak in Gajala community, Birnin kudu, Jigawa state, Nigeria September 2015

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Purpose: Cholera outbreaks are seasonal in Nigeria with recorded high case fatality rates (CFRs) of 5.1%, 1.9 % and 3.9% in 2010, 2014 and 2015 respectively. An outbreak of cholera was reported in Gajala community, Birnin Kudu Local Government Area, Jigawa State, Nigeria on September 16th, 2015. We conducted an investigation to characterize the outbreak and institute control measures

Methods & Materials: We reviewed surveillance records, conducted active case search and, described the outbreak. Stool samples were collected and examined for *Vibrio cholerae* serogroup 01/0139 using (Smart ⁷th ° II), a rapid diagnostic test kit (RDTK). Water samples were cultured. We carried out an un-matched (1:2) case control study. A case was defined as any person ≥ 2 years from Gajala presenting with acute watery diarrhea (AWD) with or without vomiting from 11th - 25th September, 2015.Similarly, a control defined as any person ≥ 2 years from Gajala not presenting with acute watery diarrhea (AWD) with or without vomiting from 11th - 25th September. We assessed the environemnt by walkthrough observation to cheek for possible food or water contamination. Data analysis was done using Epi info version 3.5.3.

Results: The index case was a commercial bus driver. Overall, 50 cases were identified, median age: 21 years (range: 2-80), more females affected, 27 (54%), overall attack rate: 10.5%. Thirty 30 (60%) of the identified cases presented with severe illness while one died (CFR: 2%). Mean duration of illness before presentation was (17.2 ± 15.2) hours. Stool samples tested positive to *Vibrio cholerae* RDT. Water samples yielded negative results. Overall the 138 respondents in the CCS 46 cases and 92 controls have median age 20 (range: 2-70). Poor knowledge of cholera prevention practices [adjusted odds ratio (aOR) = 2.5, 95% confidence intervals (CI):1.2 - 5.3]], not washing hand before eating [aOR = 2.8, 95%CI (1.2- 6.6)] and not washing hand after using the toilet [aOR= 2.63, 95% CI (1.28-5.9)] .The environmental was untidy and waterlogged.

Conclusion: A confirmed Cholera outbreak occurred in Gajala due to poor knowledge and hygienic practices. We conducted health education on cholera and hand hygiene practice which stopped the outbreak.

20.129 The cost of responding to a waterborne cholera outbreak in a village in Uganda compared to a simple hypothetical intervention

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Purpose: In September 2015, a cholera outbreak occurred in a village in Hoima district, Uganda. The Ministry of Health assembled a rapid response team, with support byCDC, UNICEF and WHO, to investigate the outbreak, establish the Kaiso Cholera Treatment Center (KCTC), and implement control measures. The team identified 120 cholera cases (with 5 deaths) and determined that drinking contaminated water from the lakeshore caused this outbreak.

We sought to determine the cost of investigating and controlling this outbreak, and compare it to a would-be simple preventive measure – constructing deep wells to provide cleaner water.
**Methods & Materials:** We collected cost data, including personnel and material costs at KCTC, health facilities, Hoima district health office, Uganda Public Health Fellowship Program, UNICEF, CDC, and WHO. We defined direct cost of responding to this outbreak as expenditure on medications, medical equipment and supplies, utilities, and allowances and transport for responders; indirect cost included salary and other compensations for responders. We did not include difficult-to-measure costs such as vehicle depreciation, building maintenance, and loss of productivity to case-persons due to illness and deaths. The cost of constructing deep wells was quoted by a U.S.-based NGO.

**Results:** The total cost incurred in investigating and controlling this outbreak was $71,769, including $21,059 in direct cost ($19,225 for allowances and transportation, $1,774 for medical equipment and supplies, and $60 for utilities), and $50,620 in indirect cost (74,484 person-hours of salary and other compensations for responders). Conversely, constructing a deep well to provide cleaner water would cost approximately $2500. Essentially, the total cost incurred in this outbreak would have been enough to construct 28 (≈71769/2500) deep wells; even the direct cost only would have been enough to construct 8 (≈21059/2500) deep wells. One such deep well would have prevented this outbreak and averted future waterborne outbreaks.

**Conclusion:** A simple prevention measure such as constructing deep wells for village residents can be substantially cost-effective for preventing waterborne diseases such as cholera. We recommend that the government should proactively implement prevention measures for waterborne outbreaks whenever possible, instead of passively responding to these outbreaks.

20.130 ISARIC – enhancing the clinical research response to epidemics

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**Purpose:** The International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC) is a network of networks that was established in 2011 by investigators from around the world to ensure a rapid research response to outbreaks of pandemic potential. By bringing together multidisciplinary research groups in more than 110 countries across different resource settings, ISARIC has supported research responses to Ebola in West Africa, MERS-CoV in the Middle East and elsewhere, and more recently, the Zika outbreak in Latin America and the Caribbean.

**Methods & Materials:** ISARIC activities have aimed to fulfill its main objectives, which are to (i) develop standardised and globally accessible research tools, (ii) set up platforms for data collection and data sharing, (iii) establish a primed infrastructure to epidemic responses, and (iv) enable research in resource poor settings.

**Results:** ISARIC is working with WHO and the IDAMS consortium on a Clinical Characterisation Protocol/Natural history protocol for Zika. CRFs for Zika have been harmonised in collaboration with other research networks (PREPARE, REACTing and IDAMS), and adapted by PAHO as part of the harmonisation process for Zika surveillance. ISARIC has established a data collection platform for SARI and VHFVs to maximize the likelihood that data are prospectively and systematically collected, shared rapidly in a format that is easily aggregated, tabulated and analysed across many different settings globally. Now ISARIC is collaborating on a Zika digital image data platform. ISARIC members have developed a standardised ISARIC/WHO Clinical Characterisation Protocol for Severe Emerging Infections; and a global short term incidence study on SARI - SPRINT-SARI (open to recruitment).

Making capacity building a reality with new networks forming around our initiatives e.g. a new East African network being led by Rwanda.

**Conclusion:** ISARIC’s role is bringing many disciplines and networks together to reach a common goal in preparedness and response to emerging/re-emerging infections and continuing to play a facilitator’s role with key partners such as WHO, PAHO, NGOs, IANPHI, local networks and individuals.

20.131 Investigation of a typhoid fever outbreak, City of Johannesburg, South Africa, January – February 2016

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Purpose: Typhoid fever is a serious public health problem in developing countries where it is endemic. We report a suspected outbreak of typhoid fever, following alerts from the National Health Laboratory services (N HLS) notification system. An outbreak response team from the NICD with the City of Johannesburg Health district personnel investigated these cases to determine the source, causative pathogen and to evaluate the contact tracing of these patients.

Methods & Materials: We describe the characteristics, possible source and causes of these typhoid fever cases. Hospital and home visits were conducted, case investigation forms (CIF’s) completed and evaluated, laboratory processing of the specimens, contact tracing and management of these cases.

Results: Nineteen culture-confirmed typhoid cases with one death (case fatality rate 5.3%) were reported systematically across the city of Johannesburg. The patients’ median age was 15 years (range 9 months - 49 years). Majority of the cases (53% [10/19]) were more than 15 years of age, followed by (21% [4/19]) of children aged 5 to 9 years, and children <5-years old respectively (21% [4/19]). Ten (53%) of the index patients traveled within the continent and abroad. CIF’s reported a travel history to Zimbabwe (n=5 [26%]), Malawi (n=1 [5.3%]), Limpopo province (n=2[10.5%]), and Bangladesh (n=1 [5.3%]). Blood and stool specimens tested culture positive for Salmonella enterica serotype Typhi (Salmonella Typhi). One of the 26 contacts traced, a potential chronic Salmonella Typhi carrier from Zimbabwe, is a likely source of infection to two children sharing a house with her. The secondary attack rate was 3.8% (1/26). Blood and stool specimens tested culture positive for Salmonella enterica serotype Typhi (Salmonella Typhi). Pulse field gel electrophoresis (PFGE) of Salmonella Typhi displayed a common profile with the Zimbabwean 2012 outbreak strain.

Conclusion: Typhoid fever is endemic in South Africa; however, we believe that most of these cases occurred as a result of Zimbabwean expatriates returning to South Africa after spending Christmas 2015 in Zimbabwe. Travelers from Zimbabwe need to be given prophylaxis treatment against typhoid upon arrival in South Africa.

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Twenty years surveillance of the re-emerging Mediterranean spotted fever in Bulgaria

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Purpose: In Bulgaria, cases of Mediterranean spotted fever (MSF) caused by *Rickettsia conorii* occurred in two epidemic waves, the first one during 1948–1972, while the second re-emerged more than 20 years later in 1994, and is still going on. The aim of this study was to trace the twenty annual surveillance of the re-emerging MSF in the largest endemic region - Plovdiv city and its suburbs (South-Central Bulgaria).

Methods & Materials: 1361 MSF patients, treated between 1994 and 2013 were enrolled in the study. MSF was confirmed by immunofluorescent assay (IFA) in the Reference Rickettsioses Laboratory. Descriptive and analytic epidemiological methods were used to determine the routes and ways of infection, seasonality, patients' age, gender and social structure.

Results: MSF spread in the region in two distinct phases: first between 1994-2003, the illness being increasingly spreading, and second from 2004 to 2013 the disease being gradually declining. The incidence was between 0.13 and 25.62, mean 10.69 per 100 000 populations (13.06±2.35 and 9.37±1.22 for phases I and II, respectively); between 1.14% and 6.25% of the infected people died (3.46% and 1.19% for phases I and II respect., mean 1.96%) The age distribution showed predominance of 40-59-years old people (31.66%), reaching peak in patients older than 60 years (37.5%). Children and adolescents under 19 years old were less affected (18.12%). Urban population was almost twice as affected as rural, regardless of the gender. The disease had summer seasonality, peaking in August. An eschar (tache noire) was found in 77.91% of the patients. Despite the epidemic decline and predominance of mild forms (43.12%), the re-emerging MSF still presents with lots of severe (11.45%) and malignant forms (8.54%). The later contributed some patients to be at serious risk of getting gravely ill with life threatening complications – encephalitis, brain edema, non-cardiogenic lung swelling, acute respiratory distress syndrome, gastrointestinal bleeding, and acute renal failure.

Conclusion: Almost two decades after it re-emerged in Bulgaria, MSF is still potentially active despite the decreasing incidence and mortality rate. The disease still presents a significant risk to life for ill people and health risk for international travelers.

CCHF Outbreak Investigation Baluchistan 2015

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Purpose: Crimean Congo hemorrhagic fever (CCHF) is a severe hemorrhagic fever with case fatality Rate up to 50%. It is a tick-borne virus belonging to family Bunyaviridae, genus Nairovirus. It is transmitted to humans through bite of infected ticks or by direct contact with viremic animals or humans.. CCHF is endemic in Africa, Balkans, Middle East and Asian countries. It's also prevalent in Pakistan with periodic outbreaks, first case reported in 1976. Right after Eid-ul-Azha, 2015 October cases of CCHF started to be reported from various districts of Balochistan including border of neighboring country Afghanistan.

Methods & Materials: Review of illness history and investigation was carried out using hospital records along with personal interviews with patients, doctors, nurses and Medical Superintendent of hospital. Hospital was visited and Active contact tracing was undertaken among family, friends and staff of Medical ICU.

Results: A total of 20 cases were reported between 1-14 Oct 2015 at isolation ward of Fatima Jinnah hospital Quetta. 16 cases (80%) were from various districts of Baluchistan and 4 (20%) from Qandhar Afghanistan. 17 (85%) cases were male and 3(15%) females. Mean age 32 years with range 14-55 years. 7 deaths reported with CFR 35%. PCR was positive of 12 patients. All cases had history of animal contact (slaughtering, Meat and waste handling).

Conclusion: Contacts traced and followed up. Animal contact is most probable cause of outbreak. Provincial CCHF cell and Rapid Response team was formulated by provincial health department on our recommendations. Close coordination with livestock department to disinfect animals while importing exporting within and outside country. Sessions on animal handling and awareness regarding CCHF at each district level planned and started. At isolation ward PPE and other latest equipment are required urgently.
20.135 Outbreak investigation of cutaneous Leishmaniasis in district Nowshera Pakistan from January to April 2014

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Purpose: On 19th March 2014, newspapers reported about presence of more than 100 Leishmania patients in different parts of district Nowshera, Khyber Pakhtunkhwa. Reportedly, most affected area was Union Council (UC) Dag Ismail Khel located close to the famous Jalozai camp hosting thousands of Internally Displaced Persons. Peoples Primary Health Initiative and Department of Health Offices in Nowshera were contacted for confirmation, quantifying of outbreak and for taking necessary actions to control them.

Methods & Materials: A case was defined as any person of any age group, gender reported to/from health facilities, community of reported UCs of district Nowshera with appearance of one or more skin lesions, typically on uncovered parts of body from 1st January – 30th April, 2014. Descriptive study was carried out, affected areas were visited, hospital records were checked and active case finding was done. Surveillance data was analyzed to identify cases, affected areas and to gather information about risk factors if any.

Results: 224 cases were identified in 11 UCs. 27% (n=60) belonged to Manki Sharif, 19% (n=42) from Kahi, 17% (n=37) from Spin Khak and 14% (n=32) from Dag Ismail Khel; were identified as the most high risk UCs. 69% (n=155) cases belonged to age group of 6-35 years. 52% (n=117) cases were females. 46% (n=102) cases reported in April while 21% (n=46) cases reported in March. All cases (100%) were clinically diagnosed as cutaneous type of leishmaniasis. Frequent history of travel, low socio economic status of the residents, humid climate, sub urban population and presence of high density of rodents in the area were identified as some of the possible risk factors.

Conclusion: Outbreak was controlled. Mapping of the possible vector sites were carried out for future preventive measures. On recommendations, existing surveillance system for Leishmaniasis was strengthened, indoor residual spray and fumigation for vector control carried out, injection glucantime were arranged, bed nets and insect repellants provision were ensured. No new case was reported in six months follow-up period.

20.136 Pertussis outbreak – Saravane, February 2013, Lao PDR

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Purpose: Pertussis is a highly respiratory infection disease caused by bacterium Bordetella pertussis. Pertussis outbreaks had been reported in Savannakhet Province, Lao PDR in 2012 and beginning of 2013. On 4/2/2013, suspected cases of pertussis were reported from Saravane Province. We investigated to confirm the causative agents, the source and to propose recommendations

Methods & Materials: Active case finding was conducted in the affected villages, their neighbors and health facilities by an investigation team. We defined a suspected case as any patient presented with cough with onset after 1/1/2013, and a laboratory confirmed case when anti-pertussis IgA antibodies were detected. We took 10 blood samples for serological testing and performed descriptive analysis using Excel.

Results: We investigated 2,596 persons, of which, forty four were suspected as pertussis and no death with an onset of symptoms between 15/1/2013 and 11/2/2013, attack rate was 1.7%. Cases first detected on 15/1/13, peaked between 24 to 27/1/2013 and started to decline on 11/2/2013. Most affected group was children under 15 years of age (93.1%). Male:Female ratio was 1.3. Eight out of 10 samples were laboratory confirmed cases (80%). The vaccination coverage was 68% in affected district, but none of these cases were received Diphtheria-Tetanus-Pertussis (DTP)-. Vaccination campaign of DTP-HepB-Hib, Vitamin A and erythromycin were adminstered and respiratory hygiene education provided.

Conclusion: This pertussis outbreak mainly affected unvaccinated children. This is a common source of person to person transmission. However, the information of whether imported case or local infection was not available. It is highly recommend that further investigation and routine childhood DTP vaccination need to be strengthened in Laos.
**20.137** Measles outbreak – Northern of Lao PDR, January 2013

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**Purpose:** Measles is an acute disease caused by measles virus that is often fatal in developing countries. Measles is vaccine-preventable disease, which in Lao PDR is given to all children at 9 months of age through the routine immunization program. On 16/1/2013, provincial clinician reported 3 suspected measles adult cases admitted to hospital. The investigation was carried out to describe the source of the outbreak.

**Methods & Materials:** Active case finding was done at the affected villages by the investigation team. We defined a suspected case as any person presented with fever and rash with onset after 1/1/2013, and a laboratory-confirmed case when specific measles IgM was detected. We took samples for serological test by ELISA.

**Results:** A total of 92 suspected cases with no death with the onset of symptoms between 1/1/2013 and 17/2/2013. Attack rate was 13.3%. Most affected age group was adult above 20 years of age (53%) and children <5 (28%). Male:Female ratio was 1.8:1. Eleven out of 34 samples were laboratory confirmed cases (32.3%). The vaccination coverage was 95% for the affected district in 2012. Measles-Rubella Supplementary Immunization Activities (MR-SIA) was conducted in 2011 and some children were receive (3.2%). However none of these cases remembered whether they received measles vaccination. Vitamin A and MR were administered together with health education during the outbreak response.

**Conclusion:** This measles outbreak indicated that majority of the affected cases are under 5 and over 20 years. Immunization coverage was high in target group of 9 months but older children and adult were not immunized. It is highly recommended that immunization strategy and SIA should be targeting to catch up these missed target groups.

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**Purpose:** In order to counter effectively the animal epidemic emergency, it is necessary that the Veterinary Public Health manages risk communication for breeders interested in the area of crisis, with timely, quick and clear information. The purpose of this paper is to describe the model of communication used and the benefits obtained in the control of African Swine Fever.

**Methods & Materials:** Following the African Swine Fever virus confirmation, the Units of Local Crisis locates the outbreak farm among those present in the database prepared by the Animal Health Service which contains updated information of all pig herds in the territory of ASL Sassari (names, addresses and telephone numbers of the owners, geographic coordinates lat / lon of farms, livestock numbers). All data is saved in Comma Separated Values (CSV) and imported on the QGIS software for the creation of a layer of all pig farms. Through geoprocessing function, two buffer zones around the outbreak of fever were created: the protection zone of 3 km and surveillance zone of 10 km. In this areas the farms were selected and through the software SMS_alert procedure were extrapolated the contact information of the farmers and sent, via a GSM modem, SMS text messages containing the recommendations and the health requirements.

**Results:** In the territory of the ASL of Sassari, in 2015, there were 7 outbreak of African Swine Fever (ASF) in domestic pigs that involved 140 pig farms in the protection zones and 765 pig farms in the Surveillance Zone. The messages are promptly arrived to all farmers concerned.

**Conclusion:** The use of the Information System SMS Alert, has fostered a risk communication to farmers effectively and enabled a rapid and immediate action of warning and their sensitization to take the best biosecurity measures to limit the spread of the ASFV. In fact, in 2015, compared with 7 primary outbreaks, there hasn’t been no secondary outbreak of ASF, health indicator of effective action by the Veterinary Public Service, and a good collaborative response by farmers.

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**20.139** Mixed species legionellosis outbreak linked to industrial cooling towers contaminated with multiple *Legionella* species, New Zealand, August-November 2015

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Purpose: Outbreaks of legionellosis associated with industrial cooling towers are typically due to \textit{L. pneumophila} serogroup (sg) 1 infection; sources of \textit{L. longbeachae} infections, by contrast, are usually considered to be gardening or horticultural materials, such as potting mix. We investigated an outbreak of legionellosis centered on a dairy-processing plant in the North Island of New Zealand to identify and control the outbreak source.

Methods & Materials: Cases with possible legionellosis were sought from disease notifications, review of workplace absenteeism and illness records, and through case finding with primary and secondary care clinicians. Possible cases were interviewed for symptoms and risk factors, and tested for legionellosis if consenting. Environmental sources epidemiologically linked to cases were sampled and tested for \textit{Legionella} species.

Results: We identified 13 patients meeting the case definitions for legionellosis, six with Legionnaires' disease and seven with Pontiac fever, with illness onset between 15/08/2015 and 19/11/2015. All except one were site workers. In three cases, \textit{L. pneumophila} sg1 infection was confirmed by culture and isolation or by urinary antigen testing. The remaining 10 cases were diagnosed retrospectively by serologic detection of antibodies to the following \textit{Legionella} species and serogroups (sg): \textit{L. pneumophila} sg1 (1), \textit{L. pneumophila} sg5 (1), \textit{L. longbeachae} sg1 (5), \textit{L. longbeachae} sg2 (1) and \textit{L. sainthelensi} (2). \textit{L. pneumophila} sg1 and \textit{L. longbeachae} sg1 were detected in cooling towers on the site. Comparison between cooling tower and clinical isolates of \textit{L. pneumophila} sg1 using SBT allelic profiles showed 100% DNA sequence homology. Disinfection of the cooling tower contamination and prevention of recurrence was implemented.

Conclusion: The DNA sequencing data provided strong evidence that the illnesses among patients with \textit{L. pneumophila} sg1 infection were likely due to exposures on the site, presumably to aerosols generated by cooling towers. The sources of infection for the remaining nine persons with legionellosis is less clear. The findings of this investigation raise the previously-unreported possibility of \textit{L. longbeachae} transmission from cooling towers, however this was not proven in this outbreak.

