19.001
Assessment of the Risk Posed to Singapore by the Emergence of Artemisinin-resistant Malaria

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**Purpose:** To assess the public health risk to Singapore posed by the emergence of artemisinin-resistant malaria across the Greater Mekong Sub-region (GMS).

**Methods & Materials:** The likelihood of importation of drug-resistant malaria into Singapore and the impact on public health of its subsequent secondary spread in Singapore were assessed to determine the overall risk. Epidemiology of malaria cases in Singapore was analysed. Vulnerability and receptivity of Singapore were examined, including the connectivity between Singapore and countries reporting artemisinin resistance (ART-R), as well as preparedness of the health authorities.

**Results:** The importation of ART-R malaria in Singapore is possible given the close proximity and significant travel volume between Singapore and the GMS countries reporting ART-R. Singapore’s vulnerability is further enhanced by its high dependency on foreign workers from neighbouring endemic countries. Nonetheless, the overall likelihood of such an event is low based on the rarity and decreasing trend of imported malaria incidence over the past few years. From 2008 to 2017, 12 out of 209 (5.7%) *P. falciparum* cases detected in Singapore had mutations associated with artemisinin resistance. All cases had recovered without complications.

**Conclusion:** This risk assessment highlights the need for a high degree of vigilance over the local and global situation to be maintained to minimise the risk and severity of the public health threat of drug-resistant malaria to Singapore.

19.002
Possible drivers for the increased West Nile virus transmission in Italy in 2018.

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**Purpose:** To assess the consistency of the observed patterns of West Nile virus (WNV) during the last six years and possible drivers for the observed increased incidence in 2018.

**Methods & Materials:** The data on confirmed West Nile Neuro-invasive Disease (WNND) human cases notified in Italy since 2012 indicate an increase of incidence in 2018 (131 cases as of 30 August). An integrated surveillance system is in place in Italy since 2008, which includes RT-PCR testing of mosquito pools, birds belonging to three target species (magpie, hooded crow, jay) and wild birds of other species found sick/dead. Data from veterinary activities are recorded in a national database, which is integrated with the data on WNND human cases. The possible correlation between the monthly numbers of positive mosquito pools and the monthly numbers of WNND cases, of positive birds of target species, of positive wild birds since 2012 have been tested.

**Results:** The monthly numbers of WNND human cases, positive birds of target species and positive wild birds were all significantly correlated to monthly numbers of positive mosquito pools: Kendall correlation coefficients (tau) equal to, respectively, 0.6466 (p=5.6e-11), 0.6983 (p=4.3e-13) and 0.5548 (p=1.5e-8).

**Conclusion:** The variations of WNV infection in mosquitoes led to similar variations in the incidence in both the vertebrate reservoirs of infection (birds) and in the accidental hosts (human beings), thus supporting the hypothesis that the increased number of human cases observed so far in Italy in 2018 is linked to a higher incidence of infection in the mosquito populations. The analysis of climatic patterns in 2018 is indicating that during the first six months of this year the temperatures were higher than usual (+1.1°C), and also rainfalls were more frequent, especially during June (http://www.meteo.it/clima-italia-2018-pioggia-caldo-temperature/). These differences in temperatures and rainfall patterns in 2018 could explain the observed increased incidence of cases in vertebrate
hosts. Further analyses are ongoing to verify this hypothesis using a more consolidate dataset, to be expected in October, after the peak of WNV infection.

19.003
Cluster of Septicaemia and Necrotizing Fasciitis Following Exposure to High Seawater Temperatures in Southeast Norway, June to August 2018

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Purpose: In the summer of 2018, remote sensing maps from the European Centre for Disease Control showed high water temperatures (>20°C) and conditions consistent with Vibriob growth in seawater along the coast of southeast Norway. On 18 July, the Norwegian Institute of Public Health (NIPH) was informed of a severe case of Vibrio vulnificus infection after swimming along the coast of southeast Norway. As V. vulnificus infections are not notifiable in Norway, we investigated if there were further cases in order to implement control measures.

Methods & Materials: A case was defined as any person with a laboratory confirmed bacterial infection who developed sepsis and/or necrotizing fasciitis after swimming along the coast of southeast Norway between 1 June and 31 August 2018. We requested that all primary laboratories and municipal health authorities inform the NIPH of serious swimming-associated infections identified during the summer. We conducted risk assessments and developed advice on how swimmers in risk groups could protect themselves.

Results: The NIPH was informed of six cases of V. vulnificus and two cases of Shewanella putrefaciens infections with onset between 17 June and 26 August 2018. Cases developed sepsis (n=8) and necrotizing fasciitis (n=7) 24-36 hours after exposure to seawater. Median age of cases was 72 years (range 51 to 88). One case had an underlying condition. All cases had pre-existing wounds before swimming. The NIPH and municipal health authorities advised people with wounds to avoid swimming along the coast of southeast Norway and people in risk groups (immunocompromised and older people) to take precautions to avoid wounds while swimming.

Conclusion: Although the incidence of V. vulnificus and S. putrefaciens infections is unknown, severe infections following exposure to seawater in Norway are considered uncommon. The absence of underlying conditions and severity of illness highlight the importance of awareness in risk groups and clinicians to ensure timely diagnosis and treatment of severe infections. Given the global increase in seawater temperatures and consequences for the upsurge in V. vulnificus growth rates, NIPH is considering making Vibrio infections notifiable in order to monitor the incidence of swimming-related infections.

19.004
Environmental Change and Malaria Risk in El Oro Province, Ecuador

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Purpose: Environmental change impacts the transmission and spread of vector-borne diseases. Significant associations between climate factors and vector-borne diseases have enabled predictive models to be developed that can be used in early warning systems to forecast and anticipate disease epidemics. Malaria is one such vector-borne disease, with a global distribution and significant health burden that is highly sensitive to climatic factors. Additionally, malaria is experiencing a resurgence in transmission, threatening elimination efforts. Malaria is also sensitive to non-climatic factors, such as land-use change, political instability, insecticide resistance and the effectiveness of public health control interventions. A greater understanding of the relative role of climate and non-climate factors in driving malaria transmission is needed to develop predictive models, particularly under future global change scenarios.

Methods & Materials: In this study, a 20-year dataset of monthly malaria cases was used to develop a spatiotemporal Bayesian modelling framework for evaluating the relative influences of climatic and non-climatic drivers of malaria incidence in El Oro province in southern Ecuador, an area historically endemic for Plasmodium vivax and P. falciparum. Between the 1980s and early 2000s, El Oro experienced a surge in malaria transmission and through an effective binational collaboration became
Effectively malaria free in 2011. However, there is still a high risk of re-emergence of malaria in the region.

**Results:** The mixed model with the best fit included the climatic variables minimum temperature and precipitation. The addition of non-climate factors, including political instability and the addition of malaria intervention activities in El Oro were also included in this model. By interacting the climate covariates with malaria intervention periods, we found the association between climate and malaria risk changed significantly following the interventions.

**Conclusion:** Distinct variation in spatially explicit random effects was found, which suggest that other environmental factors, such as land use change or area-specific intervention efforts should be explored to understand this variation. This study demonstrates that both environmental, socioeconomic and political factors should be considered when developing predictive disease models, to understand the potential environmental suitability for re-emergence in the region, given a lapse in control efforts or periods of civil unrest.

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19.005
El Nino Southern Oscillation Affects On Influenza Peak Activity Seasons: Comparison of the effects Between Korea And the USA With Surveillance Data From 2005 to 2018

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**Purpose:** Influenza affects about 10% of the population every year. In the temperate zone, influenza has a unique seasonal pattern with some variations, however, the factors affecting its seasonality and variations are still unclear. This study aimed to explore the effects of El Nino and La Nina events on the timing of influenza peak activity in both western and eastern side of the Pacific Ocean; Korea and the USA.