Purpose: As part of the outbreak control, the capacity of laboratories to respond rapidly and properly to an emergency was significant on MERS outbreak in Korea, 2015. We investigated the effectiveness of expanded diagnostic laboratories for molecular diagnosis of MERS-CoV and evaluated the status of diagnostic system for future disease outbreak in South Korea.

Methods & Materials: Molecular diagnosis for MERS-CoV initially has performed by the Korea National Institute of health using in-house and customized research-use-only(RUO) version of multiplex realtime RT-PCR targeted \textit{upE} and ORF1a gene based on WHO guideline. A total of 17 Provincial level laboratories(PHERI) were only performed screening test using RUO realtime RT-PCR kit targeted \textit{upE}, and evaluated through a national EQA program. However, with the increase of demand for test, MERS-CoV laboratory network has been continuously expanded. PHERI and 5 private diagnostic centers started to test for screening and confirmation since June 7th, and 40 designated hospitals were only performed screening test by the reason of no reporting using 4 kinds of RUO commercial kits. To assess the quality of molecular diagnostics, external quality assessment (EQA) allowing the laboratories was set out using blinded panel test and confirmatory test. In this study, a retrospective review of laboratory reporting data was carried out of all patients suspected of MERS-CoV during the MERS outbreak.

Results: KNIH reduced the test time using 1 steps of screening and confirmation, simultaneously, instead of 2 steps regarding to WHO algorithm. A total of 44,748 MERS-CoV tests had been performed, and 733 were positive. The number of confirmed cases increased gradually and peaked on the early of June, and MERS virus testing peaked in mid-to-late June(over 1000 test per a day) and waning by July. More than 76% of testing was completed within one day. Most of the specimens(80%) were sputum and lower respiratory specimen.

Conclusion: During the MERS outbreak in Korea, laboratory cooperation and expansion has been well initiated testing massive quantities of specimens from patients suspecting MERS-CoV infection. However, several improvements may allow to more successful cooperation for effective disease control and future research.
"Why didn't you see it coming?": Surveillance by and in the media—journalistic research on outbreak detection taking the example of Ebola Virus Disease in Uganda and West Africa

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Purpose: A package of papers, "Avis de décès 2013/2014": This I found at the radio station in Guéckédou/Guinea, close to the village where the spill-over of the Ebola virus had occurred. When the outbreak was detected in 03/2014, scientists and journalists arrived to trace the transmission chain of the virus. However, I went there to reconstruct the transmission chain of information: who had known what when? I also went to Uganda to see how the country had set up a completely new surveillance system after its first, severest Ebola outbreak.

For me as a science journalist focusing on NTD, Global Health, Health Systems in LMIC, the purpose of this project was double-pronged: To inform the public: Surveillance and IHR are technical terms, difficult to illustrate to lay persons. But it is important and possible to do so. To correct misperceptions: The WHO and others were blamed for taking action too late during the early stages of the outbreak. But to be able to respond to an outbreak, organizations have to know that a potential public health emergency is occurring somewhere.

Methods & Materials: Scientific approach: fieldwork in Uganda, Guinea; methods from social sciences, epidemiology, e.g. interviews with key persons, observational studies; triangulating collected data. Journalistic materials: audio/video recordings, photography.

Results: In retrospect, it became obvious that the EVD outbreak in Guinea could have been detected earlier—if the information chain hadn't been interrupted.

Online multimedia story: 30min, manuscript: bit.ly/1RP0Cq0, pageflow: http://bit.ly/1ITDvMH; both published by the German public radio news channel Deutschlandfunk on the occasion of the second anniversary of the death of "Patient Zero".

Conclusion: Journalists are strategic interlocutors in outbreak detection initiatives and IHR. They can contribute to surveillance structures or adopt a kind of epidemiological role. And sometimes journalists can even help to improve surveillance systems: After my stories were broadcast, I received a call: "The CDC in Guinea had no idea what the CDC in Uganda had done for the surveillance system and how well outbreak detection now works there. The people in Conakry just heard of your work and now reconsider their plans."

Evaluation of ProMED-mail global surveillance capability

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Purpose: A series of research questions were examined: 1) What temporal trends exist in ProMED-mail’s aggregate reporting delay? 2) Which countries are mentioned most/least frequently per capita? 3) Which ProMED feeds are most active?

Methods & Materials: Software was used to analyze posts that appeared on the primary and regional ProMED-mail feeds since ProMED’s inception in 1994 through May 2016. Using heuristics based on ProMED formatting conventions, meta-data like publication dates and source names were extracted from posts and articles were isolated. NLP techniques (e.g., toponym resolution, named entity extraction) were applied to article bodies to identify organizations and places mentioned. Finally, a summary statistics were gathered from the extracted data, and confidence intervals computed from Poisson regression and bootstrap resampling were used to assess statistical significance.

Results: 1. There is evidence of a long term upward trend in the delay between source article publication and the corresponding ProMED reports (“reporting delay”). Before 2005, the median reporting delay was 33 hours (95% CI [32, 34] computed with bootstrap resampling) compared to 44 hours (95% CI [43, 44]) after 2005. Articles in multi-article posts had a median reporting delay of 52 hours while those posted individually had a median delay of 31 hours.
2. Countries in South America, Asia, and Africa are mentioned less frequently in proportion to their populations than those in North America, Europe, and Australia. Uzbekistan has the least number of mentions per capita for an existing country with a population of over ten million.
3. The primary ProMED feed has a post rate almost as high as all the regional feeds combined. The next three most active feeds are the South Asia feed, the Middle East/North
Africa feed, and the Portuguese feed. The mean posts rates for these feeds differed by a statistically significant amount.

**Conclusion:** Various trends within the ProMED-mail dataset were identified. With further investigation, they may reveal opportunities for improving the breadth and timeliness of reports on ProMED-mail. Due to ProMED-mail's position as a prominent data-source in multiple biosurveillance systems, improvements to it could have a multiplicative effect on the field of disease surveillance at large.

20.143 Active syndromic surveillance program of arboviruses in Rio de Janeiro, Brazil


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**Purpose:** Recently, new arbovirus such as Zika virus (ZIKV) and Chikungunya virus (CHIKV) were introduced in Brazil. Despite the clinical relevance, to date there is no syndromic surveillance system focused in arbovirosis in Brazil. Here, we present the initial results of active syndromic surveillance system for arbovirus infections in Rio de Janeiro, Brazil.

**Methods & Materials:** This is a hospital-based prospective multicenter transversal study. Inclusion criteria were: Presence of fever AND/OR exanthema, with maximal duration of 7 days; PLUS: 2 or more nonspecific symptoms OR a clinical suspicion of arbovirus infection. Patients who met inclusion criteria had blood and urine tested for ZIKV, CHIKV and DENV by RT-PCR. Clinical data were collected using REDCap standardized form.

**Results:** From March 14th to May 31th of 2016 (epidemiological weeks 11 to 22) 269 patients were included in the study. Patients were predominantly female (57.7%), with mean age of 41.9 years old. One hundred ninety patients (71.1%) were PCR-positive for at least one of the arbovirus tested, 4 patients were infected simultaneously by 2 arbovirus (3 ZIKV/CHIKV and 1 ZIKV/DENV4 co-infection); 79 patients (29.4%) were PCR-negative. Among PCR-positive samples, 146 (54.3%) were confirmed for CHIKV, 36 (13.4%) were confirmed for ZIKV and 8 (3.0%) were confirmed for DENV (1 DENV3 and 7 DENV4). Over time, dengue cases were distributed between EW 11 to 13, most ZIKA cases (63.9%) between EW 12 to 15 and CHIKV cases were almost evenly distributed along the time study interval.

Median time (days) between symptoms onset and PCR confirming diagnosis were 2.0 (IQR 2.0 – 4.25), 2.0 (IQR 1.5 – 3.0) and 4.5 (IQR 3.0 – 6.5), for ZIKV, CHIKV and DENV, respectively. Fever and arthralgia were the most frequent symptoms among CHIKV (90% and 89%) and ZIKV cases (78% and 75%); fever (88%) and headache (88%) were the most frequent symptoms among DENV.

**Conclusion:** Co-circulation of dengue, Zika and CHIK has been observed Rio de Janeiro with direct impact on surveillance, diagnostic and management of the cases. Establishing an active syndromic surveillance system focused in arboviruses is paramount for a fast and precise public health response to these new arbovirus infections.

20.144 Lethality in newborn with microcephaly due to maternal Zika virus infection in Rio Grande do Norte State, Brazil: A cross sectional-study


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**Purpose:** An increase in the number of newborn with microcephaly (MCP) was noted in 2015 in regions of Brazil with high transmission of Zika virus. Many studies have been published showing causal relationship between ZIKV congenital infection and microcephaly in newborn. This study aims to assess the mortality in newborn with microcephaly due to ZIKV congenital infection from April 2015 to June 30 2016 in the State of Rio Grande do Norte (RN), a Brazilian State that has an estimated population of 3,400,000.

**Methods & Materials:** Data were obtained through epidemiological bulletin from Brazilian Ministry of Health during the period from April 2015 and up to June 30, 2016.

**Results:** There were 435 cases of microcephaly notified in Rio Grande do Norte State during the study period, of which 113 were already confirmed and 256 remain under investigation. The remaining 63 cases have been ruled out by presenting normal exams or due to presenting microcephaly by non-infectious causes. Of the total confirmed cases, 15.9% (18/113) died after childbirth or during pregnancy. The Brazilian Ministry of Health has reported that 67% (12/18) of confirmed deaths had ZIKV infection during pregnancy as the causal agent: 7 had a CT scan showing abnormalities of the central nervous system (presence of typical and indicative alterations of congenital ZIKV infection) and 5 were
confirmed by RT-PCR and/or IgM/IgG antibodies against ZIKV. The remaining 6 cases of deaths remain under investigation.

Conclusion: Our findings show a high rate of mortality (67%) in newborns with microcephaly due to ZIKV congenital infection. An increase in the prevalence of microcephaly was seen especially from September 2015, with the highest rate in November 2015 (22.3 cases per 1,000 live births). Before this ZIKV outbreak, the records in Rio Grande do Norte State between 2010 and 2014 was around 1.8 cases/year. Because of the ZIKV outbreak is still under control, it might increase the infant mortality rate in Brazil. The data presented here suggests that the current ZIKV outbreak might decrease the Brazilian's level of health. The impact of microcephalies associated to ZIKV congenital infection are still to be determined.

20.145 Authoritarian governance and the suppression of infectious disease reporting
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Purpose: Anecdotal evidence suggests that authoritarian regimes sometimes suppress the reporting of infectious disease outbreaks; the 2003 case of SARS in China is but one example. Suppression of reporting can slow and degrade the quality of public health response. However, no prior research has shown such suppression is systematic and consistent, or episodic. This paper aims to fill this gap.

Methods & Materials: I encoded a dataset cover 191 countries over the period 1980-2006. Disease reporting was measured across two distinct types of biological events: the emergence of new pathogens, and outbreaks of endemic pathogens, drawing on data from Jones et al. (2008) and Smith et al. (2014), respectively. Data on political regime was drawn from the Country Indicators for Foreign Policy project. In order to isolate the effects of regime on reporting patterns, I include variables measuring drivers of infectious disease emergence and outbreaks identified in the literature. These include ecological factors (biome type, biodiversity, pathogen richness), anthropogenic factors (population size, density, distribution, and migration), latitude, as well as other factors that might influence reporting, including surveillance capacity, scientific reporting, and GDP. All variables are measured at the country-year level. I fit negative binomial regression models to estimate the count of emergence and outbreaks events, using autoregressive terms to account for temporal trends. Rare event logistic regression models are estimated as a robustness check.

Results: Model results indicate that authoritarian governments suppress the reporting of both disease emergence and outbreak events. However, this effect is confined to only one causative agent, viruses, and is strongest for viral zoonoses. Controlling for ecological and anthropogenic factors that drive disease dynamics, authoritarian governments are significantly less likely to report viral emergence and outbreaks (p<0.01), but have a similar likelihood of reporting the outbreak or emergence of bacteria and other pathogens.

Conclusion: Prior research has shown that viruses, more than other pathogens, have been identified as a global public health threat (Elbe 2010). This research shows that some countries have responded strategically, by suppressing reporting of biological events which could draw public health attention, or blame. Surveillance in authoritarian governments should be strengthened, and historical estimates revised accordingly.

20.146 The third Clostridium difficile NAP/BI/027 strain outbreak in Mexico
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Purpose: The purpose of this study is to assess clinical characteristics and outcomes of a Clostridium difficile (NAP/BI/027 strain) outbreak at a Mexico City’s tertiary care hospital.

Methods & Materials: This is a cross sectional study, in which we studied the registered cases of Clostridium difficile infection (CDI) at “Dr. Manuel Gea González” Hospital in Mexico City from December 1st 2014 to June 15th 2016. Demographic, clinical data and outcomes (cure, recurrence or death) were registered using patient’s medical records. Patients included, had positive real time polymerase chain reaction for Clostridium difficile NAP/BI/027 strain; patients without a positive test, were excluded, as well as patients who missed information on their medical records. For the statistical analysis we used SPPS® 21 version. We calculated mean, media, percentages and rates.

Results: Annual incidence of ICD on previous years, regardless type of strain, was of 2.5, 0 and 2.3 cases per 10,000 patient-days in 2012, 2013 and 2014 respectively. Since 2015 when we detected NAP/BI/027 strain in our hospital, there has been an increase in the number of cases. We included 69 patients, 27 of them (39.1%) were positive for NAP/BI/027 strain, with a female predominance of 16 patients (59.2%) with a mean age of 53 years and a
monitored by passive telephone surveillance. Two family members were placed in isolation in a country house with their possible role of dogs in the transmission of the disease prompted outbreaks of Ebola were present. The high risk of infection for family members and the Ebola (high fever and malaise) after returning from the West African countries where Spallanzani”, in Rome. It was the first case in Italy of a patient experiencing symptoms of (Zaire ebolavirus). He worked in Sierra Leone for the Ong Emergency and he was later admitted to a high-isolation ward at the National Institute of Infectious Diseases, "Lazzaro Spallanzani", in Rome. It was the first case in Italy of a patient experiencing symptoms of Ebola (high fever and malaise) after returning from the West African countries where outbreaks of Ebola were present. The high risk of infection for family members and the possible role of dogs in the transmission of the disease prompted the use of quarantine measures (21 days). Family members were placed in isolation in a country house with their two patient-owned dogs, and they were given specific instructions. The two dogs were monitored by passive telephone surveillance. Two family members were entrusted by a

20.147 Simple online tools to visualize complex laboratory-derived surveillance outcomes – The HIV continuum experience

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Purpose: Online tools are robust for mapping illness data, and can allow for insights that are not possible with aggregate or static figures. Simple maps can be instrumental in visualizing complex surveillance data, such as laboratory-derived HIV care outcome surveillance, and may be useful for public health response to other complex emerging diseases.

Methods & Materials: HIVContinuum.org launched February 2015 and is a free online resource for visualizing HIV care continuum outcomes in 5 US cities heavily impacted by HIV - Atlanta, Chicago, New Orleans, Philadelphia, and Washington, DC. HIVContinuum.org uses laboratory-derived surveillance data from public health agencies to map HIV care outcomes at small geographic levels of detail for the following: linkage to care, engagement in care and viral suppression. The website has an intuitive user interface and allows viewing of maps by race/ethnicity, sex and age. HIV testing and care locations can also be layered over the care outcome maps.

Results: All persons newly diagnosed with HIV infection in the 5 cities from 2007-2011 had HIV care outcomes mapped for 2012. For these 5 cities, there was a total of 300 separate maps all organized within a simple map interface. This allowed us to determine that even within our most heavily impacted cities there are micro-epidemics of worse HIV care outcomes. For instance, in Atlanta we see that the core parts of the city have the largest 5-year risk of new HIV diagnoses, but that areas in South Atlanta and Northeast Atlanta have worse linkage to HIV care within 90 days after diagnosis. These areas also have less density of HIV care providers.

Conclusion: Visualization of complex surveillance data through simple intuitive online tools will increase the impact of our public health response to emerging diseases, particularly when it can identify micro-epidemics within heavily affected cities. This information can then be used by care providers, public health agencies and policy makers to better target limited resources. In the case of HIVContinuum, this data will continue to be updated and expanded to include other cities and is already being used for public health resource planning.

20.148 The first case exploring the health management of dogs owned by a man suffering from Ebola (EVD) in Italy

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Purpose: The purpose of this paper is to illustrate the health management of two dogs belonging to a patient suffering from Ebola (EVD) by Veterinary Service of the Local Health Authority in collaboration with the Department of Veterinary Medicine at the University of Sassari.

Methods & Materials: On May 12, 2015, a Sardinian nurse tested positive for Ebola virus (Zaire ebolavirus). He worked in Sierra Leone for the Ong Emergency and he was later admitted to a high-isolation ward at the National Institute of Infectious Diseases, "Lazzaro Spallanzani", in Rome. It was the first case in Italy of a patient experiencing symptoms of Ebola (high fever and malaise) after returning from the West African countries where outbreaks of Ebola were present. The high risk of infection for family members and the possible role of dogs in the transmission of the disease prompted the use of quarantine measures (21 days). Family members were placed in isolation in a country house with their two patient-owned dogs, and they were given specific instructions. The two dogs were monitored by passive telephone surveillance. Two family members were entrusted by a
veterinarian with the task of measuring the dogs’ temperatures twice daily (in the inner part of the thigh) by use of a laser thermometer and communicate by telephone any clinical signs of disease that might be observed.

**Results:** During the period of isolation, no symptoms related to Ebola were detected and the body temperatures were always maintained at physiological levels in both dogs. The same thing was observed in the two family members. As a result, quarantine measures were revoked for both for humans and animals.

**Conclusion:** Despite the lack of scientific evidence about the possibility of the human-canine virus transmission, it proved to be a good idea to adopt a biosecurity management protocol that dictated quarantine and passive telephone surveillance of both temperature and symptoms, allowing both dogs to stay alive.

20.149 AIDSVu.org: Communicating about the US HIV epidemic to diverse stakeholders

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**Purpose:** We developed an interactive, online mapping tool to allow users to visualize the US HIV epidemic, and to increase free access to datasets and educational tools. The website launched in 2010, and released its sixth annual update in June of 2016.

**Methods & Materials:** AIDSVu.org is the product of a public/private/governmental collaboration. All data mapped on the site are public, population-based, and ongoing data streams. Data on prevalent HIV infections, new HIV diagnoses and deaths among people living with HIV are obtained from the US Centers for Disease Control and Prevention. Data on social determinants of health (e.g., poverty, income inequality) are obtained from the US Census. Data at finer geographic levels (postal code or census tract level) are obtained directly from selected US city and state health departments. Maps emphasize interactive elements, and use interfaces similar to popular web tools and apps for driving directions or finding retail services. Social media content allows key HIV prevention messages to be shared easily by users, amplifying the reach of the content.

**Results:** The volume of site traffic to AIDSVu.org has grown significantly, with annual site visits growing from 40,956 in 2012 to 228,112 visit in 2015. In addition to general public users, core user groups in include visitors from academic institutions (8,069 visits from 6,460 unique visitors in 2015) and government (1,939 visits from 1,526 unique visitors in 2015). Another notable trend is the increase in user accessing AIDSVu.org resources through a mobile device: in 2015, 51% of visits were from mobile devices, and through June 2016, 60% of visits were through mobile devices. We have documented uses of the AIDSVu.org data to support ecological analyses of associations between HIV prevalence and social determinants of health, correlational analyses of social media content and HIV prevalence, and applications to optimize location of HIV treatment services based on underlying spatial distribution of people living with HIV.

**Conclusion:** Map-based information portals offer important opportunities both for the display and transparent dissemination of data from surveillance systems and for visualization of diagnosis data for newly emerging infections.

20.150 Impact of a protracted Q fever outbreak in South West Germany 2016 on the supply of blood products

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**Purpose:** The federal State of Baden Wuerttemberg is highly endemic for Q fever. *Coxiella burnetii* infection is asymptomatic in approximately 50% of cases. Between 1 January and 10 May 2016 four infected sheep flocks in a rural district lead to a protracted Q fever outbreak with 87 reported cases. To reduce the risk of blood transfusion-transmission of *Coxiella burnetii* blood donors were deferred in the outbreak area. This measure was based on the recommendation of the German Ministry of Health's National Advisory Committee 'Blood'. This study explores the effect of this long lasting outbreak on the supply of blood products.

**Methods & Materials:** Blood donation data were obtained from the database of the German Red Cross Blood Donor Service Baden-Wuerttemberg – Hessen. We analysed data on cancelled mobile blood donations and deferred blood donors between 1 January and 30 April 2016 in the outbreak area. Data on the regional need of blood products were calculated based on the population size. Overall donation rate was calculated using the mean number of donations in the period 1 January and 30 April, the last years.
Results: Between January and May 2016 approximately 2800 donations were needed to cover the needs of the affected area. 4500 donations were expected, 1875 were carried out. Overall 27 collection dates were cancelled and 256 blood donors were deferred in seven municipalities of the affected district. The action leads to a loss of overall 2720 (40%) blood donations in the district. 925 donations has to be covered by other sources.

Conclusion: The results of the investigation indicate the loss of blood donations in affected Q fever areas leading to an effect on yields of regional blood supply. We highly recommend the improvement of prevention measures to reduce the risk of Q fever outbreaks leading to the deferment of donors. Of central importance is the control of the pathogen in sheep, which also leads to a risk minimisation in non epidemic periods. Screening of blood donors on *Coxiella burnetii* DNA during outbreaks as alternative should be considered.

**20.151 Epidemiology and etiology of suspected central nervous system infections in Singapore**

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**Purpose:** Central nervous system (CNS) infections result in significant morbidity and mortality, and are of public health importance. There is limited information about the disease in Singapore. Singapore Neurologic Infections Program (SNIP) is the first prospective hospital-based national surveillance study on CNS infections in Singapore. It aims to describe the epidemiology, clinical features, outcome and possible etiologies of CNS infections as well as to assist in outbreak detection in Singapore.

**Methods & Materials:** From August 2013 through March 2016, 202 patients admitted to Singapore General Hospital, National Neuroscience Institute/Tan Tock Seng Hospital, National University Hospital, Changi General Hospital and Khoo Teck Puat Hospital with suspected CNS infections were enrolled. Demographics, clinical and laboratory data were collected.

**Results:** Among 202 patients, 163 patients had likely CNS infection (104 male:59 female; median age 48 years [range 16 to 88]). Seventy (43%) patients had comorbidities; 10 of them had prior encephalitis/meningitis. There were 104 (64%) patients who had oversea travel within 6 months and 38 (23%) patients had ill contact 1 month prior to illness onset. Common presentations include fever (72%), headache (64%), focal weakness (19%), altered mental status (19%) and dysphagia/dysarthria (17%). Forty-seven (29%) patients were severely affected, requiring HDU/ICU admissions; 2 died from pneumonia. An etiological diagnosis was identified in 94 (58%) patients. Of the identifiable etiology, 61% were bacteria, 23% viruses, 6% a mixture of fungi and parasites, 6% autoimmune and 4% brain abscess. *Mycobacterium tuberculosis* (18%) and *Group B Streptococcus* (17%) were the most commonly detected pathogens. The high number of group B streptococcus (GBS) coincided with the unusual outbreak of GBS infections in Singapore in 2015.

**Conclusion:** The etiology of more than one-third of the cases remains inconclusive. A large number of unknown cases from the study suggest limitation in current diagnostic evaluation as well as varying case definitions. With current practice, identification of CNS infection etiologies remains a considerable challenge. Better diagnostic investigations for infectious and non-infectious etiologies of cases presenting with meningoencephalitis will need to be developed and incorporated into patient evaluation. The implementation of an ongoing national surveillance system will enhance the detection of any known or emerging infections.

**20.152 Health Beliefs and Patient Perspectives of Febrile Illness in Kilombero, Tanzania**


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**Purpose:** Patient perceptions of risk and local understanding of disease transmission can influence health seeking behaviors as well as effectiveness of disease prevention strategies. This qualitative study was nested within health facility-based surveillance, evaluating the etiologies and risk factors of febrile illness. It assessed the knowledge and beliefs surrounding fever syndrome among adult febrile patients seeking healthcare in Kilombero, Tanzania.
Methods & Materials: From June 11th to July 13th 2014, 10% of adult febrile patients enrolled in the larger surveillance study, who presented with an axillary temperature >37.5 °C and symptomatic onset of <5 days, were randomly selected to further participate in a semi-structured, physician-patient interview, informed by Health Belief Model constructs. Interviews were audio recorded, translated from Swahili to English, and transcribed. Transcripts were coded using NVivo Version 11.1, and thematic content was analyzed by two separate researchers. Blood and naso-pharyngeal/oral-pharyngeal (NP/OP) specimens were collected and analyzed using both AFI and respiratory TaqMan Array Cards for multi-pathogen detection of 56 potential causative agents.

Results: Over the course of 5 weeks, 18 participants provided 188 discrete statements. When asked to identify the causative agent of febrile illness, 6 (33.3%) cited malaria and the other 12 (66.6%) offered non-biomedical responses, such as “mosquitoes” and “weather.” Major themes emerging related to severity and susceptibility to health hazards including: lack of bed net use, misconceptions about bed nets, mosquito infestation and shared animal-human food and water sources. Certain barriers to treatment were cited, including dependence on traditional healers, high cost of drugs and poor dispensary services. Several cues to action were identified, including the elimination of stagnant water in order to reduce mosquito breeding and community distribution of bed nets. Overall, we demonstrate 11.1% concurrence in consideration of fever etiology according to patients, clinicians and laboratory testing, all of which reported malaria as the causative agent of fever syndrome.

Conclusion: Our findings contribute to the important, yet limited, base of knowledge surrounding patient risk perceptions of febrile illness in a malaria endemic setting of Tanzania, and underscore the potential utility of community-based participatory research to inform disease control programs.

20.153 Regional differences in Healthcare utilization of patients with scrub typhus in South Korea, using National Health insurance claim data

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Purpose: The epidemiological features of scrub typhus is in the transient phase according to climate change and adaptation. The pattern of healthcare utilization was also changed. Patients visited general hospital>advanced general hospital>hospital by 2013, while they visited general hospital > clinics > advanced general hospital in 2014. The pattern was also heterogeneous by region. In this study we attempted to identify the regional differences in healthcare utilization of patients, and the factors affecting on this.

Methods & Materials: Using the national health insurance claim data, we analysed the characteristics of patients with scrub typhus(A75.3); temporal, regional and demographic factors. We also figure out the healthcare utilization of each patients; institutional characteristics, length of stay(LOS), and amount of health care cost. And then, multiple regression analysis was implemented to apprehend the factors affecting the length of stay and cost.

Results: Scrub typhus cases were mainly incident in the southern part of South Korea from 2010 to 2014: 457cases (24.2%) in South Chungcheng, 296(15.7%) in South Jeonlla, 187(9.9%) in South Gyeongsang, and so on. Averaged LOS was increased by about 2 days, from 2010(8.2d) to 2014(10.1d). Amount of healthcare cost was also increased by 0.1 million USD, from 2010(0.7 million) to 2014(0.8 million). LOS was the longest in Gangwon(11.7d), followed by Incheon(11.6d), North Choongcheng(9.4d). Cost was the highest in Incheon, followed by North Choongcheng, Gwangjoo. However, after adjustment of other factors such as individual characteristics of patients, both LOS and cost were the highest in South Choongcheng, where the majority of scrub typhus cases were incident.

Conclusion: The severity and fatality of scrub typhus cases are influenced by late detection and late treatment, so the cases in the low-incident area could be needed more longer and costly healthcare utilization, because of lower perception of physicians. Nevertheless, the LOS and cost were higher in the high-incident area, and the cause is thought that late detection according to lower accessibility to healthcare. This study has some limitation that claim data could cover not all of scrub typhus cases, so further study will be needed to improve this hypothesis.
The prevalence of typhoid fever from different regions of karachi

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Purpose: In the current study total 5106 cases were registered from various clinical settings from 2006- to 2011. Four hundred fifty one (8.83 %) patients were diagnosed with Enteric fever using different available protocols. The common sites selected from different Union Councils and covered various districts of Karachi. It was seen that the most thickly populated areas such as with low income group were among the most affected. It was about 36 and 34 % among the people of Lyari and Nazimabad respectively. However it was also positive in areas like Gulshan-e-Iqbal and PECHS with provision of all the basic amenities. The substantial difference in the ratio of incidence is used for control in morbidity rate of typhoid fever in endemic areas. It was 36.2% in Lyari and Saddar zones which was high in comparison to other localities.

Methods & Materials: Most available tests were performed for detection of Typhoid fever. Blood Culture, Typhidot test, Widal test and CBC. In this study Dot ELISA, ELISA and PCR was also performed for diagnosis of Typhoid fever

Results: In this study Widal was 16.2% in poor class, 12.6% in LMC, 11.9% in MC and 7.9 % in UMC. Typhidot was positive in 13.5% poor class, 9.0 % in LMC, 6.4 % in MC and 5.3 % in UMC. Blood culture due to its high cost comparatively showed high success rate in Upper Middle Class. It was 6.5 % in poor class, 3.8 % in LMC, 2.9 % in MC and 4.2% in UMC in relation to LMC and MC.

Similarly Male Children with Poor and lower Middle Class were seemed to be more affected then other socio-economic groups.

Conclusion: Poor and Lower Middle class were more affected. This requires special rehabilitation programs in the slums, kutchi abadi’s with dearth of basic facilities like;

- Clean drinking water
- Sewerage system
- Literacy programs
- Low living standard of public
- Education in Hygiene
- Hand Washing after Defecation
- Other unhygienic conditions

Motivational aspects are important in the improvement of biosecurity

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Purpose: In order to influence farmers to improve biosecurity, we need to know which factors affect their decision making.

The maintaining of professional skills might be an important motivator when farmers implement biosecurity measures. Another factor that might influence their behavior is their perceived ability to influence disease transmission.

The aim was to analyze whether maintaining of professional skills and perceived influence affect the improvement of on-farm biosecurity.

Methods & Materials: The study was based on a questionnaire sent to 3000 cattle and 1000 pig farmers in Finland. The response rate was 44% and summed up to 1656 useful answers. Maintaining of professional skills was based on the question “In what way do you maintain your professional capability” including three options: Reading agricultural magazines, attending branch related courses and lectures, and looking for information on the internet. The respondents’ perceived influence on their animal disease situation was based on the question: “Which of the following do you think you are able to influence?” giving six response options:

the disease situation on your farm,
the disease spread within your farm,
the effect of biosecurity measures on your farm,
new diseases spreading to your farm,
the disease situation in Finland,
the spread of disease from your own farm onwards

Each “yes” gave one point and each “no” zero points. These were then summed up and applied as two covariates (professional skills and influence) in the generalized linear models (GLM) that predicted the implementation of 18 different biosecurity measures on farms (e.g. quarantine, traffic arrangement, carcass container and insurance).
Results: Farmers who maintain their professional skills and believe in their ability to impact biosecurity are more willing to improve the biosecurity on their farms. Sixteen of the 18 biosecurity measures tested were statistically significantly dependent on the impact -covariate and nine were dependent on the professional skills -covariate.
Conclusion: Motivation is positively associated with increased willingness to improve on-farm biosecurity. If we manage to increase the motivation of the farmers through education etc. we could achieve a considerable improvement in biosecurity.

20.156 Detection of ambiguous vaccine-derived poliovirus type 2 in T’boli Sultan Kudarat, Philippines

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Purpose: The Keystone of the Global Polio Eradication Initiative is immunization of children with three doses of attenuated oral poliovirus vaccine (OPV). However, OPV use carries the risks of vaccine-associated paralytic poliomyelitis among recipients and the emergence of genetically divergent vaccine-derived polioviruses types (VDPVs): circulating, immunodeficient and ambiguous.

The Philippines has been declared polio-free by the World Health Organization (WHO) in 2000 and the world is paced to globally eradicate the virus in 2018. Since 1991, the Research Institute for Tropical Medicine has served as the National Poliovirus Laboratory (NPL) for the Department of Health (DOH)'s Acute Flaccid Paralysis (AFP) Surveillance Program. In 2001, type 1 circulating VDPV (cVDPV) was isolated from three acute flaccid paralysis patients and one contact from three separate communities in the Philippines. In 2015, an AFP case from T’boli Sultan Kudarat, Mindanao Island was detected and stool specimens were collected and sent to the national reference laboratory for confirmatory testing.

Methods & Materials: Stool samples were tested following WHO’s recommended algorithm for poliovirus identification: virus isolation, intratypic differentiation version 4.0 and complete sequencing of the VP1 region.

Results: The sequencing result revealed seven nucleotide base mutations (7/903 nt), which is 0.78% mutation difference from the Sabin type 2 reference strain and thus, yielded type 2 VDPV. To further determine the type of this VDPV type 2, serum sample was also collected from the case for immunoglobulin profiling and 67 stool specimens from contacts and children under five years of age in the community were tested. The results showed no evidence of immunodeficiency and no signs of circulation in the community. In accord with the findings, the isolate was classified as an ambiguous VDPV (aVDPV).

Conclusion: This is the first report of the emergence of type 2 aVDPV and the fifth case of VDPV in the Philippines. Since the Philippines has not reached the 95% coverage rate of vaccination for OPV, this aVDPV detection demonstrates the risks accompanying suboptimal routine immunization coverage. Moreover, the detection of VDPV underscores the need for vigilance in poliovirus surveillance and the exigency of establishing environmental surveillance of poliovirus to supplement the ongoing AFP surveillance.


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Purpose: Starting from 1988 and up to 2014, cases of wild poliovirus have decreased by over 99% as a result of an effective expanded immunization program and epidemiological surveillance of acute flaccid paralysis (AFP). There are only two countries that remain polio-endemic (Afghanistan and Pakistan). The Republic of Armenia was certified by the WHO as a polio-free zone in 2002. The purpose of the study is the implementation of an active epidemiological surveillance of AFP detectability among the population of Armenia by investigation of stool samples of patients obtained from AFP cases and their contacts, also from children of high-risk group (boarding schools).

Methods & Materials: Identification and isolation of viruses were performed by cell cultures methods and neutralization test. We also used the monthly and yearly report forms regarding AFP. The final classification of AFP cases was provided by the national certification
committee of the RA for polio eradication. Statistical processing was done using the method of binominal distribution of Bernoulli.

**Results:** During the years 2003-2015 AFP cases were registered in all marzes of Armenia, except for Vayots Dzor and Tavush which are considered as silent areas. There were only 2 cases in Tavush (3.8 and 4.0 respectively). After strengthening of the episurveillance there were 2 cases of AFP in Vayots Dzor marz (9.7 and 10.4 respectfully). According to the math model, the likelihood of zero cases in Vayots Dzor makes 90.9%, nevertheless one case was registered both in 2011 and 2013. This approach is a cornerstone of episurveillance. In spite of 15% likelihood (yearly) of 0 AFP registrations, the most cases were fixed in Yerevan (68) because of the high density of population and close contacts.

**Conclusion:** High level of coverage of immunization against poliomyelitis and effective epidemiological surveillance ensure that Armenia will maintain the status of polio free country. There is a need to continue active epidemiological surveillance with the purpose of early detection of AFP, otherwise the percentage of the registered AFP will be lower than the anticipated number. Vayots Dzor marz is a striking example of detection and registration of AFPs after enforcement of epidemiological surveillance.

20.158  The successful establishment of regular National Vaccination Conferences and a National Vaccination Steering Committee in Germany


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**Purpose:** The German health care system has a federal structure and based on national recommendations by the Standing Committee on Vaccination, vaccination programs are implemented on the level of the federal states. Furthermore, different governmental and non-governmental structures are involved in decision making concerning the implementation and financing of vaccination programs. Consequently, close collaboration between the federal ministry of health, 16 federal states and further important stakeholders is necessary to enhance the performance of immunisation programs.

**Methods & Materials:** Regular national vaccination conferences (NIK) have been implemented to facilitate a joint discussion of policymakers, scientists and stakeholders. Furthermore, the health minister conference enacted the implementation of a national steering committee (NaLI) presided by the host of the upcoming NIK (Lower Saxony and Bremen), to prioritise vaccination goals, agree on strategies to tackle identified challenges and guaranty continuity between successive NIKs.

**Results:** As a result of the first two NIKs, a national vaccination plan (NIP) was compiled, including an overview of the complex structures of vaccination policy in Germany and national immunisation goals. It was published in 2012 and supplemented by a national action plan for the elimination of measles and rubella in 2015. The NaLI has been successfully established in May 2016. The federal ministry with its subordinate agencies, the federal states’ health ministries and representatives of health insurance and medical associations are founding members. NaLI is supported by the committees’ office, located at the Bavarian Health and Food Safety Authority and financed in equal parts by the federal government and the federal states. First foci NaLI agreed upon are reinforced efforts to eliminate measles and rubella in Germany, alignments for the implementation of the Preventive Health Care Act and revision of the NIP, taking the WHO European Vaccine Action Plan and changes in national legislation into account.

**Conclusion:** In a federal political system, the implementation of immunisation strategies and therefore the achievement of vaccination goals can be challenging. Regular national vaccination conferences and the establishment of a national steering committee in addition to the existing structures have resulted in concrete goals and concerted foci which have the potential to enhance immunisation protection on national level.

20.159  Two third of under five year measles cases were not vaccinated against measles, Iraq, 2013-2015

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**Purpose:** Iraq noticed measles outbreak during 2013 through 2015. Almost all provinces affected. Children under five years comprised majority of reported cases. We aimed to
describe measles containing vaccine (MCV) status among under 5 year measles cases in Iraq, 2013-2015

**Methods & Materials:** We reviewed national measles surveillance data gathered during period of 2013 to 2015 already available at CDC Baghdad which is case based. We included all Iraqi cases aged below 5 years reported from all provinces and classified as confirmed by positive laboratory test, clinical compatibility or epi link. We described the cases by age, measles vaccine status, place of residence, and time of reporting. We compared confirmed cases with those tested negative as suspected regarding measles containing vaccine (MCV) status. We defined vaccinated as received at least single dose of MCV. We calculated percentages, odds ratio and confidence interval using 0.05 as statistical significance level.

**Results:** Of 3431 measles cases captured by national surveillance system, 2245 (65.5%) cases were under five years old. Of them, 1591 (70.4%) were lab confirmed while 645 (29%) were epi linked and more than half were from Baghdad and Ninawa. 144 cases (6%) were IDPs. Those aged below nine months, which is recommended age for 1st measles vaccine dose, constituted one quarter of cases. Excluding those below vaccination age, 1013 cases (61%) were not vaccinated against measles, highest in Salahaldin (82%) and Ninawa (65%), with no difference between studied years. Those who not vaccinated against measles were seven fold more prone to contract the infection compared with vaccinated children (OR 7, 95% CL: 6.8-8.8) and it is ten among IDPs (OR 10.4, 95% CI: 2.4-46). Measles cases among IDPs were three time more likely to report non vaccine status compared with non displaced (OR 3.4, 95% CL: 2.5-5.3)

**Conclusion:** Vaccination is a critical measure to prevent and contain measles outbreaks. We recommend keeping high vaccine coverage in routine and supplementary vaccination activities especially in most affected areas and IDPs. Raising pediatricians’ awareness toward under 9 months cases also considered.

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20.160 What do Lebanese women know about cervical cancer and human papillomavirus? A report on awareness levels in urban communities

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**Purpose:** To evaluate the knowledge of urban adult Lebanese women regarding the symptoms and risk factors of cervical cancer and the diagnostic tests and vaccination of human papillomavirus (HPV) infection. To measure in the same population the uptake of the cervical cancer-screening test (Pap smear) and the uptake of HPV vaccination, and determine the factors that may influence them.

**Methods & Materials:** 444 Lebanese women above 18 years of age, residing in Beirut and Mount-Lebanon, with no medical background, were recruited online and in health care facilities to fill out a 32 item questionnaire about cervical cancer and HPV. Collected data was exported to and analyzed in SPSS® v. 21.0.

**Results:** 45.7% of the women aged 18 to 25 y, residing in Mount-Lebanon (51.8%), single (49.3%), with high education qualifications (73.9%) and currently employed (49.1%) in a field not related to health (84.9%). They did not visit a general physician (64%) or a gynecologist (64.6%) regularly. 85.6% were aware of cervical cancer; HPV infection involvement in the pathogenesis of cervical cancer was identified in 53.9% of cases. 35.6% of participants had been screened by Pap smear for cervical cancer at least once whereas 9% did not know what a Pap smear was. Screening was significantly associated with cervical cancer awareness and regular visits to general health physicians and gynecologists. Only 11.7% of participants aged 18 to 35 were vaccinated against HPV. Vaccination uptake was significantly associated with cervical cancer awareness, religion, field of work and studies, and regular visits to gynecologists.

**Conclusion:** Urban Lebanese women in Beirut and Mount-Lebanon are not well informed about cervical cancer and HPV. Screening by Pap smear and HPV vaccination uptakes are non-satisfactory. Further interventions are required to improve these numbers.