**Methods & Materials:** The weekly influenza surveillance data of Korea and the USA from September 2005 to July 2018 were taken from WHO FluNET site. The El Nino southern oscillation (ENSO) were retrieved from Korean Meteorological Administration. We used the definition of El Nino as the positive sea surface temperature anomalies (≥0.5°C), while La Nina events are negative anomalies (≤-0.5°C) of 3 months moving average. The weeks with the highest activities of influenza A and B in each season were identified and compared according to the presence of the anomalies. The pandemic season of 2009/2010 was excluded from the analysis. We used the mid-p exact test for statistical significance.

**Results:** From 2005 to July 2018, there were 3 El Nino events and 5 La Nina events by the ENSO. The influenza A peak activity in study periods showed at 4.6(±4.2) and 3.6(±4.6) epidemic week in Korea and USA, respectively. However, in the season of La Nina, the peak activities of influenza A observed at 0.8(±1.4) and 5.6(±3.9) epidemic week in Korea and USA, respectively. The La Nina was associated with the early annual peak in Korea (p=0.045, mid-p exact test) and the El Nino was associated delayed the annual peak in USA (p=0.09, mid-p exact test). The peak activity of influenza B showed no statistical association with ENSO in both countries.

**Conclusion:** Both El Nino and La Nina affect the timing of influenza A peak activity, but the direction may be the opposite across the Pacific Ocean: ENSO associate early peak in Korea and La Nina associated delayed the peaks in the eastern part (USA) in northern hemisphere.

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19.006
Immunonodulatory effect of *Echinococcus granulosus* laminated layer: Impact on parasite survival

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**Purpose:** *Echinococcus granulosus* infection is among the most common parasitic infections worldwide. It usually manifests as unilocular cyst(s) surrounded by an acellular laminated layer (LL). This layer provides a physical protection to the living part of the cyst including the protoscoleces. In this study, our aim is to investigate the immunonodulatory effect of LL on macrophages phenotype.

**Methods & Materials:** We examined the effect of LL extract (LLs) on parasite survival in monocytes-protoscolecoc cocultures. Moreover, the expression of some markers of M1/M2 macrophages was analyzed. In this sense, NOSynthase 2, Arginase and NADPH oxidase activities were measured. The involvement of mannose receptor (MR), TGF-β and PPAR-γ (Peroxisome Proliferator-Activated Receptors) was also tested using Mannan (MR antagonist), Anti-TGFβ and T007 (PPAR-γ antagonist). Finally, TLR2, CD14 and CD23 expression was evaluated using flow cytometric immunoassay.

**Results:** We observe that LLs increases protoscoleces survival in vitro. Furthermore, LLs inhibits NOS2 activity and enhances Arginase activity concomitantly. MR and TGF-β seem to be implicated in these effects. Moreover, T007 significantly abolish LL inhibitory effect on NADPH oxidase suggesting PPAR-γ involvement in this effect. Finally, LLs increases TLR2 and CD14 expression and decreases CD23 expression by monocytes.
**Conclusion**: Our finding suggests that the laminated layer of *Echinococcus granulosus* induced M2 phenotype allowing parasite survival. The down-regulation of this pathway constitutes an important issue to address during anti-hydatic treatment design.
Excretion Dynamics of Henipa-related Viruses in the Egyptian Rousette Bat and Triggers for Potential Spillover

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Purpose: Henipaviruses (Hendra and Nipah) are associated with zoonotic infectious diseases of bat origin responsible for high morbidity and mortality rates in humans. The recent 2018 outbreak of Nipah virus in Kerala State, India again emphasises the public health importance. Research previously reported on an expanded geographical and host range for these viruses to the African continent and resident bat species. This study investigated the presence of Henipa- and related viruses in the Egyptian Rousette bat in South Africa as well as determining potential excretion in urine over time.

Methods & Materials: From 2012–2018, 304 spleen and 58 urine samples were collected from individually captured bats. Additionally, a total of 255 pooled urine samples were collected longitudinally over 14 consecutive months (June 2017–July 2018) from sheets placed underneath the roost. An RT-PCR assay (Tong et al., 2008) was used for detection of viral nucleic acids. Five spleens (1.64%), two individual urine (3.45%) and 16 pooled urine samples (6.27%) tested positive for paramyxovirus RNA and were further characterised using DNA sequencing and Bayesian phylogenetic analysis. The dynamics of viral excretion was investigated using a Generalized Linear Model (GLM) with a binomial error and a logit link function. The “sampling month” was set as an explanatory variable and analyses were carried out using the R software package v.3.4.1.

Results: We detected, for the first time, henipavirus sequences in bat species in South Africa. A major peak in July characterised the excretion dynamic followed by a drastic decrease. Temporal excretion data were analysed in conjunction with information on the reproductive season of the bats as well as average weather data from the region over the past five years. The July peak coincides with the dry winter period as well as the waning of maternal antibodies in the bat population.

Conclusion: This study site is surrounded by rural housing and the presence of free-roaming livestock and domestic animals. The cave is also used in traditional practices by the local population creating a conducive interface for spill-over. Further studies will be required to determine the extent of exposure and the zoonotic potential of these viruses.
Can Zambia Use Program Data for HIV Surveillance?

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**Purpose:** Zambia estimates HIV prevalence among pregnant women using periodic antenatal clinic sentinel surveillance (ANCSS). We assessed the feasibility of replacing ANCSS with routinely collected prevention of mother-to-child transmission (PMTCT) data.

**Methods & Materials:** We selected a convenience sample of eleven of 24 clinics that participated in ANCSS in 2011. PMTCT data were extracted retrospectively from paper-based registers and electronic health records (EHR) for the ANCSS data collection period. We assessed quality of PMTCT data and compared demographics and HIV test results among data sources.

**Results:** A total of 2,988 records abstracted from PMTCT data were used for analysis. EHR data were available in three sites and included 526 records for the ANCSS period. Data quality was high overall (completeness >99%; legibility >90%) for PMTCT register data. However, validity ranged from 45−76% for variables in PMTCT register data due to missing information. Challenges were encountered in obtaining EHR data, but completeness and validity were 100% for data extracted. Demographics were similar in ANCSS and PMTCT registers. Approximately 58% of women captured from PMTCT data were from urban sites, compared to approximately 75% in EHR data. Overall HIV prevalence from ANCSS (17.4%, 95% CI: 16.2−18.6%) exceeded that of PMTCT registers (14.8%, 95% CI: 13.6−16.1%) and confidence intervals failed to overlap. Overall HIV prevalence from EHR data was 15.8% (95% CI: 18.6−22.4%); wide confidence intervals overlapped with those of PMTCT register data and ANCSS overall. Similarities were found in HIV prevalence estimates between ANCSS and PMTCT among rural sites, but not among urban sites. HIV positivity was associated with women being >25 years of age, having had multiple pregnancies, having had more live births, and accessing urban (rather than rural) clinics. Associations of covariates with HIV were similar in the data sources.

**Conclusion:** Results of this evaluation suggest that PMTCT program data could be used for HIV surveillance in Zambia but only with improved accessibility and data quality. Results suggest routine PMTCT data collected by Zambia's EHR system could be used to monitor HIV trends where the system is in use and data are available for analysis.
Diagnostic Challenges With *Listeria monocytogenes* Identification From Food And Environmental Samples

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**Purpose:** An outbreak of listeriosis commenced in South Africa in 2017. The *Listeria* species responsible for the outbreak is *Listeria monocytogenes* (*Lm*), sequence-type 6 (ST-6). In response to the outbreak, testing of various food products and food processing environments for *Lm* occurred. It was critical that the diagnostic platform used, could differentiate *Lm* from other *Listeria* species. This is important as *Listeria* species and *Lm* share the same growth requirements and often co-exist in the same environment.

To ensure that the most accurate automated, diagnostic system is used for *Lm* identification, our laboratory performed a verification on the available platforms to us.

**Methods & Materials:** 50 *Listeria* species identified by whole genome sequencing (WGS) were tested on 4 platforms: (1) Microscan (Beckman & Coulter), (2) Vitek MS (Biomerieux), (3) Vitek 2 (Biomerieux) and (4) Surefast PCR kit (Congen). The *Listeria* species included 20 *Lm* strains and 30 *Listeria* species. The 30 *Listeria* species included 27 *L innocua* (a prominent *Listeria* species often associated with *Lm*), 2 *L seeligeri* and 1 *L welshimeri*.

**Results:** Among the 4 diagnostic platforms tested, no platform was ideal. All platforms overcalled *Lm* in place of other *Listeria* species. The Vitek MS misidentified *Lm* for other species and the Vitek 2 gave both *Lm* and *L innocua* options for few *L innocua* isolates. From our assessment, the worst performing platform for *Lm* identification was Microscan and the best performer was the Surefast PCR kit.

**Conclusion:** Although, isolate numbers were small, the above results suggest that further investigations are warranted. In addition, alternate testing platforms for *Lm* identification need to be investigated. Several ELISA based, fully automated technologies exist and need to be evaluated.
Bacterial Colonization of Drinking Water: Implications for an Aging U.S. Water Infrastructure

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Purpose: In 2014 the city of Flint began using water from the Flint River rather than Lake Huron in a cost-saving effort. Improper treatment resulted in corrosive water causing elevated levels of lead throughout the municipal drinking water system with grave consequences to the children living in Flint resulting in one of the country’s worst anthropogenic disasters. Factors such as decreased chlorine levels were favorable to growth of various bacteria including legionella, with 91 cases of legionella pneumonia in 2014-2015 with 14 deaths reported. In response to issues with lead, PoU filters were recommended for all households in Flint. The filters were successful in reducing lead exposure; however, their effects on bacterial infections have not been studied.

Methods & Materials: 10 homes in Flint with suspected cases of infection had water collected from the sink with the filter on, off, and from the shower. 10 Detroit homes were used as controls; water was collected from the kitchen sink and shower as only 1 out of 10 homes had a filter. 100mL sterile cups were used for water collection.

Results: Results of Flint samples are shown in table 1. No pathogens were detected from Detroit water. Residents of 7/10 homes in Flint had severe pneumonia, 1 sepsis, and 1 folliculitis. 5/10 patients died.

Table 1. Flint Water Results.

<table>
<thead>
<tr>
<th>Home</th>
<th>Filter</th>
<th>No Filter</th>
<th>Shower</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>P.aeruginosa 14CFU/mL</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>A.baumannii 6CFU/mL</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>3 sample 2</td>
<td>A. baumannii 35CFU/mL</td>
<td>ND</td>
<td>A.baumannii &gt;1250CFU/mL</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>P.aeruginosa &gt;277CFU/mL E.cloaceae 26CFU/mL</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>P.aeruginosa &gt;312CFU/mL E.cloaceae &gt;312CFU/mL K.oxytoca &gt;312cfu/mL K.pneumoniae &gt;312CFU/mL S.maltophilia &gt;312CFU/mL A.baumannii &gt;312CFU/mL</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>P.aeruginosa &gt;5000CFU/mL A.baumannii &gt;500CFU/mL</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>P.aeruginosa &gt;5000CFU/mL A.lwoffii</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>ND</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
Conclusion: The results of this study showed that even two years after switch of the water back to Huron Lake, Flint municipal water showed high levels of pathogens. These results have important implications for immune compromised patients, and other cities with aging infrastructure where PoU filters are being considered.
Tuberculosis In Chronic Renal Failure Stage V Patients At A Tertiary Care Nephology Unit In India.

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**Purpose:** There is a high incidence of TB in India. The immune dysfunction in patients with chronic renal failure Stage V (CRF-V) predisposed them to infections. Co-existing TB adds to morbidity and the treatment with anti-tubercular drugs requires knowledge of the proportion of drug excreted by kidneys and its clearance, the toxicity and the availability of alternate effective agents to cure the patient of TB. Analysis was aimed to know the incidence of TB in CKF-V patients.

**Methods & Materials:** CRF-V patients between 1st April 2015 and 1st March 2018 were retrospectively analyzed for TB. Data were collected on the demographics, clinical presentation, laboratory methods of TB diagnosis, disease site, comorbidities and outcome of tuberculosis.

**Results:** Out of a total 312 patients with CKD-V a total of 51 were screened for tuberculosis on basis of clinical suspicion by Microbiological / histopathological findings. Fever, weight loss and decrease appetite were the predominant clinical presentations. 7 patients were positive for Tuberculosis; the incidence being (13.7%) for tuberculosis. 57.2% were males & 42.8% were females. The co-morbid conditions were retroviral disease, diabetes and *Systemic lupus erythematosus* in one patient each. Extra pulmonary tuberculosis was diagnosed in 3 and pulmonary in 4. One patient was MDR and was managed on 2nd line drugs successfully. Only one patient died due to septic shock with CMV and PCP pneumoniae.

**Conclusion:** The incidence of tuberculosis was (13.7%) in CKD-V patients. Thus a high index of clinical suspicion & early diagnosis can promote a favorable patient outcome.
Norovirus Outbreaks in Abruzzo Region during 2017

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Purpose: Norovirus (NoV) is a pathogen frequently involved in non-bacterial gastroenteritis, causing diarrhea and vomiting in millions of cases worldwide annually. Transmission is mostly determined from person to person. However, NoV infection has additionally been associated with the consumption of raw shellfish or as a consequence of foodstuff contamination during processing or serving. A systematic review of outbreaks attributed to NoV between March and June 2017 in Abruzzo region was conducted to assess the contribution of NoV epidemiology in the human disease.

Methods & Materials: Ninety-eight fecal samples and 137 environmental swabs were analysed from three different settings: one restaurant, one bar and one long-term care facility. The analysis for the detection of Norovirus Genogroups I and II (NoVGI and GII) were conducted in accordance to the ISO 15216-2 2013, with modifications. The positive samples were assayed for NoV genotype, using Nested PCR.

Results: Overall, the prevalence of positive samples for the detection of NoV RNA was 44.4%. Among these, NoVGI was present in 0.4% of the samples and NoVGI in 44%. In particular, NoV genotype GII.2 was present in 4.1%, while the GII.4vSydney 2012 in 39.9% of the samples. The results obtained from the restaurant showed the presence of NoVGI.2, while NoVGI.4vSydney 2012 strain was detected in outbreaks occurred in the bar and in the long-term care facility, suggesting some correlation between these, also enforced by the same period in which the disease spread (March 2017). Different was the period of outbreaks at the restaurant (May-June 2017), supported by a diverse NoVGI subtype (NoVGI.2), confirming no correlation between the previous ones.

Conclusion: Person-to-person close contact, contaminated food and environmental surfaces were the probable transmission routes for the Abruzzo NoV outbreaks in 2017. The poor personal hygiene habits play an important role in the secondary spread of foodborne viral infections and, for this reason, it is very important to enhance surveillance systems and prevention procedures. Moreover, it would be useful to adopt surveillance systems involving food, environmental and clinical matrices, in order to facilitate activities of source attribution and for the prevention and control of potential epidemics.
Can Homegrown Feeding Program Be Sustained To Help Eliminate Soil-Transmitted Helminths: The Need For Reflection. A Study Conducted In Pilot schools, Anambra State, Nigeria.