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20.161 Private sector vaccine coverage estimates in India: Evidence from vaccine utilization data through innovative analytics

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**Purpose:** According to latest estimates of National Sample Survey (2014) private sector is major provider of healthcare in country for all illnesses including infectious diseases. However, access pattern for vaccines in private sector is largely unknown. In this context we
wanted to estimate contribution of private sector towards coverage of BCG, DPT, Measles, Hep B, Hib, PCV and Rotavirus vaccines.

Methods & Materials: We developed and parameterized a Microsoft Excel based model to estimate private sector vaccine coverage rates. The input parameters included birth cohort, vaccines sales, coverage estimates and dosing schedule. The eligible birth cohort was estimated from 2011 census of India, number of doses and dosing schedule for the selected vaccines (BCG, DPT, Measles, Hepatitis B, Hib, rotavirus and PCV vaccines) was based on recommendations of Indian Academy of Pediatrics Committee on Immunization (IAPCOI), number of vaccine doses consumed in India were estimated from IMS Health's vaccine audit data (value and volume across different brands). We then estimated the number of children immunized in private sector from IMS data followed by overall coverage estimates (public plus private) from UNICEF coverage evaluation survey and finally private sector's contribution to overall coverage rates.

Results: We observed that, among 2012 birth cohort for India, the private sector's overall contribution was 0.72% towards BCG, 1.97% towards DPT3, 3.36% towards measles and 1.97% towards hepatitis B immunization coverage among UIP vaccines. Similarly, for newer vaccines private sector contributed 1.97% towards Hib, 1.31% towards rotavirus and 0.47% towards PCV coverage. We further observed that within country private sector's contribution to immunization was significantly higher in metropolitan areas as compared rural areas.

Conclusion: The private sector is import access point for significant proportion of Indian population for access to vaccines. It is important for national government to identify strategies to engage with private sector for enhancing vaccine access and coverage. This is especially relevant from perspective of whole society preparedness for pandemic planning where private sector play an important role in not only in deployment of vaccines and other preventive interventions but also in providing access to diagnostics and critical care.

20.162 Elucidating Structure-Function Polymorphism of p7 gene of Hepatitis C Virus
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Purpose: Hepatitis C Virus (HCV) is potential virus for liver infection known to cause cirrhosis which may end up in liver failure and hepatocellular carcinoma (HCC). It has been recognized that out of the ten encoded proteins of HCV genome, p7 gene also has an antiviral potential as it is an essential HCV protein and can be obstructed by prototypic (small molecule) p7 inhibitors. Evidences suggest that p7 is potential ion channel (viroporin) that modifies several cellular pathways by interacting different binding partner proteins. However, the exact nature of these interactions is essentially vague. The present study is an investigation to resolve the binding between p7 and host binding partner proteins to the level of amino acid.

Methods & Materials: In order to address the principal objective, the structure-function analyses of p7 protein, sequences were retrieved from UniProt/NCBI data bases and subjected to multiple sequence alignment using ClustalX. Molecular models of proteins were constructed to know the structural differences between these molecules. One representative sequence from observed genotype were subjected to develop the molecular model using iterative threading and homology modelling. The models were subjected to the structural and thermodynamic refinements and used to develop the potential hexamers on the basis of geometrical complementarity.

Results: Molecular modelling shows considerable structural variations among p7 protein of different genotypes implying varying role in the HCV associated pathogenesis. This observation is further strengthen by variations in the post translation modification profile.

Conclusion: The present study will provide composite picture of the structural variations in p7 gene and their potential role in pathogenesis. Augmented with the further studies the present investigation may elucidate the potential targets for therapeutic intervention.

20.163 Predictors of recurrent fast breathing in children under five years of age in a low income community in southern Pakistan: a nested case control study
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Purpose: To establish predictors of recurrent fast breathers in children under 5 years of age from a low income community from Karachi, Pakistan.

Methods & Materials: A case control study nested in an ongoing equivalence trial (RETAPP) comparing standard antibiotic with no antibiotic (placebo) for management of children with isolated fast breathing pneumonia in a periurban slum area in Karachi, Pakistan (Trial
Virological evaluation and clinical impact of field vaccination against lumpy skin disease in cattle

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Purpose: The objective was to evaluate the clinical impact of the field vaccination against lumpy skin disease (LSD) in cattle, and to investigate the duration of viremia and excretion of the vaccine LSDV strain with saliva, nasal swabs and bulk-tank milk (BTM).

Methods & Materials: The study was conducted in a commercial dairy cattle farm (215 animals) immunized against LSD with a Neethling strain-based commercial live vaccine for cattle (Onerstarpoort Biological Products SOC Ltd). Twenty-seven animals were randomly selected for repetitive blood, saliva and nasal swab samplings. Specimens were collected for virological testing (qPCR and DNA sequencing) the day before the immunization, every two or three days until day 17 post-vaccination (p.v.) and at days 22 and 29 p.v. All vaccinated animals were being examined for signs of malaise and lesions compatible with LSD. All suspect cases were recorded and blood samples were obtained. Nodule (lump) biopsies were collected from representative cases for virus detection and identification. Milk yield was calculated, based on BTM measurements.

Results: Viremia was detected between days 6-15 p.v. at 63% of the sampled animals. Saliva, nasal swabs and BTM samples were negative at all sampling points. Pronounced swelling (aseptic inflammation) was observed at injection-site at about 12% of the animals, starting at day 6 p.v., and was resolved after 2-4 days. Small-sized skin nodules were developed between days 8-18 p.v. mainly on the neck, scapula and thorax, only in adult cows, and at about 10% of the vaccinated animals. Resolution of the lesions was observable 10 days after development. The vaccine virus was also identified in aspirates obtained from the injection-site lesions, as well as in nodule biopsies. Daily milk production was reduced during the first 12 days p.v. (max. up to 16%).

Conclusion: Apart from drop in milk production and injection-site reactions, immunization against LSD resulted in the occurrence of mild and transient LSD-like signs in a considerable number of animals. In this regard, the application of diagnostic techniques for the discrimination of infected from vaccinated animals (DIVA) is of utmost importance for the field evaluation of LSD cases in vaccinated cattle herds.

Major mumps outbreak in the South Bohemian Region, a question for the buster vaccination

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Purpose: Nationwide vaccination against mumps has been carried out in the Czech Republic since 1987. In the time before vaccination, outbreaks occurred in 5-6-year intervals. Since introduction of mass vaccination major outbreaks occurred in 1995, 2006 and 2012, mostly in vaccinated population.

In 2016, public health authorities notified mumps outbreaks in several regions of the Czech Republic with the highest occurrence in South Bohemia. We investigated an outbreak in South Bohemian region with the aim to understand the factors of re-emergence.
Methods & Materials: The data are collected from general practitioners by the notification system EPIDAT. We analysed surveillance data by time, place and person and additional characteristics, particularly vaccination status. We compared the notification data with results in serological survey 2013. Case definitions: suspect case (11 %) – compatible symptoms, probable case (57 %) – compatible symptoms and epidemiological link to a confirmed case, confirmed case (32 %) – compatible symptoms and laboratory confirmation.

Results: Outbreak has been detected in week 5 2016 during proms and peaked in week 12 with 158 cases. Until week 22 we notified 1415 mumps cases, 56 % males, median age 18 years, range 0-75 years, 462 confirmed cases, 1 % vaccinated once and 80 % vaccinated twice, 19 % not vaccinated or with not known status. Highest attack rate was in 15-19 years old (1893/100000), then in 10-14 years old (866/100000) and in 20-24 years old (658/100000). Most cases were vaccinated (81 %). Complications were reported in 58 cases (4 %), 38 orchitis, 12 meningitis, 36 of them were vaccinated. Relatively high incidence in young adults corresponds with the results of the serological surveys from 2013 when antibodies were found only in 33 percent of the age group of 15 - 19 years.

Conclusion: The unusual incidence of mumps, resulting in an outbreak, was probably caused by weaning immunity after vaccination, primarily in the age group of 15 - 19 years. Our findings suggest that the third vaccine dose at the end of elementary school may increase herd immunity and prevent further outbreaks.
Purpose: Influenza vaccination effectiveness is generally lower in adults aged 65 years and above compared with healthy adults below the age of 65. This is generally attributed to weaker immune response in the elder population as a result of immunosenesence. However, the reasons for this phenomenon are not well characterised. The aim of this systematic review is to identify and summarise documented determinants of seasonal vaccine effectiveness in older adults.

Methods & Materials: A literature search was conducted on bibliographic databases PubMed and EMBASE to identify relevant articles that investigate the determinants of influenza vaccine effectiveness in older adults up to July 4, 2015. Studies were eligible if they investigated potential determinants of seasonal influenza vaccination effectiveness in reducing the risk of laboratory-confirmed influenza, influenza-related hospitalisation or complications, and death or excess mortality in adults aged above 65.

Results: Out of 37 relevant studies that were identified through the search process, eight studies that assess potential determinants of influenza vaccine effectiveness in older adults were identified. Six of the selected studies investigated vaccine factors, one assessed host factors, and one described factors involving vaccination policies and strategies as potential determinants of vaccine effectiveness in adults above 65 years old. The determinants that were significantly associated with influenza include vaccine type and route of administration, time elapsed after vaccination, frailty, and pneumococcal vaccination. However, vaccine effectiveness was evaluated using varying endpoints in these studies as investigators are interested in different primary outcomes, including laboratory-confirmed or diagnosed influenza, influenza-related hospitalisation or complications, and death or excess mortality.

Conclusion: Documented determinants of influenza vaccine effectiveness in older adults can be broadly categorized into vaccine factors, host factors, and vaccination policies and strategies. Nevertheless, published studies that assess the impact of these determinants are relatively scarce and have lower internal validity as they are observational in nature. There is a need for more well-designed observational studies to identify and investigate the determinants of vaccine effectiveness in elder individuals, as they are at a higher risk of developing serious complications of influenza. The identification of these determinants will facilitate efforts to improve influenza vaccination in elder individuals.
Increased rates over years show the awareness of health care workers to report AEFI. However, underreporting is still present and further efforts need to be done to receive appropriate information on time, maybe to establish also sentinel active surveillance systems to detect serious adverse events.

20.169 High prevalence of serotype 3 in pneumococcal isolates causing complicated pneumonia and empyema in Portugal (2010-2015)
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**Purpose:** In spite of the use of pneumococcal conjugate vaccines (PCVs), there are some reports of increasing incidence of complicated pneumonia, accompanied by significant pleural effusion or empyema, caused by *Streptococcus pneumoniae*. Some serotypes seem to be more frequently associated to this clinical presentation, such as serotypes 1, 3, 5, 7F and 19A. We aimed to characterize the pneumococcal population causing complicated pneumonia and to evaluate the role of molecular techniques in the enhanced detection of this important pathogen in pleural fluid samples.

**Methods & Materials:** Pleural effusion or empyema samples from pediatric patients, recovered in Portugal between 2010 and 2015 were included. When traditional culture methods revealed *S. pneumoniae* these were serotyped. In culture negative cases, samples were analyzed by conventional and RT-PCR for *S. pneumoniae* detection and serotyping.

**Results:** 109 out of 152 cases of complicated pneumonia were confirmed to be due to *S. pneumoniae*. The majority of cases (n=92) were diagnosed by molecular methods. The most frequent serotypes were serotypes 3, 1 and 19A together accounting for 62% (n=68) of the isolates. Some of the patients included in this study had infections due to vaccinal serotypes, in spite of being age appropriately vaccinated.

**Conclusion:** The high prevalence of the additional serotypes included in PCV13 is probably due to a higher propensity of these serotypes to cause complicated pneumonia, although the longtime use of PCV7 may have contributed to the decline of PCV7 serotypes. The persistence of some PCV13 serotypes in spite of vaccination is of concern. Ongoing surveillance studies are essential to understand the dynamics of the pneumococcal population after the inclusion of PCV13 in the National Immunization plan in Portugal.

20.170 Coverage of poliomyelitis vaccination in Lagos Mainland Local Government Area
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**Purpose:** Poliomyelitis is a debilitating disease. On 25th September, 2015; Nigeria was delisted from list of poliomyelitis endemic countries and will be eligible for World Health Organisation certification in 2017. In March, 2015, a reported case of acute flaccid paralysis (AFP) in Lagos State, Nigeria was laboratory-confirmed negative for poliomyelitis virus. While awaiting the laboratory result, we conducted a survey among children 0-59 months (under-five) to obtain vaccination history and assess ‘missed’ opportunities for poliomyelitis vaccination.

**Methods & Materials:** A descriptive cross-sectional survey on AFP was conducted among 80 under-five children in Otto-ido, Lagos Mainland local government area, Lagos state in March, 2015. Otto-ido is a small settlement located along a major railway line in Lagos state. Data on demographic characteristics, doses of oral polio vaccine (OPV) at routine immunisation (RI) and during supplemental immunisation activities (SIAs) and underlying factors was collected using a Pro forma. Data analysis was performed using Epi-info software.

**Results:** The mean age of sampled children was 31 ± 19 months. Three (3.8%) were natives of Lagos state, 64 (80.0%) were from other states while 13 (16.3%) were foreigners. Overall, 13 (16.3%) had zero dose for OPV. During RI, 52 (65.0%) had zero dose and the main reasons were religious belief (65.4%, n = 34) and lack of felt need (7.7%, n = 4). During SIA, 14 (17.5%) had zero dose and the main reason was religious belief (64.3%, n = 9). Being a child of northern origin was associated with zero dose for OPV (odds ratio: 66.5, 95% Confidence Intervals: 12.3 – 359.2)
Conclusion: Non-immunisation of the children with OPV was probably influenced by religious belief and region of origin. We recommend involvement of religious and traditional leaders in the planning and implementation of immunisation activities to help reduce non-compliance. We distributed posters in the local language to each household in the community to create awareness on poliomyelitis. Mop-up immunization was done to vaccinate all eligible under-five children in the community. Poliomyelitis survivors should be engaged especially during immunisation campaigns to educate caregivers without felt need for poliomyelitis immunisation.

20.171 Adverse effects following immunization of pneumococcal vaccine in nepal

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Purpose: Pneumonia is one of the top 3 killer diseases of children under the age of five globally. In Nepal, it accounts for 13 percent of under-5 child deaths. Children under the age of five are most at risk of developing and dying from pneumococcal diseases. Nepal government has started the PCV vaccination through Routine immunization Schedule since January 2015

The purpose of the study was to identify the adverse effects following immunization of PCV at grass root level health facilities in Nepal

Methods & Materials: This was cross sectional descriptive study conducted in fifteen randomly selected health posts in Kathmandu. 637 mothers were involved in Client Exit Interview.

Results: All most all mothers reported redness, tenderness and/or swelling at the injection site within 6 hours of injection. Fever (>100°F), irritability and disturbed breast feeding after 6 hours of injection was reported by 92% mothers. Drowsiness and problems with sleeping was reported by 76% mothers. About half (48%) of the mothers complained about loose motion and 32% of them reported as vomiting following 6 hours of immunization. Fever (>102 F) was reported by 6.7% mothers. Fever with convulsion was complained by 3.2% mothers. Mild allergic reactions were reported by 9.3% mothers.

Conclusion: Most commons adverse effects are local effects like redness, tenderness and swelling at injection site. Among severe effects Diarrhea and vomiting is more common. Further study based on stronger study design needs to be conducted to establish the cause effect relationship between PCV and adverse effects

20.172 Clinical characterizations of children with bacterial meningitis (BM) in the Republic of Kazakhstan

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Purpose: Bacterial meningitis (BM) is characterized by severe clinical manifestations, and significant rate of residual effects. It is continue to be a common cause of morbidity and mortality worldwide In Kazakhstan, according to statistics Consumer Protection Committee (CPC) BM recorded in all regions of the country but this epidemiological data was never been analyzed linked with clinical information.

Study’s aim: To determine morbidity characteristics of BM in children <14 years in various regions of Kazakhstan during 2014-2015.

Methods & Materials: Data obtained from CPC of Kazakhstan for BM was analyze for the country and by region in children <14 years. The introduction of Hib vaccine in 2008 into the national vaccination schedule and during 2010 - 2015 the 13-valent pneumococcal conjugated vaccine (PCV13) was gradually introduced in the country. Applying the methods of modern biomedical statistics: Analysis of extensive performance analysis and intensive indicators. Statistical analysis was performed using Statistics 9.0.

Results: N. meningitidis was the most common bacteria , 70% of all cases, followed by pneumococcus, 10-15%. The highest incidence of BM in 2015, determined in Almaty 24.81%, in Astana 24.05%, but in comparison with the year 2014 these figures decreased to half. In the other regions of the country incidence rates, of disease was absent in 2014, Akmola (0.6%), West Kazakhstan (0.67%), Kyzylorda (1.27%), Mangystau (0.52%), and
North Kazakhstan (1.73%). During 2015 a significant increase of incidence of BM was observed compared to 2014 year in Almaty region, from 0.75% to 6.18%.

Conclusion: In the Kazakhstan Republic among children <14 year in 2015 compared to the year 2014 established decrease of BM from 5.52% to 4.49% for 100,000 population, while in other regions there is a consistent trend of increased morbidity.

20.173 Epitope based DNA vaccine design using epitopes predicted from Zika virus polyprotein

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Purpose: Recent outbreaks of Zika virus have made the need for developing a vaccine imperative. The polyprotein sequences of the virus have been used for finding epitopes and designing an epitope based DNA vaccine using bioinformatics tools has been attempted in this study.

Methods & Materials: NCBI Protein database was used as a source of polyprotein sequences of Zika virus. The sequences were studied for conservancy by MegAlign tool of DNASTAR Lasergene 8.0. NetMHCPan, NetMHCIIpan and IEDB analysis resource tools were used for finding Class I MHC binding peptides, Class II MHC binding peptides and B cell epitopes respectively. From the pool of binding peptides for MHC I CTL epitopes were predicted by CTLPred and NetCTL servers. The selected epitopic peptides were analyzed for similarity to human protein by BLASTp tool. AlgPred server was used for finding the presence of allergenic peptides. The peptides were joined in tandem differently for MHC I, MHC II and B cell epitopes to generate three peptide sequences. The peptide properties were predicted by ProtParam of ExPasy tools. These peptides were reverse translated using Human Codon Usage table by Sequence Manipulation Suite. These gene sequences were then separately cloned into pVAC vector and the vector map was generated PlasMapper server.

Results: 114 full length sequences of Zika virus polyprotein were retrieved; partial sequences were omitted for the study. The alignments revealed high degree of conservancy among the sequences and therefore consensus sequence was used for predictions. The MHC I & II peptides that were selected had no similarity to human protein sequences, were represented only once in the final sequence, were the strongest binders for the respective allele and had no allergenic properties. For the B cell epitopes only sequential or linear epitopes were predicted and the epitopes in the final sequence had all the properties as mentioned for MHC I & II except for allelic specificity. Cloning using human codons ensures the expression in humans.

Conclusion: The DNA vaccine vector generated in the study has been rationally designed using existing principles of epitope based vaccine design and could be used after lab validation studies.

20.174 Evaluation of monoclonal antibodies against pfMSP10 for the prospective use on in vitro growth inhibition assays of peruvian P. falciparum isolates

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Purpose: The aim of this study is to evaluate monoclonal antibodies (mAb) generated against pfMSP10 (Merozoite surface protein 10) and their functional role to inhibit the invasion of Peruvian P. falciparum isolates into red blood cells (RBC) by using the Growth inhibition Assay (GIA) in vitro.

Methods & Materials: Seven mAb and one polyclonal antibody were synthetized and evaluated by Western Blot (WB) against the recombinant MSP10 protein (rMSP10). Synchronized and purified schizonts from P. falciparum 3D7 and their concentrated supernatant were obtained by ultrafiltration. Detection of pfMSP10 protein was also evaluated in synchronized ring stage of P. falciparum cultures from 1 to 12 hours post-invasion. IFA assays were also carried out.

Results: Only one mAb and the polyclonal antibody showed a strong reaction band against rMSP10 and no cross reaction bands against non-infected RBC. Results by WB showed the presence of an approximately 70 kDa band in purified schizonts and rings stages parasites from 1 to 12 hours post-invasion, the binding of this antibody to mature schizonts was corroborated by IFA.

Conclusion: In conclusion, we have identify one mAb and a polyclonal antisera capable to detect pfMSP10 protein in P. falciparum parasites. GIA in vitro assays are underway using
Both passive administration of mAb 33D2 and active immunization of mice with modified NS1 result in high titer to homologous virus serotypes and in vivo. We found that both mAb 33D2 and modified NS1-WD immune sera could neutralize DENV infection in vitro via complement dependent and independent manners. In addition, both passive administration of mAb 33D2 and active immunization of mice with modified NS1-
20.177 Application of Real Time PCR technology to determine dengue virus serotypes in routine clinical samples

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Purpose: Dengue is one of the leading causes of morbidity in tropical and subtropical regions. Infection with any of the four-dengue virus serotypes (DENV1-4) results in a wide range of clinical manifestations. Early detection/typing using sensitive assay could support patient management. This text describes a real-time RT-PCR (rRT-PCR) assay in a single reaction for serotyping and identified dengue serotypes in clinically suspected dengue patients.