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Purpose: Infection by soil-transmitted helminths (STHs) persists among pupils in Nigeria. Evaluation on the current infection status in pupils where Home Grown School Feeding Program (HGSFP) is been implemented was conducted one year after.

Methods & Materials: A total of 1677 consenting primary pupils aged 6 to 11 years from schools in three senatorial zones of Anambra State, Nigeria were enrolled. Kato-Katz technique was employed for examination of fresh faecal samples collected from pupils. Pupils were later treated with 400_mg Levamisole for direct worm count. Structured questionnaire was administered to assess knowledge, attitude and practice (KAP) towards helminthiasis.

Results: Results showed that of 1677 pupils, 49.4% were males and 50.6% were females. Prevalence of 4.94% was observed which was not significant (p>0.05) with respect to sex but significant (p<0.05) with age. STH ova observed were Ascaris lumbricoides (60.24%), Trichuris trichiura (12.05%), Ancylostoma spp. (12.05%), (15.66%) Co-infection was observed, but varied. The highest incidence of coinfection was between A. lumbricoides and Ancylostoma spp. (10.84%). Assessing intensity of A. lumbricoides in pupils after treatment, 36 female pupils voided 37 worms while 31 male pupils voided 64 worms. Of 101 voided worms, male worms were 49 and females 52; comprising 83 adult worms and 18 young worms. Assessment of KAP were significantly (P<0.05) related to transmission. Low prevalence of infection recorded is attributable to efforts of HGSFP.

Conclusion: Constant monitoring, surveillance and sustenance of water, sanitation and hygiene practices in HGSFP can help in the elimination of soil-transmitted helminths. Community involvement through health education is also advocated to reduce cases where siblings may be infected.
19.014
Situation of paratuberculosis and tuberculosis in small herds in an area with limited surveillance in northern Colombia.

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**Purpose:** Paratuberculosis and tuberculosis are diseases that can occur in ruminants, which produce granulomatous lesions in the intestinal tract and in the respiratory tract respectively. Although the epidemiological knowledge of these diseases is indispensable for public health decision-making, the situation is uncertain in the context of small herds in the Colombian Caribbean region.

**Methods & Materials:** In this study, the prevalence of paratuberculosis and tuberculosis was determined in the village La Peña, south of the department of the La Guajira (Colombia). 100 bovines and 74 ovines were evaluated for paratuberculosis and 72 bovines for tuberculosis. Samples of fecal material (paratuberculosis) and nasal exudate (tuberculosis) were collected, a parasitological examination was performed on the fecal samples and after a concentration process for both types of sample, bacilscopy and DNA extraction were performed with determination of the concentration and quality through the Nanodrop 2000, a Speed Oligo test was modified for detection of the Mycobacterium tuberculosis complex (CMT) for fecal DNA samples and a conventional PCR was applied for detection of Mycobacterium avium subsp paratuberculosis using primers for the insertion sequence IS900 (IS-900 F 5'tgatctggacaatgacggttacgga3 '/ IS-900 R 5'cgcggc acggctcttgtt3'). Collectively, the concentrated samples of nasal exudate were cultured in Lowenstein Jensen and Ogawa Kudoh medium

**Results:** The prevalence of paratuberculosis in bovines reached 10.2% and in sheep 4.05%, with 57.14% positive herds. Likewise, a tuberculosis prevalence of 11.11% was obtained, a figure higher than the one reported by the surveillance agency for 2017 with 55.5% of cattle positive for bovine tuberculosis.

**Conclusion:** There was a correlation between the signs and symptoms of paratuberculosis and molecular detection, however, no correlation was found with age. In contrast, in animals with cultures positive for CMT, no relationship was found with the signs and symptoms. These results expose the epidemiological complexity of these diseases in small Colombian livestock and corroborates that passive surveillance of these may be generating sub registers that underestimate the real problems in this type of area.
Pathogen Identification in Travelling Patients with Severe Acute Respiratory Infections from the Middle East to the Philippines, 2014-2016

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Purpose: Out of more than 2400 patients with severe acute respiratory infections who travelled to the Philippines from the Middle East since 2013, only two cases were confirmed positive for the Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Considering that these respiratory infections were imported into the country, it is important to determine the pathogen/s causing the disease to mitigate potential outbreaks that may overburden the country’s health care system.

Methods & Materials: Archived upper and lower respiratory samples (n=203) collected from 2014 to 2016 that tested negative by MERS-CoV RT-qPCR were tested using a multiplex PCR respiratory panel to detect human viral, bacterial, and fungal pathogens. The samples that tested negative for the multiplex panel were subjected to next generation sequencing (NGS). The NGS reads were analyzed using sequence-based ultrarapid pathogen identification (SURPI) computational pipeline and Centrifuge metagenomic sequence classifier to classify the reads according to their origins for further pathogen identification and to search for divergent viruses.

Results: Ninety percent of the samples were found to be positive for at least one respiratory pathogen. The most common bacteria detected were those associated with nosocomial and community-acquired pneumonia such as Klebsiella pneumoniae, Staphylococcus aureus, and Haemophilus influenza while the common viruses detected were Rhinovirus, Adenovirus, and Influenza A and B. In the 4 samples run on NGS, some of the pathogens identified were also associated with nosocomial and community-acquired infections and are important multi-drug resistant pathogens such as Pseudomonas aeruginosa, Streptococcus pneumoniae, Acinetobacter sp., Corynebacterium resistens, and Mycobacterium tuberculosis complex. Some viral and bacterial species that were supposed to be covered by the multiplex assay such as, K. pneumonia, Legionella longbeachae, L. pneumophila, Bordetella spp., Moraxella catarrhalis, Influenza A and C, and Human Metapneumovirus were also detected, highlighting the comprehensive capabilities of NGS in pathogen detection.

Conclusion: Respiratory pathogens constitute important causative agents of SARI in travellers. The results puts a spotlight on the need for identification of respiratory pathogens from traveling patients to enhance monitoring of imported infections, especially those with antimicrobial resistance to mitigate possible outbreaks from novel, emerging and re-emerging infectious diseases.
19.016
Flu Near You: Crowdsourcing Influenza-Like Illness Reporting in the United States Comparing the 2016-17 and 2017-18 influenza season with participant-reported symptoms

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Purpose: The United States Centers for Disease Control and Prevention (CDC) estimates that influenza sickened over 30 million people in the United States during the 2016-17 influenza season. While vaccination remains one of the best defenses against it, early detection is crucial to halting its spread. Recognizing symptoms early can allow public health officials, and the communities they serve, to take steps to reduce transmission.

Flu Near You is an online participatory disease surveillance system that allows volunteers in the United States and Canada to report their health information using a brief weekly survey. The system collects symptom data, which it publishes to the website, and offers an interface to compare its data with data from the CDC sentinel influenza network. Participating in Flu Near You is simple – individuals can enroll in the Flu Near You system by signing up at www.flunearyou.org.

Methods & Materials: Here we compare self-reported symptoms during the 2017-18 influenza season to the 2016-17 influenza season, to determine any differences in symptom reporting and healthcare-seeking behavior observed between the two years. Symptoms include: fever, headache, diarrhea, fatigue, nausea, rash, cough, sore throat, body ache, chills/night sweats, shortness of breath, and runny nose. Influenza-like-illnesses (ILI) include: fever and cough and/or sore throat.