Methods & Materials: This study included, clinically dengue suspected residual serum samples sent for routine dengue testing from September 2015 through May 2016. Considering patient data, 100 samples were selected (at day of illness 2-10), and were tested using multiplex rRT-PCR assay that included previously validated primers, probes and cycling conditions. In a single reaction (tube), all primers and probes accomplish both reverse transcription and cDNA amplification. Reaction targets, highly conserved 5’ un-translated region and capsid gene, improved sensitivities. Genomic RNA from each serotype was included as a control strain. Viral RNA was extracted using QIamp Viral RNA Mini kit. The assay carried out using SuperScript III Platinum One-Step qRT-PCR kit, and performed on Applied Biosystems 7500 instrument. All the samples were tested serologically for dengue infection; 80 serum samples with capture Dengue IgM ELISA and 20 serum samples using dengue NS1 rapid immunochromatographic assay.

Results: Total 100 samples were tested, and 96(96%) determined the serotype. 76(79%) DENV-1, 14(15%) DENV-4, 03DENV-3 and 03 were DENV-2. 04(04%) did not detect /indicate the serotype. All 80 and 20 samples gave evidence for positive dengue virus infection by showing anti-DENV IgM antibody and dengue NS1 antigen respectively.

Conclusion: This multiplex assay allows detecting dengue serotypes in clinical samples. Study reveals all 4 serotypes were circulating during the study period and predominant circulating serotype for the season was type 1 dengue virus, whereas dengue virus type 4 responsible for a lesser extent. Data convinced, improved sensitivities has the ability to detect viral RNA in relatively late samples in the course of illness and also with detectable anti-DENV IgM, thereby lengthening the period of time for molecular diagnosis.

20.178 Exploiting Nigerian lichens biodiversity for alternative panacea to global malaria endemicity

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Purpose: Major setbacks to the eradication of malaria endemicity globally have been resistance to the conventional drugs and drawbacks in vaccine development. In recent times, attention is being shifted towards the discovery and development of plant-based drugs in combating the disease. The therapeutic potentials of Nigerian Lichens- the algae -Fungi symbiotic form of life are yet to be fully exploited. This study investigated the antiplasmodial and cytotoxic potentials of the foliose lichen Flavoparmelia caperata epiphytic on oil palm tree

Methods & Materials: Antiplasmodial activity was done using the PLDH assay method and the TOX8 test kit from Sigma-Aldrich was used for the mammalian cell toxicity assay with chloroquine and emetine used respectively as reference drug for comparison. The constituents of the extract were separated using chromatography techniques while the structure of purified compound was elucidated using nuclear magnetic resonance spectroscopy.

Results: A novel antiplasmodial depsidone ester (1) (IC50 =25μg/mL) with insignificant mammalian cell toxicity (IC50 >100 μg/mL) was isolated and characterized from NMR spectroscopic analysis.
Conclusion: This study reports for the first time the potential of Nigerian lichens in the management of malaria infections.

20.179 Spatial clustering of malaria cases using active and passive case surveillance in a highland area in Kenya

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Purpose: Epidemiological characteristics of clinical malaria may differ from asymptomatic infections, thus both cross-sectional parasite screening and longitudinal clinical case surveillance are necessary for malaria transmission monitoring and control.

Methods & Materials: In order to monitor malaria transmission, surveillance of clinical malaria from two years of active case surveillance in three cohorts of 6,750 individuals, asymptomatic parasitaemia cases of 5,300 individuals and clinical cases in three study areas were carried out in the western Kenyan highlands in 2009 and 2010. Age distribution, seasonality and spatial clustering were analysed.

Results: There was a significant difference in the age distribution of clinical cases between passive and active case surveillance, and between clinical case rate and asymptomatic parasite rate. The number of reported cases from health facilities significantly underestimated clinical malaria incidence. The increase in asymptomatic parasite prevalence from low to high transmission seasons was significantly higher for infants (<two years) and adults (≥15 years) (500% increase) than that for children (two to 14 years, 65%), but the increase in clinical incidence rates was significantly higher for children (700%) than that for adults (300%). Hotspot of asymptomatic infections remained unchanged over time, whereas new clusters of clinical malaria cases emerged in the uphill areas during the peak season.

Conclusion: Different surveillance methods revealed different characteristics of malaria infections. The new transmission hotspots identified during the peak season with only active case surveillance is an important observation with clear implications in the context of malaria elimination. Both mass parasite screening and active case surveillance are essential for malaria transmission monitoring and control.

20.180 Coxiella burnetii in companion animals and ticks: Serological and molecular screening

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Purpose: Dogs and cats are potential sources of infection for some zoonotic diseases such as Q fever, caused by Coxiella burnetii, a multiple host pathogen. Ticks are considered C. burnetii vectors in wild and domestic cycles. This study aimed to screen for C. burnetii in pets and in ticks collected from infested animals.

Methods & Materials: A cross-sectional study was conducted from 2011 to 2014. Sera obtained from pets (n=180) were tested for C. burnetii antibodies using a commercial ELISA adapted for multi-species detection. Ticks were identified morphologically and C. burnetii in ticks was screened by PCR assay targeting IS1111.

Results: An exposure to C. burnetii was observed in 17.2% (95%CI:5.8-35.8%) of cats and in 12.6% (95%CI:7.7-19.0%) of dogs. The exposure occurred in cats living in rural habitat, but there were no differences in the exposure between dogs living in urban or rural habitats. Ticks (n=91) were identified as Rhipicephalus sanguineus, Ixodes ricinus and Dermacentor reticulatus, being the former the most common. None was positive for C. burnetii DNA.

Conclusion: The higher exposure in cats might be explained by their hunting activities and contact with wildlife. An increase of antibody positivity in dogs was observed over the last 20 years in Portugal, but we could not associate wild, domestic animals and ticks as a source of infection. This study revealed that ticks do not seem to be involved in the transmission of C. burnetii. This highlights the need of more research to clarify the potential sources of infection in dogs and the role of ticks in C. burnetii transmission.

20.181 The changing patterns of Dengue and malarial infections -study from a Hospital in Mumbai India

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Purpose: A definite change in the trend of Dengue and malarial infections, their clinical features and outcomes has been noticed recently. The present study, in a Mumbai hospital, during three consecutive monsoons, was carried out to observe and compare the changing patterns of Dengue and malarial infections, in Mumbai, India.

Methods & Materials: A comparative, retrospective cross sectional study of Dengue, Malaria and their coinfections was carried out during three consecutive monsoons (June to November; 2013-2015) in a Mumbai hospital. Febrile patients, during this period, were investigated for both Dengue and Malaria simultaneously. Elisa (NS-1/IgM) and peripheral smear examination was done to confirm Dengue and Malaria, respectively. Clinical comparison of signs and symptoms, severity and outcomes was systematically carried out.

Results: During 2013, of the diagnosed acute febrile cases, 41 were Malaria, 39 being P. vivax and 2 mixed Malaria. 52 cases of Dengue were confirmed. 2014 saw a total of 55 malaria cases, 23 being P. falciparum and 16 cases of P. vivax and mixed Malaria each. During the year, 84 Dengue cases were detected. 2015 saw a surge of acute febrile illnesses. 117 cases were of Malaria, 107 being P. vivax and only ten positive for P. falciparum. No mixed Malaria cases were encountered; whereas Dengue cases escalated to 206. During 2014, 16 (10.25%) coinfection (Dengue and Malaria) cases were noted, whereas in 2015, 28 (6.7%) were coinfection cases. No coinfection cases were observed during 2013. Mortality during 2013 and 2014 were three each, all being P. vivax during 2013 whereas one was coinfection and two malarial deaths in 2014. Recovery was total in 2015.

Conclusion: Within the three consecutive years, it was observed that Dengue cases exceeded Malaria, as a major cause of monsoon related febrile illnesses. Within the malarial infections, P. falciparum appears to be on the decline. P. vivax has increased in incidence and severity, thus not considered benign any longer. Focussed malaria control probably led to fewer malaria cases. Dengue formed the largest group, the surge being probably related to increased Aedes breeding sites. Changing clinical trends require close monitoring. Enhanced surveillance and public health measures can contribute to better disease control.

Purpose: The first confirmed human case of WNV infection in Bulgaria was presented as a West Nile neuroinvasive disease. The first confirmed case in Bulgaria

20.182 West Nile virus neuroinvasive disease: The first confirmed case in Bulgaria

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Methods & Materials: For the etiological diagnosis of WNV infection specific serological tests were applied for detection of IgM in CSF and IgM and IgG in serum. WNV RNA was detected by real-time RT-PCR. Full genome sequencing was performed.

Results: In the summer 2015, a 69-year old man with cardio-vascular disorder and a history of mosquito bites and no recent travels outside Bulgaria, developed a febrile syndrome, tremor, and weakness, followed by neurological disturbances with coma and lethal outcome. CSF examination showed mild lymphocytic pleocytosis. WNV-specific IgM antibodies were detected in CSF and WNV-specific IgM and IgG antibodies were found in serum. WNV RNA was detected in a urine sample. Sequencing of the full viral genome and phylogenetic analysis demonstrated that the virus belonged to Southern-European WNV lineage 2 clade and had high sequence similarity with WNV strains circulating in Greece and in Hungary.

Conclusion: This case report demonstrates the presence of WNV lineage 2 in Bulgaria and supports public health interventions for vector control and prevention of WNV transmission because of the risk of severe neuroinvasive disease and the high mortality rate, especially in elderly patients with co-morbidity.

20.183 Severe clinical forms of Mediterranean Spotted Fever: A case series from an endemic area in Bulgaria

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Methods & Materials: A retrospective study was conducted at the Department of Infectious Diseases, Stara Zagora University Hospital (Southeastern Bulgaria) between April 2015 and June 2016. For the analyzed period 54 cases had clinical and laboratory data for
Mediterranean spotted fever (MSF). Raoult diagnostic criteria were used for the evaluation of severity. For the etiological diagnosis serological tests were applied. MSF-specific IgM and IgG antibodies were detected in serum by indirect immunoenzyme assay (ELISA IgG/IgM, Vircell, Spain). *Rickettsia conorii* ELISA IgG Sensitivity 85%, Specificity 100% and *Rickettsia conorii* ELISA IgM Sensitivity 94%, Specificity 95%. Statistical analyze was done by MS Excel 2007 and SPSS Statistics, version 19.0.

**Results:** Seventeen patients presented as severe forms. The predominant gender of them was male, 82% were men and 18% women. The median age of the analyzed group of seventeen cases was 59 years (range: 14-78 years). Ten patients developed hepatic disorder, three had neurological signs, and three cases evolved pulmonary involvement. Laboratory data found thrombocytopenia in 14 patients, mean value of thrombocytes count (PLT) for the whole group was 108.1±55.4x10⁹/L. Liver enzymes were elevated with mean value of aspartate aminotransferase (AST) 154.9±88.4IU/L and alanine aminotransferase (ALT) 162.0±83.6IU/L. Acute phase reactant as C-reactive protein (CRP) had mean value of 143mg/L (range: 9-230mg/L). Kidney function was impaired in some cases, the calculated mean value of Creatinine for the studied group was 147.3µmol/L (range: 85-313µmol/L) and mean value of Urea was 10.4mmol/L (range: 4.3-27.4mmol/L).

**Conclusion:** Bulgaria is an endemic area for tick-borne diseases. Annually cases of Mediterranean spotted fever are reported. Severe and "malignant" forms of MSF are not rare. Typical clinical and laboratory markers for severity would be actively searching for. Early diagnose and proper treatment is the key to avoid complications and patient’s recovering.

20.184 One Health approach gap analysis of leishmaniasis detection, prevention, and response in the Republic of Albania

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**Purpose:** One Health approach gap analysis of leishmaniasis surveillance, prevention, and response to evaluate critical capacities in Albania for early detection, case management, vector and reservoir control, and intersectoral collaboration.

**Methods & Materials:** A cross-sectional study, SWOT analysis, and community interviews were conducted between April - November 2015, by adapting the *WHO European region strategic framework for leishmaniasis control in 2014–2020.*

**Results:** Visceral leishmaniasis (VL) due to *L. infantum* is the main form of the disease in Albania with 30-100 cases annually, of which 70% are in children under 14 years of age, and among children 70% are from 1-4 years old. There is under-reporting of cases from the district to national levels. There is no active surveillance for canine leishmaniasis and no population control measures for street dogs. There is vector surveillance and the main vector is *Phlebotomus neglectus* with *P. tobbi* playing a secondary role but the vector control programme for sand flies is not well established.

Ambisome was used for paediatric cases in the main university hospital centre, but it is not supplied in the whole country and currently there is no treatment available for adults. Case detection and clinical management protocols are out-dated with low capacity for early detection at regional or district level. Availability of kits and reagents for diagnosis were lacking in hospitals with no access to PCR or Rapid Diagnostic Tests (RDTs).

Albania has not established a One Health control program as the disease is not perceived as a public health priority. Public awareness of leishmaniasis is low in all areas.

**Conclusion:** The gap analysis highlighted the need to improve early detection and treatment of VL in Albania, ensuring that all patients have access to appropriate anti-leishmania medication.

One Health principles should be applied to improve inter-agency cooperation (i.e. MoH/MoA, municipalities) for the better control of leishmaniasis in vector and reservoir species. There is a need for similar gap analyses in other countries in the region together with capacity-building activities such as training workshops and joint surveillance activities to better control vectorborne and zoonotic diseases.
20.185  Gap analysis of leishmaniasis detection, prevention, and response lessons learned for vectorborne diseases in Albania, Jordan, and Pakistan
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Purpose: The gap analysis of leishmaniasis detection, prevention and response utilised the Strategic Framework for Leishmaniasis Control in the WHO European Region 2014–2020 to determine critical capacities for reducing the burden of visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL).

The objective of the gap analysis was to identify constraints in implementing the WHO strategy in three different environments. Connecting Organizations for Regional Disease Surveillance (CORDS) facilitated this project by bringing together two of its networks, Albania from the Southeast European Consortium (SECID/SEEHN), and Jordan from the Middle East Consortium (MECIDS), along with a partner from Pakistan (POHA).

Methods & Materials: A multidimensional, cross-sectional approach was utilized in each country study, from April – November 2015, which included: An objective description of the current status of leishmaniasis surveillance, treatment and control activities. A critical evaluation of the effectiveness of these activities, identifying weaknesses/opportunities for improvement. Collation and analysis of clinical case data to map endemic zones and calculate incidence rates; vector distribution and prevalence rates in reservoir hosts were similarly reported. 1-2 week study tour and in-country workshops. On-going dissemination and collaboration activities via Leishmaniax.net platform.

Results: Detailed reports were produced for each of the three countries as well as a combined summary report.

All three countries currently lack the funding, personnel, and resources needed to address existing and emerging vectorborne disease (VBD) threats including dengue and Zika. Deficiencies were identified in all critical capacities: legislative, institutional, administrative, financial, technical, research, and awareness. In each country, effective and affordable anti-leishmania treatment is frequently unavailable in the impoverished urban and rural communities where leishmaniasis is most prevalent.

In Pakistan, anthroponotic cutaneous leishmaniasis (ACL) is endemic in Afghan refugees and the host communities where they live, putting an insupportable burden on health service providers. A similar problem will develop in Jordan and other countries in the region unless immediate and effective measures are taken to curtail the spread of ACL among Syrian refugees.

Conclusion: For each country, we propose the establishment of a national VBD unit with adequate funding, staff, and resources to conduct integrated vector control interventions against diseases such as malaria, dengue and leishmaniasis.

20.186  Assessment of the anti-feeding and insecticidal effects of the combination of dinotefuran, permethrin and pyriproxyfen (Vectra® 3D) against Triatoma infestans on rats
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Purpose: This study, based on the rat model, was designed to explore the anti-feeding and insecticidal efficacy of a topical ectoparasiticide dinotefuran-permethrin-pyriproxyfen (DPP) against Triatoma infestans bugs, a vector of Trypanosoma cruzi for which dogs are domestic reservoir hosts.

Methods & Materials: Twenty adult male Sprague-Dawley rats were randomly divided into two equal groups: an untreated control group and a DPP treated group. Each rat was exposed under sedation to 16 T. infestans bugs at mixed life stages for one hour on days 1, 7, 14, 21 and 28 post-treatment. The anti-feeding and insecticidal efficacy of DPP was estimated after one hour of exposure. Insecticidal efficacy was assessed after incubation of the insects for 24 hours after the end of the exposure period.

Results: The anti-feeding efficacy of DPP was 96.75%, 84.71%, 80.5%, 81.52% and 42.67% on days 1, 7, 14, 21 and 28, respectively. Insecticidal efficacy evaluated at one hour and 24 hours after exposure on days 1, 7, 14, 21 and 28 was 100%, 91.25%, 82.5%, 80.03% and 29.14% and 100%, 100%, 100%, 96.07% and 49.92% respectively.

Conclusion: Throughout the trial, there was a significant difference between the treated and control groups for both anti-feeding and insecticidal efficacy. Our study demonstrates that a
single administration of DPP spot-on at an equivalent to the minimal recommended dose on rats has a powerful effect against T. infestans starting from day one and lasting for at least three weeks.

20.187 Molecular biology research regarding lyophilized *Francisella tularensis* revitalized after 40 years

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**Purpose:** *Francisella tularensis* is a highly contagious Gram-negative bacteria that causes tularemia or "rabbit fever" and is contagious to humans. There are four known subspecies of *Francisella tularensis*, two of them are the most studied: A Type strain that is the more virulent (found in North America) and B Type (subspecies *holarctica*, also referred to as *palearctica* strain, found in Europe) that is the less virulent.

**Methods & Materials:** Lyophilized strains were rehydrated in nutrient broth, cultured in *Francisella tularensis* specific medium (CHAB-PACCV) and passed on nutrient medium. Microbiological diagnosis (including optical microscopy) was confirmed by immunoassay (Tularemia biothreat Alert kit, Tetracore) and molecular tests: Real Time PCR with TaqMan *Francisella tularensis* detection kit, Applied Biosystems for two genes (*fopA* and *tul4*) and TicKitqPCR (in house kit) for one target insertion sequence-like element (IS*Ftu*2).

**Results:** The methods have confirmed the presence of *Francisella tularensis* strain in revitalized samples after 40 years of storage.

**Conclusion:** Our researches have been based on the revitalization of lyophilized strains of *Francisella tularensis* in order to obtain the positive controls required for the in house real time PCR kit for CCHFv and TBEv and *Francisella tularensis* and *Borrelia burgdorferi s.l.* agents transmitted by ticks.

20.188 Environmental barrier in the fight against malaria in Nigeria

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**Purpose:** Malaria is endemic in Nigeria and the pathogenesis of the disease is becoming more complex as many therapeutic options have not reduced the morbidity and mortality associated with it. Relapse rates and therapeutic failures prevail. The screening of homes with mosquito nets and the recommendation of insecticides treated nets have not reduced the incidence of malaria in many communities in Nigeria. This study in line with the above evaluated the impact of the environment in the fight against malaria.

**Methods & Materials:** Ado-Odo-Ota the second largest local government area in Ogun State, an industrial conclave and a home to three Universities and the largest Church auditorium (Faith Tabernacle with 50000 seat capacity) was used for this study. The study was conducted through informed interviews use of structured questionnaire and visits to 5 towns and 12 villages. A total of 3500 respondent data were analyzed. Environmental factors considered were conditions of roads, drainage system, refuse disposal plan and availability of public amenities.

**Results:** Data of 3500 subjects in Ado-Odo-Ota revealed that 20% suffer from malaria attack monthly, another 18% experience an episode once every month while 15% reported recurring attacks twice monthly following artesunate/amodiaquine or artemether/lumefantrine combination therapy. Of the 3500 subjects, 85% claimed to suffer from malaria at least once a year. Assessment of the environment of the 17 rural and semi-urban settlements identified pools of stagnant water in bad roads of street, heaps of refuse in residential quarters and highways, dilapidated infrastructure and poor or absence of drainage system as factors aggravating the proliferation of mosquitoes in the environment.

**Conclusion:** We concluded that drug in the absence of adequate environmental clean-up and provision of well maintained infrastructure will have little or no impact in the fight to eradicate malaria.
Serological evidence of Chikungunya virus (CHIKV) in Mozambique: Is CHIKV endemic in Mozambique?

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Purpose: Non-malaria febrile illness have increased in Quelimane city, situated the center of the country, where undifferentiated acute febrile illnesses corresponded to 63% in 2015. Surveillance system for acute febrile illness was established in Coalane Health Center in February 2016 to investigate the etiology of undifferentiated fever. In March 2016, was noted that frequency of antibodies against Chikungunya virus (CHIKV) was unexpectedly high. An investigation team was deployed from National Institute of Health to conduct an investigation and assess the magnitude of the disease.