Results: During the 2017-18 influenza season, 51.6% participants reported at least one symptom through FNY that was either self-reported (users) or submitted on their behalf (household members). Compared to the 2016-17 influenza season, 44.5% participants reported at least symptom through Flu Near You. We also observed that 22.7% participants with at least one symptom sought medical attention during the 2017-18 influenza season, compared to 26.5% participants during the 2016-17 influenza season.

Conclusion: We observe an increase in symptom reporting during the 2017-18 influenza season to date, compared to the 2016-17 flu season. We also observed a decrease in participants experiencing symptoms who seek medical attention. We hope to provide an opportunity for the public to engage directly, as well as continue to be the trusted source for community-level disease surveillance.
Elucidation Of Specific And Cross Reactive Zika Virus Antibody Target Regions


Purpose: Since the viremia in flavivirus infected patient is transient, detection of antibodies directed to flaviviruses is essential to identify the infected cases. Although in case of ZIKV, antibodies persist for longer period in serum, the cross reactivity to other flaviviruses is high, which hinder the development of a highly specific serological assay. In this study, we applied peptide microarrays to map individual ZIKV antibody targets on the ZIKV polyprotein with potential in differential diagnosis.

Methods & Materials: A total of 1643 15-merere overlapping oligopeptides encompassing the full amino acid sequences of ZIKV proteins of African, Brazilian, USA and French Polynesian origins were produced via SPOT synthesis and printed onto glass slides. Thirty-five serum samples from Zika outbreaks in Brazil, Senegal and Cape Verde as well as EU returning travelers were tested with the ZIKV chips. In addition, well-characterized archived serum samples of Dengue, Yellow Fever, Tick Borne Encephalitis, West Nile viruses were employed to identify any cross-reactive antibody targets.

Results: Sixty-eight antibody target regions were uncovered, 13 of which could be classified as ZIKV-specific. In addition, the 55 targets responsible for the cross reactivity to other flaviviruses were identified. The antibody targets are scattered across the entire ZIKV proteome with no striking preference of envelope over non-envelope proteins.

Conclusion: Our results pave the way for possible differential flavivirus assay. Further work is needed to identify the kinetic of antibody targets in relation to antibody viral enhancement, virus neutralization or associated with the neurological syndromes.
Validation Of A New Real-Time PCR Based Kit For Detection and Typing Of 5 Human Pathogenic Plasmodium Species, Including P. knowlesi In Whole Blood Specimen


Purpose: Malaria remains an important cause of morbidity and mortality worldwide. Even in settings in which malaria is not endemic, infections can be seen in individuals who have travelled to or emigrated from regions with ongoing malaria transmission. Human infection is caused by five Plasmodium species including P. knowlesi, which has emerged as a widespread cause of zoonotic human malaria in Southeast Asia, associated with a high risk of severe disease. Clinically, Plasmodium species are managed differently and it is important to identify the causal agent at species level.

The purpose of this study was the validation of a new real-time PCR based Kit for detection and typing of human pathogenic Plasmodium species.

Methods & Materials: A collection of 105 whole blood samples from individual patients was included in the study. All samples were previously analyzed and typed by an established in-house workflow at the Centro National de Microbiologia, Madrid, Spain.

The collection comprised 5 times 15 specimen pre-typed as P. falciparum, P. vivax, P. malariae, P. ovale, P. knowlesi, respectively, 15 specimen previously tested negative for human pathogenic Plasmodium species and 15 specimen previously tested positive for other parasites causing diseases with similar symptoms like malaria.

The specimen were thawed, nucleic acid was extracted using the QIAamp DNA Blood Mini QIAcube kit (Qiagen) and eluates were analyzed with the RealStar® Malaria Screen&Type Kit (altona Diagnostics) on an Rotor-Gene 6000 (Corbett-Research). The Internal Control (IC) of the RealStar® kit was added during the nucleic acid extraction process.

Results: All 30 specimens pre-tested negative for human pathogenic Plasmodium species were tested negative with the RealStar® Malaria Screen&Type Kit. The IC showed a valid result in all samples.

74 out of 75 specimen pre-tested positive for a human pathogenic Plasmodium species were tested positive and typed identically to the pre-typing results with the RealStar® Malaria Screen&Type Kit.

Conclusion: Our study shows, that the RealStar® Malaria Screen&Type Kit provides a reliable assay with high diagnostic sensitivity and specificity for the detection and typing of human pathogenic Plasmodium species. It can be a useful tool in the management of malaria patients.
19.019
Development of a Loop-Mediated Isothermal Amplification Method to detect Zika Virus

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Purpose: The emergence of Zika virus disease is a worldwide public health issue. South America is one of the most affected areas. Many methods to detect Zika virus (ZikV) has been developed. However, the low sensitivity, high cost and laborious procedures make of these methods very impractical, especially in South America. New diagnostic tools that involves minimal laboratory capacity is urgently required.

Methods & Materials: We developed a novel method based on reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) to detect ZikV. The ZikV RT-LAMP primers were designed based on the NS5 region using a multiple alignment of 64 ZikV complete genomes. Dried LAMP reagent was used for the reaction. Initial specificity and sensitivity assays were performed. Seven RNA templates of different arboviruses were analyzed by the RT-LAMP and only the samples positive for zika virus returned positive. Additionally, serial dilutions of one of the ZikV RNA templates we tested by RT-LAMP and real time RT-PCR (CDC-US).

Results: Comparison results showed that RT-LAMP is 1000 times more sensitive than real time RT-PCR; revealing detection limit as low as 0.0007pg. Finally, we evaluated 250 serum samples using the RT-LAMP and the results were compared with the RT-PCR and obtained a sensitivity of 96.77, a specificity of 100, a positive predictive value of 100 and a negative predictive value of 99.2.

Conclusion: Our novel ZikV RT-LAMP proved to have good performance and reliability. Furthermore, confirmation of the utility as a point-of-care testing provides a low-cost alternative for rapid diagnosis of ZikV in primary health care facilities or local hospitals with resource-limited laboratories.
Assessment of Event Based Surveillance in Cross Border Areas in South East Europe

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Purpose: This work presents preliminary results from a multi-country assessment conducted by the Southeast European Center for Surveillance and Control of Infectious Diseases (SECID) Network for the Connecting Organisations for Regional Disease Surveillance (CORDS) supported by The Rockefeller Foundation on national and local level event based surveillance (EBS), with a focus on cross border areas.

Methods & Materials: An online and paper-based assessment tool with qualitative and quantitative indicators was developed to assess EBS at national and local levels (cross border sites) for selected countries. Supplementary methods using qualitative approaches of structured interviews of key informants and a document review were used to identify different characteristics of EBS including objectives, guidelines and definitions, cross-sectorial collaboration, stakeholders and human capacity. Additional data was captured through a standardised event log where all the events occurring in the last 12 months were captured and indicators on event detection, verification and response were measured.

Results: The evaluation was conducted in three national level and fifteen cross border sites of Albania, Macedonia and Kosovo. Different operating practices for capturing, registering and monitoring public health events were identified. Rumours and mass media are screened routinely for any potential outbreaks and events are recorded mainly on paper-based log-books. Over a third of the events identified are reported by the non-health sector, 30% by community phone calls, 20% by rumours, 10% from media and in 5% of cases the notification is done in person by the community. Cases of diseases and outbreaks have been detected in 83% of investigated events. Most of the health events (26%) are related to food safety, followed by the environmental domain (22%) and 17% from the animal health domain.