Methods & Materials: A cross sectional study was conducted between May 21st and June 3rd at 4 medical centers and 2 neighborhoods of Quelimane city. Acute febrile patients with history of arthralgia were consecutive enrolled. To assess presence of sequelae, non febrile person with previous history undifferentiated febrile illness and arthralgia within the prior 12 months were also enrolled. Acute sample were screened for IgM anti-CHIKV as well as for NS1, IgM and IgG anti-Dengue.

Results: A total of 66 patients were enrolled at 4 health centers and 9 at neighborhoods of Quelimane city and of these, 60 (90%) had history of fever for fewer than 5 days. The median age of study participants was 28 years (IQR: 6–62 years). Headache, cold and arthralgia were the most frequent symptoms reported in 59 (98.3%), 51 (77.2 %) and 48 (72.7%) of the patients respectively. A total of 29 (43.9%) were positive for IgM anti-CHIKV antibodies, 21 (31.8%) had malaria, 3 had positive dengue NS1 results. The rate of co-infections between CHIKV, dengue and malaria was: CHIKV-malaria: 11 cases (37.9 %), CHIKV-dengue: 1 case
(3.4 %), malaria-dengue: 1 case (5%) and malaria-dengue-CHIKV: 2 cases (6.89 %), respectively. Patients with CHIKV antibodies where from a total of 15 neighborhoods of Quelimane city. A total of 11 (37.9%) patients with IgM anti-CHIKV antibodies received antibiotics.

**Conclusion:** The investigation provides serological evidence that CHIKV is highly prevalent in Quelimane city suggesting that the virus represents and important but unsuspected cause of febrile illness in this city and should be considered in the differential diagnosis of acute febrile illness.


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**Purpose:** Arbovirus of the genus Alphavirus and Flavivirus can cause febrile illness and sometimes neurological disease in both humans and animals. West Nile Virus (WNV), Eastern Equine Encephalitis Virus (EEEV), Western Equine Encephalitis Virus (VEEV) and Venezuelan Equine Encephalitis Virus (VEEV) are endemic in the Mesoamerican region and the local appearance of these viruses is regulated by the amount of vectors and reservoirs. The objective of this research was to establish the etiologic agents associated with the presentation of arboviral neurological disease in Costa Rican horses from 2009-2016.

**Methods & Materials:** The clinical cases were attended mainly by official veterinarians and technicians from the Costa Rican National Animal Health Service (SENASA) as part of the surveillance program for infectious diseases. Blood samples from diseased horses were collected. The sera were centrifuged, cooled and sent to the laboratory. Information about the horses, owner, and GPS location of the farm were recorded. All sera were assessed by IgM capture ELISA, using a 1:400 dilution. Reagents and reference sera were purchased from NVLS, Ames Iowa, or donated by CDC, Fort Collins, USA or by Gorgas Institute, Panama. The peroxidase conjugates were MAbs: 2A2C-3 /6B6C-1. The substrate was TMB (Sigma). A serum was considered positive when the OD of the test serum was twice the mean OD value over the negative serum with viral antigen.

**Results:** Between 2009 and 2016, 181 outbreaks of neurological disease in horses were studied and a total of 284 serum samples were analyzed by IgM capture ELISA, resulting in the diagnosis of 4 cases of EEEV, 131 cases of VEEV and 35 cases of WNV. Most of the positive cases were located in the Northwestern region of Costa Rica in the low lands, in the provinces of Guanacaste, Alajuela and Puntarenas. In addition, all of the positive cases occurred during the rainy season (May to January). Most of the positive cases were unvaccinated horses.

**Conclusion:** Our results clearly demonstrate that the arboviral encephalitis affecting horses in Costa Rica show a defined seasonal and geographical pattern, representing a valuable clue for the prevention and implementation of a vaccination program to reduce their incidence.

20.192 Gap analysis of leishmaniasis detection, prevention, and response lessons learned for vectorborne diseases in the Hashemite Kingdom of Jordan

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**Purpose:** The gap analysis of leishmaniasis detection, prevention, and response utilised the Strategic Framework for Leishmaniasis Control in the WHO European Region 2014–2020 to determine critical capacities for reducing the burden of zoonotic cutaneous leishmaniasis (CL), which is endemic in Jordan, and preventing the spread of anthroponotic CL among Syrian refugees and their host communities.

**Methods & Materials:** A multidimensional, cross-sectional study was conducted between April - November 2015, which included surveillance, analysis, intersectoral collaboration.

**Results:** Zoonotic CL (ZCL) due to *L. major* and *L. tropica* is endemic in Jordan. The fat sand rat, *Psammomys obesus*, is the main reservoir host. The annual average incidence rate of CL is 3.02/100,000 with evidence of under-reporting due to low awareness of the importance of
early diagnosis and proper treatment of cases. Visceral leishmaniasis (VL) is rare in Jordan with less than 20 cases reported over last 50 years. In Syria, anthropoontic CL (ACL) due to *L. tropica* is hyper-endemic with incidence rates of over 800/100,000 in some governorates. In 2015, there were over 630,000 Syrian refugees in Jordan, of which only 17% were living in refugee camps. The number of reported CL cases in Syrians living in Jordan increased from 12 cases in 2012 to 187 cases in 2015. Without effective surveillance and control measures, there is a strong likelihood of ACL spreading in impoverished communities hosting Syrian refugees in Jordan and elsewhere in the region. Currently, no anti-leishmania drugs are licensed for importation and sale in Jordan. Supplies are therefore erratic; occasionally, medication is unavailable.

**Conclusion:** In order to prevent ACL becoming established in Jordan, health services capacities for early detection, treatment and active follow-up of cases in refugees must be strengthened at peripheral levels and in refugee camps through better intersectoral cooperation. The establishment of a national vectorborne disease unit would enable leishmaniasis surveillance and control activities to be harmonized and integrated with other vectorborne disease programmes, thereby optimizing the use of available personnel and resources. Appropriate and effective strategies for the control of sand fly vectors need to be developed and implemented together with practical measures to limit the spread of ZCL from reservoir species.

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20.193 The global population genetics of Dengue viruses revealed through temporal and spatial mapping of viral genetic variation

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**Purpose:** Dengue viruses are a genetically diverse group of mosquito-borne RNA viruses that cause more than 300 million infections yearly worldwide. Numerous phylogenetic studies have shown that within each Dengue virus serotype, viruses can be clustered into Asian and American lineages based on their sequence similarity. However, the precise genetic variation that determine these groups, the frequencies and distributions of variants and the reasons behind these observed polymorphisms remain poorly understood and complicated by rapid molecular evolution of the viral genome.

**Methods & Materials:** To reveal viral genetic variation with regard to space and time, we developed an automated pipeline that periodically scours public databases for viral genetic sequences together with its sequence annotations, and isolation metadata to maintain a comprehensive list of sequences with spatial and temporal information. Retrieved sequences were computationally serotyped based on sequence similarity to known references, and were aligned using MAFFT to determine insertions, deletions, nucleotide substitutions, and amino acid substitutions. For sequences with isolation date and location, we analyzed genetic variation in the context of isolation location and year of sampling to map the emergence of extinction of viral gene tic polymorphisms.

**Results:** We found that intra-serotype lineages, known as Dengue genotypes, possess distinct sequence profiles of conserved and variable sites that can be used as genetic markers for identification. Within Dengue serotypes, we also found numerous co-occurring mutations that indicate that epistatic interactions play a role in viral evolution. We speculate co-occurring variants in structural proteins are related to maintaining the stability of the encoded viral protein. Finally, we show that long-distance transmissions can cause decreased genetic diversity in the destination due to domination of the newly imported lineage.

**Conclusion:** Here we compiled a comprehensive database of viral genetic variation tied to temporal and geographical information to reveal the fine-scale molecular evolution of Dengue viruses worldwide across time. Our integrated map will help track the global spreading of Dengue virus variants to retrace the source of outbreak in new locales and reveal the effect of nascent Dengue vaccination on viral evolution.

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20.194 Standardisation of Zika virus nucleic acid testing

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**Purpose:** Standardisation of diagnostic assays enables laboratories, doctors and patients to have confidence in the results and provides inter-laboratory comparisons. For emerging infectious diseases, standardisation is critical, particular when assay development is very active and a wide range of tests are being applied by diagnostic laboratories. NIBSC has worked closely with the WHO, over the past 20 years, in the development of International
Reference Reagents and Standards for nucleic acid amplification techniques used in infectious disease diagnosis. As part of our response to the Zika virus outbreak in South America, we have developed two Zika virus reference materials: 1) to be used to calibrate assays and 2) to run alongside clinical samples in nucleic acid amplification based diagnostic tests. These materials enable effective monitoring of intra-laboratory variation on a daily basis and allow standardised reporting of laboratory measurements of virus load between laboratories.

**Methods & Materials:** A MR766-like African Zika virus isolate was propagated in Vero cells and heat treated prior to (1) freeze drying at a high concentration in plasma as a candidate International Standard (as part of a collaborative study at the Paul-Ehrlich Institute) or (2) freeze drying in a trehalose excipient at a low concentration for an in-run control after reconstitution in an appropriate clinical matrix.

**Results:** Heat treatment at 60°C for 1 hour reduced virus infectivity by more than 8 log_{10} to below detection limits. No viral replication was observed after 3 passages of this material on susceptible cells. Both lyophilised formulations were stable when stored at -20°C. Accelerated degradation studies at a range of temperatures from +45°C to -20°C indicate that the plasma formulation is highly stable. Reconstitution of the virus in plasma or urine resulted in an apparent reduction in sensitivity of the assay.

**Conclusion:** The availability of these new Zika virus external in-run controls will contribute to effective standardisation of diagnostic assays and reduce both intra- and inter laboratory variability of reported results. Further reference reagents derived from South American strains of virus are being produced.

20.195 A case of West Nile viral encephalitis with reversible hearing loss in an immunocompetent patient

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**Purpose:** We present a unique case of a healthy man who presented with moderate to severe bilateral sensorineural hearing loss as well as flaccid paralysis due to West Nile viral (WNV) encephalitis. Upon diagnosis, the patient was aggressively treated with oral steroids that significantly improved his hearing loss. To our knowledge, this is a rare case of reversible sensory loss due to WNV encephalitis.

**Methods & Materials:** A 45 year old man, previously healthy and normoacousic presented to the emergency department (ED) with a fever of 102.7 F, abdominal pain, nausea, vomiting and weakness. In addition, accompanying family members describe him as having visual hallucinations. The patient had been seen in the ED one day prior and was discharged home with a diagnosis of viral syndrome. He reports eating raw meat about two weeks before symptoms began, and admits to swimming off the coast at an urban island daily. The patient worked at the Parks Department.

Initial blood count and metabolic panel were within normal limits. Urine and blood cultures was ultimately negative. Abdominal ultrasound revealed mild hepatomegaly. A non-contrast CT of the head was negative. The patient was admitted with a diagnosis of gastroenteritis and treated with ciprofloxacin and metronidazole.

Over the next three days, the patient became progressively confused and agitated and was transferred to intensive care unit. Despite broad spectrum antibiotics, the patient continued to spike fevers as high as 103 F. Lumbar puncture revealed high protein and pleocytosis suggesting viral encephalitis; intravenous acyclovir was initiated. High titers of IgM antibodies against WNV in CSF confirmed the diagnosis.

On fourth day, patient complained of hearing loss, confirmed by an audiogram. At this time, oral prednisone was initiated for five days and tapered over another week.

**Results:** The patient recovered hearing loss completely as was confirmed by a follow-up audiogram as an outpatient in two months.

**Conclusion:** WNV has variable clinical presentation but hearing loss is relatively uncommon. This is a rare description of complete recovery of bilateral of sensorineural hearing loss with use of steroid therapy in an immunocompetent individual and should be considered in select cases, given lack of potent therapeutic options.
**Aedes aegypti** mosquito vector of dengue in the Arabian Peninsula: Ecology, phylogenetics and control

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**Purpose:** *Aedes aegypti* mosquito is the vector of dengue in Saudi Arabia and Yemen; where many epidemics have occurred in the last two decades. The epidemiology of dengue and risk of disease outbreaks are associated with fluctuations in *Ae. aegypti* mosquito populations and climatic conditions, especially rain fall and ambient temperature. Understanding vector population ecology and phylogenetics are essential for dengue control plans.

**Methods & Materials:** Entomological surveys were carried out in Jeddah, western Saudi Arabia for *Ae. aegypti* larval-pupal collections. For molecular phylogenetics studies, genes markers were used, mitochondrial (COI and ND4), nuclear (rDNA-ITS2) and polymorphic microsatellite loci for single nucleotide polymorphisms (SNPs) and Neighbour-Joining (NJ) phylogenetic trees analyses.

**Results:** Most of larval water types were man-made artificial sites, and belonged to 10 water container or habitat categories for different uses (average productivity: 83.9±103.6 larva/site, 5.4±7.4 pupa/site). SNPs and NJ analyses of single and concatenated marker sequences identified different molecular haplotypes unique to Saudi Arabian *Ae. aegypti* populations and numerous haplotypes shared with *Ae. aegypti* from other zoogeographic regions. Microsatellite loci showed that *Ae. aegypti* from Saudi Arabia is most closely-related to Asian populations, although they are genetically distinct. These results indicate the presence of considerable genetic variation among the studied *Ae. aegypti* populations from Saudi Arabia and other world zoogeographic regions.

**Conclusion:** This is the first report on *Ae. aegypti* phylogenetics from the Arabian Peninsula, the most eastern part of the Afrotropical zone, and bordering the Palaearctic and Oriental zones. These results are important for better understanding of dengue transmission and implementation of control programmes at both the national and global levels.

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**First molecular identification of Dirofilaria spp. (Onchocercidae) in mosquitoes from Serbia**

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**Purpose:** Dirofilariosis is a common and widespread veterinary health issue in several European countries with notable zoonotic potential. The causative agents are *Dirofilaria immitis* and *Dirofilaria repens* nematoda species which are transmitted by different mosquito vectors. Similar to other mosquito-borne infections, the knowledge about mosquito species involved in disease transmission is crucial for the complex understanding of local transmission cycles.

**Methods & Materials:** Since there is no available data on mosquito species, potentially involved in disease transmission from Serbia, 6369 female mosquito individuals representing 11 species were retrospectively tested for *Dirofilaria* nematodes, collected within the framework of a municipal mosquito monitoring programme from 13 localities in Vojvodina province, Serbia, in 2013. After species identification by morphological taxonomic keys, nucleic acid extractions from mosquito homogenates were tested for *Dirofilaria repens* and *Dirofilaria immitis* using molecular biological technics.

**Results:** Altogether, 8.33 % of tested pools showed positivity, composed of five mosquito species, mainly, *Culex pipiens* and *Aedes vexans* the most abundant species of the area. *Dirofilaria immitis* (80 % of infected mosquitoes) and *Dirofilaria repens* (20 %) were both detected from multiple localities (n = 6, both urban and rural areas), during the whole period of mosquito breeding season (from May to August with a maximum rate in July), which provides the first data on local transmission characteristics regarding mosquitoes from the Balkans.

**Conclusion:** Since urban territories were found to be positive also, our results need to be concerned by veterinary and human health professionals as well and call increased attention to the importance of mosquito surveillance, particularly in human-associated areas.
Malaria cases were reported all year round. Of the 238 functional public health facilities, 68% (160) were functional and provided services. The available surveillance data analyzed was found to be complete and of good quality. Six stakeholders reported delay in health facilities' monthly data submission and reporting to the area councils. The available surveillance data analyzed was found to be complete and of good quality. The stakeholders also reported that the system used Integrated Disease Surveillance and Response (IDSR) tools for planning, monitoring and evaluating malaria control programmes. In the Federal Capital Territory (FCT), the 2013 malaria surveillance system is well-established and is being met.

Purpose: O. Tsutsugamushi is a rare entity of central nervous system infection but can result in life-threatening conditions and requires timely treatment. We investigated the clinical and laboratory findings of patients with O. Tsutsugamushi-related meningitis/meningoencephalitis and their outcome.

Methods & Materials: Patients diagnosed with meningitis/meningoencephalitis who were admitted to the Department of Neurology, Seoul National University Hospital, Korea, between January 1, 2000 and December 31, 2015 were enrolled. O. Tsutsugamushi infection was diagnosed with serologic testing. Clinical and laboratory information was obtained by the electronic medical records of Seoul National University Hospital.

Results: Sixteen cases were identified, of whom ten were diagnosed with meningitis and six with meningoencephalitis. Thirteen patients (82%) had headaches and nine patients (56%) had fever, though six out of the ten patients with meningitis only reported headaches without fever. Eschar was found in two of the patients with meningoencephalitis but none in patients with meningitis. Cerebrospinal fluid (CSF) WBC counts were higher in patients with meningitis (mean ± S.E.M., 354.2 ± 132.8x10^3/μl) than in patients with meningoencephalitis (44.8 ± 36.0x10^3/μl) (p-value = 0.020). Four out of the six patients with meningoencephalitis showed no or mild (<10x10^3/μl) pleocytosis. One patient showed no WBC in the CSF on the day of admission despite dysarthria and personality change occurred 6 days earlier but showed delayed pleocytosis (16x10^3/μl) 2 days after admission. Critical care was needed in 1 patient due to hemorrhagic fever with renal syndrome accompanied with scrub typhus. All patients were treated with doxycycline and recovered to normal without significant neurologic deficits.

Conclusion: O. Tsutsugamushi should be suspected as an etiology of meningitis/meningoencephalitis in endemic areas and empirical treatment should be started on clinical suspicion despite the lack of CSF pleocytosis, especially in cases of meningoencephalitis. Repeated CSF studied may show delayed pleocytosis supporting the evidence of meningoencephalitis. A considerable proportion of patients only reportedly headaches, therefore the lack of febrile illness should not delay the diagnosis of meningitis. Neurologic recovery is excellent with minor sequelae if treated rapidly and systemic infection is well-controlled.

Evaluation of Malaria surveillance system in the Federal Capital Territory, Abuja, Nigeria, 2009-2013

Purpose: Malaria constitutes a major public health problem. Malaria surveillance system can be used for planning, monitoring and evaluating malaria control programmes. In the Federal capital territory (FCT), malaria is the commonest reason for hospital out-patient attendance despite the existing surveillance system and prevention programmes. We evaluated malaria surveillance system in FCT to describe the attributes (simplicity, flexibility, representativeness, stability, quality, timeliness) and determine whether the set objectives for establishing malaria surveillance are being met.

Methods & Materials: The evaluation was conducted using the "CDC's Updated Guidelines for Evaluating Public Health Surveillance System, 2001". Using semi-structured, interviewer-administered questionnaires, we obtained information regarding the malaria surveillance system and its key attributes from 28 stakeholders (2 FCT Disease surveillance and notification officers, 6 monitoring and evaluation officers, 12 focal persons or health facility in-charges). We reviewed data tools, and analyzed available malaria surveillance data for 2009–2013 using descriptive statistics. We determined the number of malaria deaths and case fatality rate.

Results: Eighteen stakeholders reported that the malaria surveillance system was flexible. The stakeholders also reported that the system used Integrated Disease Surveillance and Response (IDSR 003) forms that were easy to fill and case definition that were easy to apply. Six stakeholders reported delay in health facilities’ monthly data submission and reporting to the area councils. The available surveillance data analyzed was found to be complete and malaria cases were reported all year round. Of the 238 functional public health facilities,
106(45%) reported timely, private health facilities did not submit reports. A total of 307,662 malaria cases were recorded over a 5 year period. Of these, uncomplicated malaria constituted 82.9% of cases. Out of these 82.9%, 25% of cases occurred in children 12-59 months of age and 24% were malaria in pregnancy. The overall case-fatality rate was 2/100,000. The World Health Organization supports government officers with robust financial and technical assistance.

**Conclusion:** Malaria surveillance system in FCT was found to be simple, flexible, useful, of good quality and acceptable to stakeholders. Mortality due to malaria was low over the 5-year period. However, improvement is needed in the stability, representativeness and timeliness to strengthen the surveillance system.

20.200 Surveillance strategies of Lyme borreliosis in Belgium

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**Purpose:** The surveillance pyramid for vector-borne diseases is used to describe the epidemiological situation of Lyme borreliosis in Belgium.

**Methods & Materials:** Three sources contribute to routine surveillance of Lyme disease in Belgium. A network of sentinel laboratories performs laboratory surveillance by weekly reporting the number of positive serological tests for *Borrelia burgdorferi*. The yearly number of persons hospitalized for Lyme disease is monitored through the hospitals’ minimum clinical datasets. Finally, the incidence of erythema migrans (EM) is estimated based on repeated studies carried out by a sentinel network of general practitioners (GP). In addition, a seroprevalence study on 3200 serum samples representative of the Belgian population was carried out in 2015 and surveillance of the vector of Lyme disease in Belgium was initiated in June 2015, with the launch of an online citizen-based platform (TiquesNet) for the reporting of tick bites.