Conclusion: In the assessed countries there was evidence of ongoing EBS, but a well-designed system was lacking. Guidelines and Standard Operation Procedures for event reporting, verification, investigation and response are in the process of development. The collaboration with other sectors is good but there is a lack of joint agreements for collaboration and data sharing. Routine data sharing between countries is operative mainly through epidemiological information exchange at national level but is missing at local cross-border areas.
19.021
Rapid Community Dog Assessment in Rabies Endemic Area

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Purpose: Rabies is prioritized as an important zoonotic disease which alarmingly ignites major public health and socio-economic concerns in Thailand. Hua Hin and Pranburi Districts, popular seaside resort towns in Prachuap Khiri Khan Province, were engulfed in rabies outbreak frenzy with 25 animal rabies cases reported in Pranburi during 2016 - 2017. To effectively implement rabies prevention and control schemes in the endemic area of Pranburi (population of 75,026 in 765.4 km² or population density of 96.7/km²), community dog population must be quickly and realistically accessed.

Methods & Materials: Therefore, a simple tool using smartphone application, Epicollect5, was employed to determine community dog population. Following relevant dog information were collected; photos, characteristics, sex, age, and GPS coordinates for place of presence. Both free-roaming and owned dogs were recorded in the center of Pranburi District (3.75 km²), which covers areas of communities, factories, schools, temples, agricultural farmland and unused land (overgrown bushes and/or patches of woodland). Free-roaming dog count was conducted within randomized 15 blocks from 65 blocks of Pranburi District central area on 8 consecutive occasions using photographic capture-recapture and sample block selection according to WSPA guidelines. Beck’s method was used to estimate the size of free-roaming dog population. Door to door survey was conducted to access owned dog number.

Results: In the surveyed area, free-roaming dog population was 395 (95% CI: 387-403) or 1,983 (95% CI: 943-2,022) calculated for total area. The free-roaming dog density was 527 dogs/km². A total of 1,509 owned dogs were found in the total area, which 50.5% of owned dogs were confined. The average number of owned dog was 2.26 dogs/household (95% CI: 2.08-2.44).

Conclusion: The results from this study provided fundamental information for community dog population management and rabies prevention/control campaign in Pranburi District. Photo capture-recapture method is a valuable approach for free-roaming dog estimation. Smartphone application can assist dog population monitoring system with ease in field work situation.
Active And Syndromic Surveillance For Mass Gatherings: BOOM Festival 2018, Portugal

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Purpose: To describe the implementation and results of an active syndromic surveillance team in a planned and recurrent international mass gathering (MG) allowing early detection of outbreaks, and thereby, allowing intervention by local, national and international authorities.

Methods & Materials: A field team of 17 health-care workers performed active and syndromic surveillance at Boom Festival 2018 between 21st and 29th of July 2018, in Portugal. Data including demographic characteristics, signs, symptoms, and environmental exposure was collected during admission at Boom Medical Service (BMS). Paper records were transcribed and analyzed with Microsoft Excel® 2016 (v 16.0). Descriptive analysis was performed and reported daily to relevant stakeholders.

Results: Approximately 30,000 people from 147 countries attended the BOOM Festival, and 3,100 healthcare episodes occurred, including 451 (14.5%) complaints of gastrointestinal symptoms. During the Festival, there was a considerable increase in the incidence of gastrointestinal symptoms and an early warning alert for a possible outbreak of acute gastroenteritis was issued to the national health authorities on 24th of July.

Interventions were carried out in collaboration with local public health authorities to control the outbreak and to address possible common-sources of infection. Contaminated food or water were suspected, and a person-to-person transmission was not excluded since several cases mentioned attending another MG and symptom onset before arrival at the festival. The concern about an early stage outbreak was valid since 62.5% (n=282) of the cases with gastrointestinal symptoms were reported between 26th-29th of July.

Conclusion: Active and syndromic surveillance are essential for timely data collection and analysis in MGs, delivering a comprehensive snapshot of daily incidents, enabling rapid response to possible outbreaks and providing early-warning alerts for stakeholders. Nevertheless, enhancements are needed: data sharing and improved communication channels between stakeholders and similar international MG are necessary to create an effective syndromic surveillance system.
Assessment of Event Based Surveillance in Cross Border Areas of Six Regional Networks

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Purpose: With its six member Networks, Connecting Organisations for Regional Disease Surveillance (CORDS – www.cordsnetwork.org ) aims to contain outbreaks at the source and keep communities safe from the spread of infectious diseases in animals and humans. One of its strategies is to promote innovations that strengthen event-based surveillance (EBS) at community level. Supported by a grant from The Rockefeller Foundation, and a collaboration with Ending Pandemics, the CORDS member Networks have started to assess EBS systems and practices, with a focus on cross border sites in selected countries in Sub-Saharan Africa, Southeast Asia, the Middle East and Southeast Europe, in order to share current collection methods of human and animal health events and identify areas of improvement.

Methods & Materials: A mixed methods design was employed. A standardised assessment tool was developed in web and paper-based formats to include quantitative and qualitative indicators to be assessed at national, regional and local levels. The tool assessed multiple domains in relation to EBS systems and processes, data collection tools and sources, event verification and response and intersectoral collaboration and cross border surveillance. Supplementary data collection methods of structured interviews with key stakeholders, document reviews and observations were used to obtain information. Networks received online training on the tool and trained data collectors within their Networks. Pre-testing and piloting of the tool was undertaken within Networks and experiences shared at a Network wide meeting. The main implementation phase is currently underway.

Results: Fifty-three cross border sites are included in the assessment across 14 countries (Indonesia, Laos, Thailand, Vietnam, Burundi, Kenya, Tanzania, Uganda, Israel, Jordan, Palestinian territories, Albania, Kosovo and Macedonia). We present the results of the process of implementing this methodology at a multi-country level and the opportunities and challenges of this approach and potential for scale-up at a wider level.

Conclusion: Cross border areas remain particularly at risk from disease outbreaks, however there is poor understanding of EBS practices within these settings. This research provides a standardised methodology to assess EBS systems across regions of the world with a focus on One Health and the use of digital tools.
Purpose: The main purpose of this study was to estimate the cost of various interventions and to quantify the economic impacts of rabies in Tunisia

Methods & Materials: The different calculated costs are divided into 3 categories:
- The total cost of the disease management which includes: The cost of vaccination, the slaughter of stray dogs and the cost of human rabies control.
- The cost of surveillance: seding samples, cost of investigations.
- The direct and indirect economic loss include the human outbreaks and livestock loss.

The data was collected from the statistics of the ministry of agriculture, the ministry of interior and the ministry of public health.

Results: The cost of human post exposure treatment (PET) between 2012 and 2016 was estimated to 13,428,336 Tunisian dinar (td). The total cost of the mass vaccination campaign of dogs and livestock is about 1,298,513,827 td. During this period, 157,852 dogs were slaughtered with an approximate cost of 134,174,200 dt. The total cost of animal rabies control including mass vaccination, laboratory analysis, surveillance cost and dog slaughter, during these 5 years, is estimated to be approximately 2,029,929,030 td. The economic Losses of livestock animals caused by rabies were estimated to be about 250,000 td between 2012 and 2013.

Conclusion: The total cumulated cost of all prevention and control measures is about 15,710,016,400 td. Despite these efforts, rabies remains enzootic and still threatens human and animal health.
Harmonisation Of Research Data For Congenital Zika Syndrome: Need For Core Data Sets For Epidemic-prone Infectious Diseases.

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Purpose: To reduce the delay in the clinical research response during outbreaks; we sought to define core variables for research on Congenital Zika Syndrome (CZS) based on the experiences of research responders in Recife, Brazil - a Zika affected region.

Methods & Materials: We employed a qualitative methodology with interviews and document review. Two sets of case record forms (CRFs) designed at the start of the Zika epidemic were reviewed; one developed by the Microcephaly Epidemic Research Group (MERG) in Recife and the other by ISARIC (International Consortium for Severe Acute Respiratory and Emerging Infections Consortium) and her collaborators. Semi-structured qualitative interviews were conducted to explore the views of the MERG team on core domains for research on CZS. Thematic discourse analysis was used to look for similarities and differences in the data.

Results: The document review indicated that the CRFs complemented each other and merging the domains from both sets of questionnaires produced a relatively comprehensive data set. The participants proposed a minimum set of domains for observational research on CZS and divided these into clinical and epidemiological domains based on the type of data generated. Cross-cutting themes which emerged were the importance of the research context and timing of research on the structure, construct and content of items in each domain. Participants underscored the need for dynamic CRFs at the start of an epidemic; because outbreaks are rapidly evolving, and questionnaires need to be adaptable to accommodate new information during the outbreak.

Conclusion: Developing a standardised case record form for clinical research involves various steps, each of which takes time. The domains recommended lend themselves to other arbovirus infections or infections with the potential for mother-to-child transmission. The study strengthens the need for an agreed core set of variables for various clinical syndromes and unique issues such as pregnant women and children. A methodology on how to decide on the data variables would be useful. A future study could be to identify what the generic research questions might be, to further refine the core data set in advance of an outbreak, given that CRFs should ideally be linked to predefined research questions and protocols.
Rapid Outbreak Identification Using Point Of Need Nanopore Sequencing

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Purpose: Polymerase Chain Reaction (PCR) is considered as gold standard tests for molecular detection of pathogens. The PCR relies on the use of known sequences of specific target genes. A false negative PCR result might be obtained in case of new variant of a known pathogen or emerging diseases. Alternatively, Next Generation Sequencing (NGS) can be applied to identify the pathogen responsible for the outbreak through sequencing all nucleic acids existing in the collected sample. In addition, NGS gives insight about the origin and variant of the causative agent. In this study we have established a protocol for rapid identification of the cause of unknown fever as well as the origin of infection.

Methods & Materials: A RNA mock sample with fever of unknown origin was tested using a sequencing protocol relying on multiple displacement isothermal amplification and oxford nanopore sequencing. The yielded data were analysed using offline BLAST search data bases to facilitate its implementation at low resource settings.

Results: The procedure took around 400 minutes including sample preparation and data analysis. In total, 63,678 sequence files were yielded. After running a local BLAST against a viral genome database, approximately 4% of the reads were identified as ZIKV. The complete ZIKV genome sequence was recovered with 2454 reads with an average read length of 685 bases. The in-depth sequence analysis revealed that it was more related to ZIKV isolated from Senegal.

Conclusion: In conclusion, the protocol enables rapid and reliable virus identification and serotyping. The procedure has been conducted with a mobile suitcase laboratory, which is easy to use at the point of need in endemic countries. However, the protocol is still cooling chain dependent and the cost per sequencing run are very high.
Analysis of Measles Surveillance Data in Balochistan Province, Pakistan 2017/18

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Purpose: To estimate disease burden secondary to recommendations for future strategies

Methods & Materials: 1st January 2017 to 31st August 2018 available surveillance data sets and clinical notes were analyzed. WHO case definitions for measles followed any person in whom a clinician suspects measles infection or any person with fever and maculopapular rash and cough, coryza or conjunctivitis while Presence of measles-specific IgM antibodies are laboratory confirmed. The demographic, clinical and laboratory data was extracted and analyzed by Epi info 7. The MCV coverage data was also analyzed.

Results: 3444 Measles cases identified, out of them 390 cases were laboratory confirmed. Male were 1837 (54%) while Female were 1589 (46%) and ages were varies between 02 months to 720 months and mean age of cases was 3.8 years. 96% cases were either unvaccinated or incompletely vaccinated. 116 deaths (Case Fatality Rate =3.4%) reported due to post measles complications. Complications were diarrhea 890 (26%) pneumonia 1070 (31%) and encephalitis 156 (4.5%). 187 (25%) cases were hospitalized. The trend shows that the peak number of reported cases was reached in November (n=848).

Highest number of cases were reported from Quetta district which accounted for 40% of cases (n=437). Total 74 Measles outbreaks were reported during 2017/18.

Conclusion: Province has poor routine measles immunization coverage, outbreak occurred in low vaccinated population and rapidly spread through a susceptible population. In response to the analysis of surveillance data, measles campaign and mop-up vaccination was suggested. Efforts to strengthen routine immunization coverage to more than 95%, using strategies like outreach, door to door services and regular pulse immunization should be adopted. Community awareness sessions, Defaulters tracking, enhanced surveillance and measles ‘catch-up’ mass immunization campaign are recommended to interrupt chains of transmission. It is recommended to conduct supplementary immunization activities throughout the province and permanent involvement of lady health workers program in routine immunization.
19.028
The development of the stochastic model for vivax malaria occurrence based on the climate factor in the Republic of Korea

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Purpose: We developed stochastic model for vivax malaria occurrence based on the climate factor in malaria risk areas.

Methods & Materials: We used the national surveillance database of registered malaria cases in period of 2001 to 2013 and malaria vector surveillance database in period of 2009 to 2013 from Centers for Disease Control and Prevention, Republic of Korea. Daily weather data were obtained from the Korea Meteorological Administration. Because global circulation indices ultimately affect climate variability on the Korean Peninsula, we adopted major oscillation of the oceanic climate variability in this study period. The effects of climatic factors in Anopheles mosquitoes transmission, effects of Anopheles mosquitoes in short-term incubation malaria transmission, and effects of climatic factors on long-term incubation malaria transmission were analyzed using Generalized linear Poisson models. We adjusted for seasonal variation, between-year variation, and other climatic factors.

Results: 1. A 1 ℃ increase in temperature was associated with a 41.4~44.4% increase in Anopheles mosquitoes after a 1~3 week lag, 2. A 100 mm increase in rainfall was associated with a 8.5% increase in Anopheles mosquitoes after a 7 week lag, 3. A 100 increase in Anopheles mosquitoes was associated with a 0.4~1.2% increase in short-term incubation malaria cases after a 2~4 week lag, 4. A 1 ℃ increase in temperature was associated with a 7.2~9.5% increase in long-term incubation malaria cases after a 1~4 week lag, 5. A 1 unit increase in El Nino Modoki was associated with a 39.8% increase in long-term incubation malaria cases after a 6 week lag, 6. A 1 person increase in short-term incubation malaria cases was associated with a 6.8% increase in long-term incubation malaria cases after a 31 week lag.

Conclusion: It is suggested that Anopheles mosquitoes associate with temperature and relative humidity, short-term incubation malaria cases association with Anopheles mosquitoes, and long-term malaria cases association with El Nino Modoki.
19.029
Potential immune-mediated genes expression induced by dengue antibodies enhance zika virus infection in THP-1 cell line.

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Purpose: Dengue virus (DENV) and zika virus (ZIKV) belong to the same family of flaviviridae. Due to high homology of amino acid sequences between DENV and ZIKV (42.9%, 42.5% and 57.0% identical for capsid, prM and E regions, respectively), Anti-DENV antibodies (DENV Ab) routinely show cross-reactivity in serological assays with ZIKV. In this study, we report the ability of sub-neutralizing DENV Ab to enhance (ADE) the ability of three different strains of ZIKV (One African and two Asian genotypes, Thailand and Philippines) to infect THP-1 monocytes cells. The potential immune mediators underlying ADE mechanism were also demonstrated.