**Results:** Since 2007, between 1200 and 2300 positive serological results are reported every year, with a stable positivity rate of about 2%. The consultation incidence for EM at a GP in 2015 was estimated at 10.3 (95% CI 8.8-12.1) per 10 000 patients and did not significantly increase compared to 2003-2004. From 2003 to 2013, between 200 to 300 patients are hospitalized for Lyme disease every year. We estimated the overall seroprevalence of *Borrelia* IgG in the general population at 1.06% (95% CI: 0.67-1.67%), using a 2-tiered strategy. Finally, TiquesNet registered over 5000 tick bites between July and December 2015, spread over the country but with highest incidence in the provinces with the most suitable habitat for ticks and reservoir hosts.

**Conclusion:** Because of their complex nature, monitoring of vector-borne diseases needs to integrate different strategies, in which surveillance of both the disease and the vector are important. In Belgium, surveillance systems are in place at a national level for all levels of the surveillance pyramid of Lyme disease in humans. Up to 2015, none of the routine surveillance sources identified a significant increase in the incidence of Lyme disease. However, surveillance and research activities for the vector part are scarce and fragmented, and should be further developed.

20.201 Socio-ecological mapping of *Ae. albopictus* spatial and temporal patterns and associated public health risks in New York City

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**Purpose:** *Ae. albopictus*, also known as the Asian tiger mosquito, is an invasive mosquito of consequence for people living in temperate urban areas such as New York City (NYC). Its daytime biting behavior is cited as a major limitation of outdoor enjoyment in the summertime and its ability to transmit multiple arboviruses including all serotypes of dengue, chikungunya, and zika raise additional concerns. We posit that climatic factors mediated by socio-ecological factors define risk contours of *Ae. albopictus* mosquito vectors, viremic hosts, and local arboviral disease transmission in NYC.

**Methods & Materials:** NYC is a major hub for international travel with many imported arboviral cases, our first aim is to characterize the distribution of returning cases using data of imported chikungunya cases during the 2014 epidemic. With entomological data collected by the New York Department of Health’s Vector Control, we track the invasion, establishment, and inter-annual variability of *Ae. albopictus* abundance in NYC. Using high temporal resolution satellite based climatological and high spatial resolution land cover characteristics
as well as census derived socioeconomic variables, we aim to quantify the relationships between socio-ecological conditions and the abundance of *Ae. albopictus* mosquitoes and viremic hosts in NYC. We use general linear mixed effects models to determine influential socio-ecological drivers.

**Results:** A priori, we hypothesize that diffuse vegetation within neighborhoods of single-family homes provides the most suitable habitat for *Ae. albopictus* mosquitoes and neighborhoods with a high percentage of immigrants from Latin America will represent the greatest number of returning arboviral cases in NYC. We find significant associations between climatic conditions, vegetation patterns and the spatial distribution of *Ae. albopictus* as well as census derived socioeconomic variables on the distribution of returning chikungunya cases in NYC.

**Conclusion:** When climatic conditions are optimal, areas where these factors overlap will be at greatest risk for local arboviral transmission. Understanding the relationships between vegetation and *Ae. albopictus* abundance is essential to inform future urban greening initiatives billed to reduce environmental health disparities and deliver ecosystem services. This research will inform vector control and public health campaigns needed to minimize local spread of arboviruses.

20.202 West Nile virus circulation in Djibouti
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**Purpose:** West Nile virus (WNV) is a mosquito-borne member of the genus *Flavivirus*, responsible for meningoencephalitis in humans and horses. Birds are the natural reservoir of the virus, which is maintained in a sylvatic mosquito–bird–mosquito transmission cycle. WNV has been detected in Africa including Djibouti, but scientific data is often poor and not sufficient to understand the circulation of the virus.

**Methods & Materials:** In January 2016 we sampled 23 wild and domestic ruminants and 10 equids, located in the DECAN Association, in Djibouti, Republic of Djibouti. Moreover, from 2012 to 2014, we collected blood samples on 79 military working dogs before and after a four months mission in Djibouti.

All the sera were kept deep-frozen until being processed in our lab. Each sample from the DECAN Association was tested by seroneutralization. Dogs samples were tested for IgG against WNV using an in-house ELISA with inactivated WNV as antigen. Serum samples were considered positive if the optical density at 450 nm was >3-fold the mean of that for negative antigen. Because of the antigenic cross-reactivity among flaviviruses, all ELISA positive samples were further confirmed by Western blot.

**Results:** From the DECAN Association, 13/23 (56.5%) of wild and domestic ruminants and 9/10 (90%) of the equids were seropositive. In dogs, 4/14 (28.6%), 0/33 (0%), and 16/32 (50%) seroconverted, respectively in 2012, 2013, and 2014.

**Conclusion:** These results highlight an important circulation of the West Nile virus in Djibouti, with variations according to the years. Among the French soldiers deployed in Djibouti, one clinical case of West Nile virus infection was reported earlier this year, one in 2015 and 3 in 2014. Seroconversion in animal populations, more particularly in dogs and horses, is an useful tool to detect an active circulation of the virus and an increased risk for humans.

20.203 Health seeking practices and associated factors among malaria suspected patients at an urban hospital in Kampala Uganda
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**Purpose:** To determine the proportion of patients that seeks health care within 24hours of symptom onset and find out patients’ knowledge and health seeking practices in relation to malaria. Improved community awareness of the importance of seeking early diagnosis and treatment especially within 24 hours of symptom onset prevents most malaria cases from progression to severe fatalities; reduce transmission rates and the overall malaria burden.

**Methods & Materials:**
A descriptive cross-sectional study was conducted at Naguru Hospital in Kampala.
Data was collected using a semi-structured questionnaire from all malaria suspected patients (OPD or admitted) who had had a malaria test or clinical diagnosis of malaria, to cover a total of 380 patients. The data was entered in computer using Epi Data and analyzed with SPSS.

**Results:**
The results showed that 73% of the cases sought treatment from a trained health-worker or qualified facility within 24 hours (Had good health seeking behaviour). Having an education level of secondary and above, and a distance of less than 3km from facility were more likely to influence good seeking behaviour. No correlation was seen between seeking behaviour and age or level of income. Most patients (82%) were knowledgeable about malaria.

**Conclusion:** The studied population had generally good health seeking behaviour and knowledge about malaria but a similar study in remote/non-urban settings is recommended for comparison.

20.204 Emergence risk and surveillance of West Nile virus infections in Romania

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**Purpose:** The circulation of West Nile virus (WNV) takes place in cycles between mosquitoes and birds as main hosts, and the mammals including humans as tangential hosts. The circulation of WNV is endemic in Romania on large territories. The human infections appear continuously as sporadic cases and occasionally severe outbreaks and represent an important public health problem in Romania. The investigations for the evaluation of the emergence risk with a view to apply preventive and control measures were carried out.

**Methods & Materials:** The values of the seroprevalence of specific IgG antibodies against WNV in its vertebrate hosts (domestic and wild birds and horses) by specific ELISA techniques were evaluated. The virus detection (by RAMP test and/or RT-PCR), virus isolation (by inoculation on suckling mice) and identification (by a multi-level protocol based on RT-PCR, nested and sequencing) from mosquitoes were performed. The confirmed human cases of neurological infections were registered.

**Results:** The high seroprevalence of specific IgG antibodies against WNV detected in its vertebrate hosts showed the enzootic circulation of the virus on very large territories in Romania. The virus was detected in females of several main vector species in Romania, including Culex pipiens, during the annual transmission periods, and in C. pipiens males (showing the vertical transmission of the virus) and over-wintering females of this species (confirmation of the WNV passing over winter in Romania). The virus was detected also in Coquillettidia richiardii, Ochlerotatus caspius and Anopheles maculipennis s.l. The virus was isolated several times from Culex pipiens collected in Bucharest and 1 Decembrie rural area. Human WNV cases were confirmed every year after epidemics in 1996.

**Conclusion:** The enzootic circulation of West Nile virus is registered in Romania in very large territories where Culex pipiens and other vector species are present and/or dominant. All these data together with the permanent emergence of human cases confirm the endemic presence of this virus in Romania.

20.205 A health facility based serosurvey of circulating Dengue in Trans nzoia, Western Kenya

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**Purpose:** Dengue virus, responsible for outbreaks of febrile illness in Kenya, has become endemic in certain parts of the country. In those infected, it often presents with pyrexia, and is frequently misclassified as fever of unknown origin during diagnosis. Data on the prevalence of dengue in Kenya is scarce and its real burden is unknown. In this study we aimed to describe the prevalence of dengue fever virus exposure in patients presenting with febrile illness visiting three health facilities serving the same region in Trans Nzoia County, Western Kenya.

**Methods & Materials:** Patients presenting with fever were tested for Malaria, typhoid and other common causes of fever at Andersen Medical Center, Kitale District Hospital, and Endebess Health Center. For those who tested negative and consented, a questionnaire on sociodemographic characteristics and exposure status was administered. Remnant whole blood samples were then taken to the laboratory where they were tested for Dengue 2 virus.
IgG and IgM to identify past infections and recent exposure using Enzyme Linked Immunosorbent (ELISA) assays. For those that were antibody positive, Plaque Reduction Neutralization Tests (PRNTs) were performed.

**Results:** A total of 53 (4.2%) of the 1258 samples screened by ELISA were positive for dengue 2 virus exposure. Six (11.3%) of the 53 positive samples by ELISA had neutralizing antibodies screened by PRNT. Patients presenting at Kitale level 5 referral hospital were 3.3 (95% CI 1.7-7.4) more likely to be seropositive compared to patients from Andersen Medical center. There was no statistical difference between patients from Andersen and Endebess health facilities. Gender and age were not found to be statistically associated with seropositivity.

**Conclusion:** This study demonstrates that Dengue virus is circulating at low levels in Trans Nzoia County in Western Kenya. The possibility of an outbreak in the near future must be considered and should drive preventative measures.

20.206 Application of the social determinants of health to assess dengue control in an urban area of Colombia

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**Purpose:** Colombia has implemented the World Health Organization guidelines in dengue endemic areas and applied some strategies for educating people, engaging communities and society sectors, and promoting community participation towards dengue vector control. It seems that in spite of these strategies there are other social factors influencing the vector preference to breed in households and subsequently there are failures in its control. This research applied the social determinants of health framework to assess its effect in dengue vector control in a Colombian urban area.

**Objectives:** Evaluating participants’ knowledge and practices toward dengue vector control according to WHO guidelines in an urban endemic area of Colombia. Assessing the role of the social determinants of health in dengue vector control.

**Methods & Materials:** Mixed-methods approach. An urban endemic area was selected for applying a knowledge, attitudes and practices survey in a random sample of households. To reach more vulnerable households, public participation engagement and snowballing sampling was applied. A total of 425 households were included. Knowledge related to dengue symptoms, vector identification, and control measures were assessed, as well as practices towards water storage and water containers cleaning. Structural and intermediate social determinants of health (migration, urbanization, and housing) were also investigated. Qualitative approaches explored attitudes and practices from the community and authorities. Quantitative data were analyzed using parametric and non-parametric methods, and categories of analysis were used for qualitative information.

**Results:** The majority of the participants knew about dengue with certain inaccuracies about disease severity, treatment, and transmission. They also acknowledged their role in controlling dengue. A culture of water storage in diverse containers was found among participants, in spite of the existence of a good piped water network in the city. Practices towards maintenance of artificial water containers varied among households with different socio-economic status, but in general good practices towards cleaning prevailed. Vector occurrence within households seemed more influenced by social determinants such as migration and types of housing.

**Conclusion:** In general, participants had good dengue knowledge and practices towards vector control. However, the vector distribution seemed to change accordingly to certain social determinants of health such as migration, urbanization and housing.

20.207 Entomological surveillance for West Nile and Usutu viruses in Sardinia (Italy) during 2015

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**Purpose:** West Nile Virus and USUV are a re-emerged health problem in Europe. In Sardinia outbreaks of West Nile Disease have been reported since 2011 while in 2013, USUV was detected for the first time in mosquitoes. The aim of this study was to identify the mosquitoes involved in viral transmission and their seasonal abundance in the framework of the entomological activities defined in the Italian surveillance plan for West Nile Disease (WND) in 2015.
Methods & Materials: Entomological surveillance was conducted using CDC Light Traps operating for 24 hours in 35 geo-referenced sites every two weeks, from April until October 2015. Sampled mosquitoes were identified at the species level and then pooled according to species, sex, presence or absence of blood in the abdomen, station and date of sampling, with a maximum of 50 individuals per pool. Mosquito pools were assayed by two real-time RT PCR for the detection of WNV lineage 1-2 and USUV RNA.

Results: During 2015 a total of 5,345 adult mosquitoes representing 17 different species were collected. The most abundant species were O. caspius with 1,454 specimens (27.2%), C. pipiens 994 (18.6%), C. theileri 764 (14.3%) and O. detritus 732 (13.7%). The seasonal abundance peak of O. caspius and O. detritus were recorded in October with a maximum of 859 and 324 individuals, respectively. C. pipiens and C. theileri showed a peak in July with 208 and 577 adults, respectively. Overall 1,004 pools were sorted and tested for WNV and USUV. WNV lineage 1 was detected in a single pool of 3 not-engorged females of C. pipiens collected in September in Bari Sardo municipality. USUV was detected in 4 pools consisting of not-engorged females of C. pipiens captured from August to October in Siniscola (6), Oschiri (8), Alghero (3) and Olbia (7) municipalities.

Conclusion: The entomological surveillance was able to detect circulation of WNV lineage 1 and USUV RNA confirming the vector role of C. pipiens in Sardinia.

20.208 Phosphoenolpyruvate carboxykinase modulates the glucose deprivation induced virulence in Leishmania donovani

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Purpose: Is there any role of gluconeogenesis in modulation of virulence in L. donovani parasites under glucose-starved (GS) environment?

Methods & Materials: Glucose starved and glucose replete L. donovani parasites were used in this study. Percentage of metabolically active parasites and their rate of proliferation were determined. Virulent characteristics of GS L. donovani were determined by microscopic analysis, complement mediated lyses and expression of LdSHERP (small hydrophilic endoplasmic reticulum protein). Role of gluconeogenic flux in virulence of GS parasites was investigated using phosphoenolpyruvate carboxykinase (LdPEPCK) null mutants (Δpepck) generated by homologus gene replacement by formation of autophagosomes and stress response against glucose starvation. Autophagy induction was determined by protein level and puncta fluorescence of GFP-ATG8 and the level of protein kinase A regulatory subunit (LdPKAR1) was determined. Fluorometric measurement of reactive oxygen species in Δpepck GS parasites was performed, determination of NADPH/NADP+ balance, and level of ascorbate peroxidase and peroxiredoxin was determined. The in vitro rate of infection of Δpepck GS parasites was determined by microscopic analysis.

Results: Glucose starvation demonstrated a decrease in parasites viability and proliferation, however, was reversed by supplementation of gluconeogenic amino acids. The characteristic metacyclic features of GS parasites, along with an increase in LdPKAR1 mediated autophagy, clearly marked enhanced virulence under starvation. Glucose starvation driven oxidative stress showed upregulated antioxidant machinery with increased infectivity and greater parasitic load in primary macrophages. Interestingly, LdPEPCK, a gluconeogenic enzyme, exhibited the highest activity under GS to regulate viability of L. donovani by alternatively utilising amino acids. Δpepck decreased virulent traits and resulted in lowering of parasitic load but increased autophagosome formation. Furthermore, Δpepck parasites failed to activate the Pentose Phosphate Pathway shunt, abrogating NADPH/NADP+ homeostasis, conferring increased susceptibility towards oxidants following glucose starvation.

Conclusion: This study revealed that L. donovani undertakes metabolic rearrangements via gluconeogenesis under glucose starvation for acquiring virulence and its survival in the hostile environment.

20.209 Drug resistance patterns of CQ resistance transporter, Multi drug resistance 1 gene and Kelch 13 gene of Plasmodium falciparum isolates from North India

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Purpose: Since 2009, ACT (Artesunate plus sulfadoxine/pyrimethamine) has been adopted as the first-line treatment in view of increasing CQ drug resistance and in order to treat all
Methods & Materials: A total of 4,341 patients were screened for malaria by microscopy from May 2013 to January 2015 who visited PGIMER. A total of 34 P. falciparum cases were diagnosed positive using microscopy and further confirmed using Nested-PCR by targeting 18S rRNA gene. The clinical isolates were further subjected for drug resistance molecular markers in three genes including Pfcrts, Pfmdr1 for resistance to chloroquine and Kelch 13 propeller domain for resistance to AS. Nested polymerase chain reaction was used for the amplification of targeted genes and further sequencing was performed except for the PfMDR gene where PCR-RFLP method was used. The sequencing data was further analyzed using various bioinformatics software such as Clustal X2.1, ExPASy translate tool and BLAST.

Results: After restriction digestion of Pfmdr1 gene with Apol enzyme 8% samples were found to have N86Y mutation and rest of the samples 92% were of wild type. Upon sequence analysis of PfCRT gene in our clinical isolates we found that all our isolates belongs to the either of the three chloroquine resistant haplotypes (SVMNT, CVIET and CVMNT) maximum of which were of SVMNT type (81%). Whereas in K13, there was absence of mutation in the propeller part of the gene in all the clinical isolates.

Conclusion: This population genetic studies revealed the presence of higher nucleotide diversity in the PfCRT gene as compared to the Pfmdr1 gene in our isolates which strongly suggests the presence of chloroquine resistance in our clinical isolates. The presence of the wild type genotype of K13 in our clinical isolates strongly suggests the effectiveness of the drug in the study areas.

20.210 A Review of Dengue deaths in 2013 in Vientiane Capital and Champasack Province of Lao PDR

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Purpose: The epidemic curve shows a peak at Epi-Week 28 with a downward trend in later weeks. The number of reported cases and deaths was double from the big outbreaks in 2010. This study aims to identify the causes of high case fatality dengue during the 2013 outbreak in Vientiane capital and Champasak province in order to make recommendations for further case management improvement.

Methods & Materials: This is a retrospective study of dengue deaths from 7 hospitals during the dengue epidemic in 2013. Data was collected by chart reviews of adult and paediatric patients using a standardised form The dengue case management forms were reviewed. An Epidata software and Microsoft excel are used for data analysis.

Results: A total of 44,171 cases and 95 deaths were reported (case fatality rate-CFR 0.21%) as of Epi-Week 52. The majority mortality [61/95 (64.2%)] of deaths were reported from Champasack (n=35) following Vientiane Capital (n=26); and most of the deaths was under 15 years of age. Median and mode between symptom onset and first presentation to hospital was 4 days. Patients visited physicians too late when they developed high fever. Patients had infusions at home before going to the hospital (34%). Regarding the health care services, most of the death cases were admitted to hospital late (with fever on day 4) with severe bleeding (59%), fluid overloading due to prior IVF therapy (41%) and eventually becoming fluid overloaded and dying from respiratory failure rather than shock. Late or incorrect diagnosis for dengue with warning signs is common among the deaths cases and lack of constant monitoring of vital signs (due to the shortfall of doctors and nurses).

Conclusion: This is the largest dengue epidemic year in Lao PDR since dengue was made a notifiable disease, with ah high number of fatal cases. Therefore, ongoing training to healthcare providers should be implemented to improve diagnosis and case management outcomes. Further community-based survey on the health-seeking behavior of patient may indicate whether traditional practitioners or other practices remain as risk factors.

20.211 Phylogenetic analysis on emerging Arboviruses in Iran
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Purpose: Aarboviruses are emerging tick and mosquito-borne viruses that cause human infections in Iran. To address the risks posed by arboviruses, ongoing arbovirus surveillance is fundamental. Crimean-Congo hemorrhagic fever virus (CCHFV) is an unexplained disease with high case fatality rate in the North India. Therefore the present study was aimed to determine the frequency distribution of molecular markers associated with resistance to CQ and AS among P. falciparum clinical isolates from North India.

Methods & Materials: A total of 4,341 patients were screened for malaria by microscopy from May 2013 to January 2015 who visited PGIMER. A total of 34 P. falciparum cases were diagnosed positive using microscopy and further confirmed using Nested-PCR by targeting 18S rRNA gene. The clinical isolates were further subjected for drug resistance molecular markers in three genes including Pfcrts, Pfmdr1 for resistance to chloroquine and Kelch 13 propeller domain for resistance to AS. Nested polymerase chain reaction was used for the amplification of targeted genes and further sequencing was performed except for the PfMDR gene where PCR-RFLP method was used. The sequencing data was further analyzed using various bioinformatics software such as Clustal X2.1, ExPASy translate tool and BLAST.

Results: After restriction digestion of Pfmdr1 gene with Apol enzyme 8% samples were found to have N86Y mutation and rest of the samples 92% were of wild type. Upon sequence analysis of PfCRT gene in our clinical isolates we found that all our isolates belongs to the either of the three chloroquine resistant haplotypes (SVMNT, CVIET and CVMNT) maximum of which were of SVMNT type (81%). Whereas in K13, there was absence of mutation in the propeller part of the gene in all the clinical isolates.

Conclusion: This population genetic studies revealed the presence of higher nucleotide diversity in the PfCRT gene as compared to the Pfmdr1 gene in our isolates which strongly suggests the presence of chloroquine resistance in our clinical isolates. The presence of the wild type genotype of K13 in our clinical isolates strongly suggests the effectiveness of the drug in the study areas.
important arboviral disease in Iran caused by infected tick bite, contact with blood or tissues of infected livestock and nosocomially. Dengue Fever virus (DFV) and West Nile virus (WNV) are mosquito-borne *flavivirus*.

**Methods & Materials:** Since the establishment of the Arboviruses and Viral Hemorrhagic Fevers Laboratory at Pasteur Institute of Iran in 2000, all probable human sera for CCHFV, DFV (mainly travelers) and WNV were tested serologically and molecularly. To conduct phylogenetic analyses, genomes obtained from Iranian patients were sequenced and analyzed by MEGA-6 software.

**Results:**
- CCHFV: Of 3104, 960 cases have been confirmed for CCHF and 135 deaths. The highest rate of CCHF infection has been observed in Southeastern regions of Iran and slaughtermen and farmers. Phylogenetic studies have shown that clade IV (Asia 1 and 2), clade V (Europe-2) and clade VI (Europe-1) are circulating in Iran.
- DFV: Of 300, 3 (1%) were both serologically and RT-PCR positive. All three PCR positive samples had travel history to Malaysia. The phylogenetic tree demonstrated that all sequences fell into DENV-1 (genotype I and III).
- WNV: Among 632 tested patients; three (1.2%) of them were RT-PCR positive for both serum and CSF. Phylogenetic analysis of one sequence showed that Iranian WNV sequence grouped within lineage 2.

**Conclusion:**
- CCHFV: CCHF has been mainly seen in certain professions and regions in Iran. Given gender, CCHF in male is more than female, which seems due to male implication in high-risk professions. Phylogenetic studies have been demonstrated that different genomic variants of CCHFV are co-circulating in Iran.
- DFV: Phylogenetic analysis showed that the origin of dengue virus among three Iranian travelers were in company with their travel history to South-east of Asia (Malaysia). It seems that travelers play an essential role in the epidemiology of dengue infections in Iran.
- WNV: Data expresses the existence of WNV lineage 2 in Iran. In this regard, awareness of ministry of health and physicians for dealing with this infection is necessary.

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20.212 Acute febrile illness in children under 5 years: Magnitude and associated factors in Muleba district, northern Tanzania, 2013

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**Purpose:** Acute Febrile Illness (AFI) is common among children in Tanzania. Causes of AFI are difficult to distinguish clinically, and malaria is often considered to be among the leading causes of AFI among hospital admissions. We conducted diagnostic evaluation and determined factors associated with AFI among children <5 years in Muleba.

**Methods & Materials:** We conducted a cross-sectional study in 28 randomly-selected health facilities. We recruited children <5 years with both reported history and confirmed fever lasting <72 hours. Blood was collected and tested for malaria using mRDT and microscopy while other cause of AFI was detected using real time polymerase chain reaction (RT-PCR). A questionnaire containing demographic and risk factor information for AFI was used to collect data. Data was entered cleaned and analysed using Epi Infor 3.5.1. Univariate, Bivariate and Multivariate were done where aOR was used as the measure of effect with 95%CI.

**Results:** The median age of 300 recruited children was 22.5 (range, 6-59) months with males constituting 51% of study respondents. Several causes of AFI including Parasites (58%), bacteria (1.3%) and viruses (0.3%) were detected with overall identification of 73.3% (220/300). Of the samples taken 56% (168/300) tested positive for malaria, 0.3% for dengue, 1% for rickettsia, 0.3% leishmania, 0.3% for bartonella, 0.3 for cremean congoland hemorrhagic fever, 0.3% Trypanosome brucei. Bed-net ownership was 88% (264/300), with 66.3% (175/264). Of bed-nets being in good physical condition, and 86% (227/264) slept under bednet every night. Of those without bed-net 72.2 % (26/36) had AFI. Children with incomplete immunization for their age were 1.8(95% CI: 0.789 to 4.199) times more likely to experience AFI than those with complete immunization.

**Conclusion:** Pathogens other than malaria were identified among children with AFI in Muleba. Approximately a quarter of children with AFI had no malaria. There is a need to fully explore other causes of AFI other than malaria in order to promptly manage the patients that presents with AFI.
Tetracyclines modulate cytokine production and induce autophagy in THP-1 cells

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Purpose: We previously reported that excess production of cytokine is associated with the increase in severity in rickettsial infection. Recently some researcher reported that autophagoy has the pivotal role for biological defense mechanism in various infections. However, few reports are found which clarified that rickettsial infection induced autophagy. Tetracycline (TC) is the first line of treatment in rickettsial infection. This study elucidated that minocycline (MINO) and tigecycline (TGC) suppress cytokine production and induce autophagy in addition to acting as antibiotics. Using our established method, we elucidated modification of signaling pathways by MINO and TGC.

Methods & Materials: In the THP-1 human monocytic leukemia cell line, we used the Multi Plex Immunoassay to measure TNF-α and IL-8. To elucidate modulation of signaling pathways by MINO and TGC, the protein levels of phosphorylations of p38/MAPK and NF-κB were determined by Western blotting. Addition of LPS with or without TC, the induction of autophagy was examined. We examined LC-3-II protein and p-mTOR. Furthermore we examined the effects of mTOR inhibitor.

Results: Using the Multi Plex kit, we confirmed that LPS induced cytokines production in THP-1 cells. TC downregulated the release of cytokines. MINO inhibited TNF-α to 16.0% and 10.7% at 1h and 2h, respectively, and inhibited IL-8 to 43.6% and 30.1%. TGC inhibited TNF-α to 14.0% and 66.6%, IL-8 to 32.2% and 97.1% respectively, compared to control levels. NF-κB inhibitor and p38 inhibitor suppressed of TNF-α and IL-8 production. LPS stimulation increased LC-3-II protein, indicating that autophagy was induced. Adding TC with or without LPS, LC-3-II proteins were increased in THP-1 cells. mTOR inhibitor induced autophagy.

Conclusion: We clarified that NF-κB and p38/MAPK signaling pathways have a notable effect to regulate cytokines production. We certificated that TCs modulated autophagy induced by LPS in THP-1 cells. It is suggested that the modulation of cytokines production and autophagy induction might prevent the severity in rickettsial infection.

Socioeconomic and behavioral risk factors for infection of visceral leishmaniasis from Sudan

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Purpose: A descriptive cross-sectional community based study was conducted at Barbar Elfugara village with an objective to study socioeconomic and behavioral risk factors among head of the households regarding infection with visceral leishmaniasis in Gedaref state.

Methods & Materials: A sample size of 224 head of households in the area was determined using statistical formula. Data were collected using questionnaire, observation check list and reviewing records and were analyzed using Statistical Package for Social Science (SPSS) and the association between different variables were checked using Chi-square test, at a level of significance was taken of 0.05.

Results: The study showed that male were infected more than twice female among head of the households (P.V=0.03), the infection is high among the age group (<20 yrs) (42.9%), most of the infections were founded among illiterate head of the households (30.1%). The proportion of infection is more significant among farmers (28.2%), and the infection is associated with large families (more than 6 member) (31%). The study revealed that there is a significant relationship between Kala azar infection and the respondents' monthly low income (P.V=0.05). The study indicated that the majority (97.3%) of head of the households aware about kala azar disease and most (93.6%) of them aware that kala azar is an infectious disease. Also the majority (90.8%) of them knew that the disease is transmitted by an insect but about (55.5%) of them aware the vector is sand fly. A group (34.5%) of head of the households aware that the time of biting is dusk and the majority (74.5%) of them showed poor knowledge about the breeding site. The majority (91.1%) of head of the households knew the ITNs, but small group (17.4%) of them use it.

Conclusion: In general, the present findings showed that even though the respondent’s knowledge about the disease was good but their overall behaviour and practice about prevention and control of getting VL infection were still poor. Based on the findings the study recommended that; Health education and social interventions are essential to fill the gap between the good knowledge of the respondents and their behaviours and practices.
Concurrent outbreak of Chikungunya virus in the Philippines caused by 2 genotypes 2011 to 2015

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Purpose: Chikungunya (CHIKV) is a mosquito-borne infection that caused large outbreaks in tropical countries. Prior to 2011, an outbreak in an agricultural village was reported in 1996. We report the re-emergence and continuous transmission of Chikungunya in the Philippines for the last 4 years concurrently caused by 2 genotypes.

Methods & Materials: Blood samples collected from patients presenting with fever, rash, and joint pains were tested for Chikungunya IgM. Samples collected <5 days after onset of symptoms with negative IgM were tested for CHIKV RNA. The partial E1 gene was amplified using one-step RT-PCR and followed by direct Sanger sequencing. Phylogenetic analysis was performed using neighbor joining method using Kimura-2 parameter model (K2+G) on the partial E1 gene (733nt) by MEGA 6.05.

Results: Of the 7,097 serum samples collected from 2011 to 2015, 50% have detectable anti-Chikungunya IgM. CHIKV RNA was detected from 259 samples while 31 samples were sequenced for partial E1 gene. Most of the Philippines strains were grouped into Asian genotype and clustered into the same branch, with high similarity from Indonesian and Malaysian strains. Likewise, East/Central/South African (ECSA) genotype was detected in some provinces such as Davao, Bohol, and Zamboanga collected from 2011 to 2015. The ECSA have the alanine to valine substitution in the codon 226 (A226V) which increased the transmissibility of the virus.

Conclusion: Chikungunya has caused outbreaks throughout the country with continuous transmission in new and existing areas. Both the Asian and ECSA genotype with the A226V mutation was reported in the country. Additional information on the transmission dynamics of Chikungunya and social behaviors during epidemic and inter-epidemic periods Philippines would be helpful in explaining the sustained transmission of the virus after a massive outbreak.

Evaluation of dengue NS1 antigen rapid diagnostic test

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Purpose: The objective of this study was to evaluate the sensitivity and specificity of a novel dengue NS1 antigen rapid diagnostic test (RDT) and to compare its accuracy with available laboratory methods (RT-PCR, IgM, IgG and NS1 antigen ELISA).

Methods & Materials: Retrospective study evaluated the sensitivity and specificity of SD BIOLINE Dengue Rapid NS1 antigen test in the diagnostics of acute dengue virus infection. The diagnosis was confirmed by RT-PCR (Shanghai ZJ Bio-Tech), detection of NS1 antigen by ELISA (Bio-Rad Laboratories) and serology (PanBio).

Results: A total of 143 stored serum samples from 102 cases of dengue and 20 controls (malaria, chikungunya, enteric fever) were tested with the aforementioned kit. Dengue NS1 antigen was detected by evaluated RDT in 118 (82.5%) and by ELISA in 129 (90.2%) out of 143 serum samples from patients with dengue fever (p=0.084). There were 112/123 (91.1%) RDT positive and 120/123 (97.6%) ELISA positive samples from patients who presented in 0-10 days from symptoms onset (p=0.051). All non-dengue samples were negative using both RDT or ELISA.

Conclusion: This study evaluated test performance characteristics of one of commercially available dengue NS1 antigen RDTs. These immunochromatographic tests are easy-to-use, rapid and cheap, however, the sensitivity was lower in comparison with ELISA, thus the results of RDTs should be interpreted cautiously and negative samples with clinical suspicion should be retested by more accurate tests such as ELISA (NS1) or RT-PCR. However, RDTs can be useful for initial assessment and diagnostic work-up.
20.217 Non-coding RNA determines flavivirus transmission by mosquitoes
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**Purpose:** Flaviviruses like West Nile, Zika, yellow fever and dengue virus produce an abundant non-coding subgenomic flavivirus RNA (sfRNA) in infected cells. SRNA results from stalling of the host 5'-3' exoribonuclease XRN1/Pacman on conserved RNA structures in the 3'UTR of the viral genomic RNA. SfRNA production is conserved in insect-specific, mosquito- and tick-borne, as well as no-known-vector flaviviruses, suggesting a pivotal role for sfRNA in the flavivirus life cycle. Here we investigated the function of sfRNA during West Nile virus (WNV) infection of Culex pipiens mosquitoes and evaluated its role in determining virus transmission.

**Methods & Materials:** An sfRNA-deficient WNV was generated and compared to wildtype WNV for replication rates in cell lines. Infectivity and transmissibility was assessed in mosquitoes by measuring virus titers in the body and saliva.

**Results:** The sfRNA-deficient WNV displayed similar growth kinetics as wildtype WNV in both RNAi-competent and RNAi-compromised mosquito cell lines. Importantly, we demonstrate that the sfRNA-deficient virus displayed significantly decreased mosquito infection and transmission rates in vivo when administrated via the blood meal. Infection and transmission rates were not affected by sfRNA after intrathoracic injection.

**Conclusion:** This study identified sfRNA as a key driver to overcome the mosquito midgut infection barrier. This is the first report to describe a key biological function of viral, non-coding RNA in mosquitoes, providing an explanation for the strict conservation of sfRNA production in all flaviviruses.

20.218 Novel laboratory methods to study/describe the molecular characterization of African swine fever virus isolates for the purposes of genotyping in Georgia
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**Purpose:** Since pig farming is inexpensive/efficient, pork and pork products are essential to the food supply of Georgia. Recently, swine in Georgia suffered from African swine fever (ASF), an exotic and especially dangerous communicable disease. Spread of this infection is a global concern and may reoccur in Georgia. The causative virus, ASF virus (ASFV), is a large, double-stranded, enveloped, icosahedral virus; it is the only DNA arbovirus. Molecular and genetic study (VP72-based) revealed 23 genotypes of the virus. According to the current data, outbreaks are usually caused by genotypes I and II. The outbreak in Georgia was caused by genotype II (Dixson et al., 2008), which has a high potential for spread. There are several approved diagnostic methods used by the Laboratory of the Ministry of Agriculture (LMA) for testing for ASFV including an enzyme-linked immunosorbent assay and a virus specific polymerase chain reaction. The goal of the study was to implement new ASFV genotyping methods at LMA, thereby providing the opportunity to conduct laboratory based molecular/genetic study of ASFV for the first time in Georgia.

**Methods & Materials:** We selected twenty ASFV-containing samples for testing, which were collected in different regions of Georgia from 2007-2009. Three loci of the ASFV genome were used for the new genotyping method: 1) C-terminus of B646L, which encodes protein p72 (Bastos et al., 2003); 2) E183L which encodes protein p54 (Gallardo et al., 2009); and 3) central variable region primers.

**Results:** As expected, band sizes in the twenty ASFV positive samples were typical for genotype II viruses.

**Conclusion:** This research allowed us to conduct laboratory-based studies to identify molecular and genetic characteristics of ASFV in Georgia for the first time. If necessary, LMA will conduct similar studies in the future in Georgia. In case of recurrence of the disease, we will be able to determine the genotype of the circulating virus and be able to identify any changes in the virus genotype.
**Purpose:** This work describes the entomological surveillance carried out in Piedmont, Liguria and Valle d’Aosta (Northwestern Italy) before the occurrence of human cases. This diagnostic approach is focused to all major mosquito-borne flaviviruses of medical interest.

**Methods & Materials:** During the vector season, mosquito traps were located fortnightly in selected sites according to risk factors. Adult females were counted, identified to the species level and pooled by collection site, date and species with a maximum of 100 specimens. After RNA extraction, pools were analysed by Real Time RT-PCR distinctive for WNV Lineage 1 and Lineage 2 and USUTU virus (USUV). Furthermore, a Flavivirus End-point RT-PCR was performed on pools collected in higher risk sites for the introduction of exotic invasive species and pathogens (international airports, ports and connection points, infectious diseases hospitals). Positive pools were sequenced to confirm the specificity of reaction and sent to the National Reference Centre for Animal Exotic Diseases (CESME, IZSAM) for confirmation.

**Results:** Since 2011, about 2,700 pools were analysed. The analysis revealed USUV circulation in Piedmont since 2011 and in Liguria since 2014. In 2014, WNV Lineage 2 was detected in Piedmont (Alessandria province) and Liguria (Genoa province). In 2015, the same virus was found in four different Provinces of Piedmont, showing an expansion of its activity and, at the end of the vector season, the first human case was confirmed. Following WNV detection in mosquitoes, as provided by national legislation, veterinary and human health control measures were activated (screening of equine sera and blood transfusion).

**Conclusion:** The surveillance network allowed to early detect the presence of mosquito-borne flaviviruses potentially pathogenic for humans and animals and provided useful information to public authorities, in order to apply control measures. With particular reference to WNV, our results showed that entomological surveillance can detect the virus in mosquitoes much earlier than in humans, as reported in other Italian regions. Moreover, considering the emergence caused by other flaviviruses of medical interest like Zika or Dengue, the ability of this surveillance protocol to detect *Flavivirus* spp. could represent an important tool for Public health authorities.

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**Purpose:** Zika virus (ZIKV) is an emerging mosquito-borne *Flavivirus* causing birth defects and severe neurological complications in adults. Dendritic cells (DCs) act as major initiators and regulators of the immune response towards viral infections. There is at present very limited data on the interaction of ZIKV with DCs and possible immune evasion mechanisms. Here, we analyze the replication of ZIKV in DCs and their antiviral response with a particular focus on the comparison between the African and Asian strains of ZIKV.

**Methods & Materials:** Human monocyte-derived DCs were generated from the PBMCs of blood donors and infected with Asian (H/PF/2013) and African (M/Uganda/1962) ZIKV strains. Viral RNA load and interferon (IFN) type I and III responses were assessed by RT-PCR. Viral shedding was determined by a titration assay and markers of DCs activation/maturation and ZIKV-induced cell death were measured by a flow cytometry approach.

**Results:** We found that DCs were more susceptible to the Asian ZIKV strain in comparison to the African strain. Viral loads over time were similar for both strains. However, virus shedding by infected DCs was higher after infection with the African vs. Asian strain. Interestingly, infection with the Asian ZIKV strain induced lower IFN responses than the African strain. Neither activation of DCs nor cell death was induced after infection by both virus strains.

**Conclusion:** DCs are more susceptible to infection with the Asian ZIKV in comparison to the African strain. In addition, the IFN responses after infection with the Asian strain of ZIKV are reduced in comparison to the African strain, which may explain the increased susceptibility of...
DCs to this strain. Although these results provide important information on how the ZIKV strain currently circulating in the Americas may evade the innate immune response, further investigation of ZIKV cellular targets are urgently required.

20.221 Molecular exploration of genetic resistance in riverine buffalo
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**Purpose:** Bovine tuberculosis (bTB) is a neglected endemic zoonoses, causing lot of mortalities every year. Previous methods of controlling bTB, like vaccination, antibiotics, have raised the concern of animal product consumers regarding microbial resistance. These measures have not been proved effective in developing countries, so disease prevalence in these areas is increasing continuously. In a report from WHO, Pakistan have been declared Eight out of ten countries with the highest incidence of bTB. So, there is dire need to find new therapeutic and preventive measures against Mycobacterium infection. Identification of selection signatures for genetic resistance is promising new alternate to combat bTB. Many of countries have opted this new approach and have reported useful data in cattle but very limited efforts have been put into the river buffaloes. River buffaloes of Indo-Pak region are world famous for their superior genetic potentials and inter-breed variations that provides substantial basis for identification of significant selection signatures.

**Methods & Materials:** Present research was planned to explore IFNg gene in river buffalo for its association with bTB. Interferon gamma (IFNg) is a key responder cytokine in Mycobacterium infection. For its genetic characterization, blood was collected from tuberculin negative (n=267) and tuberculin positive (n=194) animals. DNA was extracted and Sanger’s method of DNA sequencing was used. Sequence comparison of two groups provided a total of five variations. Significance of each variation was tested by Hardy Weinberg equilibrium (P<0.05). Association was performed by one way ANOVA.

**Results:** Results illustrated only one variation found significantly associated with better immunity against bTB. For purpose of some additional and supportive information phylogenetic analysis was also being performed by neighbor joining method with bootstrap value-1,000. Tree indicated that river buffaloes are in closest proximity to Bos taurus and its genetic distance from other species may also be seen in Figures provided.

**Conclusion:** Genetic markers identified in this study can be useful in future breeding selection programs against bTB resistance.