Methods & Materials: Antibody dependent enhancement of ZIKV infection was tested using THP-1 cell line. Serial ten-fold dilution of pooled DENV infected sera or flavivirus negative human serum were separately mixed with each strains of ZIKV prior to infect THP-1 cells. Cells and supernatant were harvested to determined the level of intracellular ZIKV RNA and infectious virus production by quantitative RT-PCR (qRT-PCR) and plaque assay, respectively. The expression of immune-related genes including IRF-I, MDA5, RIG-I, ATG5, DAK, NOS2, SOCS3, IL-6 and IL-10 was evaluated by qRT-PCR using specific primer.

Results: Although non-significant differences in intracellular viral RNA were observed, significantly higher infectious ZIKV production were found when incubated in the presence of sub-neutralizing DENV Ab. We further investigated the ability of DENV Ab to facilitate ZIKV cell entry. DENV Ab increased cell entry of all ZIKV strains tested. Using quantitative RT-PCR, we measured various immune cytokines and mediators that may have contributed to the DENV Ab enhancement of ZIKV infection (ZIKV-ADE). Up-regulation of IL-10 was observed in ZIKV-ADE as early as 12h post infection, whereas expression of MDA-5 and IRF-1 was down-regulated in the presence of DENV Ab expression comparing to ZIKV infection alone.

Conclusion: The results from this study illustrate potential immune mechanisms underlying DENV Ab enhancement of ZIKV-ADE, in vitro. This information is of particular relevance to DENV and ZIKV vaccine developments and in areas where both DENV and ZIKV are endemic.
The incidence of malaria which exponentially increasing according to the proximity of the demilitarized zone in South Korea: 2011-2015

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**Purpose:** We tried to estimate whether the mosquito infected with malaria in the North Korea region crossed the DMZ and still have the influence to infect residents in South Korea. In the absence of credible data on malaria incidence in the North Korea, malaria incidence data near the demilitarized zone (DMZ) in the South Korea is a good window to look into the incidence of malaria in North Korea. So the data in this study were based on epidemiological survey on malaria patients from Korea Centers for Disease Control and Prevention (KCDC) in South Korea.

**Methods & Materials:** Total 3,150 malaria cases were reported in South Korea from 2011 to 2015. About 35% of all malaria cases were civilian infections in their residence. In this study, except for soldiers, veterans and civilians whose infection route is travelling, 1,139 civilian infected with malaria in their residence have been studied. They accounted for 36.2% of the total reported numbers. In 1,139 cases over 5 years, the number of cases of malaria infected civilian residents per 100,000 population was calculated at the level of eup·myeon·dong which is the basic administrative unit.

**Results:** As a result of analyzing only cases of residents from 2011 to 2015, the malaria rate decreased as the distance from the DMZ increased. And the incidence of malaria in civilian increased exponentially rather than linearly increasing as the distance from the DMZ decreased. This pattern was similar in the two administrative area, and the R-squared values in the exponential distribution model were 0.40 and 0.52, respectively, indicating high goodness of fit.

**Conclusion:** As you can be seen from the our results, the malaria occurring around the DMZ between the South Korea and North Korea in the 2010s is still most likely caused by anopheles mosquitoes crossing the border. In order to solve this problem, there is a limit to the efforts made within the territory of the South Korea, so it is essentially required to carry out the malaria prevention project in the border area with North Korea.
West Nile Virus In North-Eastern Italy: Overview Of Surveillance Activities In 2018

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Purpose: West Nile virus (WNV) has circulated in north-eastern Italy in 2008, and is considered to have become endemic in the area. In 2009, a national surveillance programme was implemented, and following re-modulated in Veneto and Friuli Venezia Giulia (FVG) regions, north-eastern Italy, since 2011. The aims of the regional plan were the early detection of WNV seasonal reactivation, to inform Public Health control measures. Hereby we report the results of the surveillance plan in Veneto and FVG for 2018.

Methods & Materials: A risk-based approach allowed to define the area where WNV was more likely to re-activate, considering the epidemiological situations of previous years. Active surveillance was performed on a representative sample of residential horses by searching IgM antibodies, indicating recent infections with WNV. Entomological surveillance consisted in bi-weekly mosquito captures and PCR-testing pools of the known mosquito vectors of WNV (Culex pipiens, Ochlerotatus caspius, and Aedes albopictus). Mosquitoes were collected by CDC-CO₂ or gravid traps, identified, pooled by species/date/location, and examined by RT-PCR. Passive surveillance was performed on found-dead wild birds, and syndromic horses in surveillance area.

Results: As of 31 August 2018, in Veneto, 1847 equine sera were screened, revealing 92 IgM sero-positive equines in 69 holdings. A total of 119,835 mosquitoes of 14 species were collected at 55 capture sites, and 1747 pools examined. One hundred fifty-three Cx. pipiens and 10 Oc. caspius pools tested positive for WNV. Seven non-migratory wild birds, out of 154 sampled, were positive for WNV. In FVG region, of the 186 equine sera tested in 79 farms, 8 resulted positive. A total of 9937 mosquitoes of 9 species were collected at 17 sites, and of 260 examined pools, 9 Cx. pipiens pools tested positive. Three wild birds out of 25 found-dead were PCR-positive.

Conclusion: In 2018 WNV re-appeared more than one month earlier than in previous years. Possible reasons could include the peculiar weather conditions, which might have influenced mosquito abundances and/or host-pathogen dynamics. The combination of veterinary and entomological surveillances into a broader integrated plan that includes screening of blood donors, proved valuable for early detecting WNV circulation, serving as trigger for enhancing public health measures.
Spatio-temporal and socio-demographic patterns of Chikungunya, Dengue, and Zika infections in Mexico in 2016-2017

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Purpose: Chikungunya, Dengue, and Zika viral infections are vector-borne diseases that are endemic in Mexico. Here we analyze the relationship between onset timing and climate data and socio-economic variables across the country.

Methods & Materials: We collected weekly incidence data, daily climate data, and socio-economic status on each state of Mexico in 2016-2017. The data sources are Mexican surveillance system, the Weather Underground, OECD.org, and INEGI Mexico. We measured the direct distances from six southernmost states of Mexico (i.e. Campeche, Chiapas, Oaxaca, Quintanaroo, Tabasco, or Yucatan) to all other states and compared them with the timing of the state-level curves. Then we performed step-wise multivariate analysis on weather and socio-economic variables to find the ones that best predict the onset timing of the three infections.

Results: Chikungunya and Dengue both showed "south-to-north" spreading patterns especially in the states that are located along the coast lines of Mexico. Low socio-economic status generally explained a significant fraction of the variation in epidemic onset compared to weather and geospatial variables (with the coefficients of determination, R², on onset weeks of chikungunya, dengue, and Zika epidemics being 0.279, 0.501, and 0.788, respectively). Across diseases, these socio-economic status variables included maternal mortality rate, quality of housing, unemployment rate, and air pollution.

Conclusion: The coastline and south-to-north patterns of spreading, as well as socio-economic factors, may be good predictors of epidemic onset for these vector-borne infectious diseases.
Degradation of ZIKV and DENV Envelope Protein through Selective Targeting to ERAD Pathway

19.033

Purpose: In the last decades, flaviviruses have contributed majorly in the global mortality and disabilities, posing a dire need for designing novel strategies to combat these infections.

Methods & Materials: The endoplasmic reticulum associated degradation pathway (ERAD) is the quality control mechanism of the cell for proteins within the secretory pathway which detects terminally unfolded or misfolded proteins and recruit them to proteasomal degradation through recognition by SEL1L protein. We have developed a strategy to exploit the ERAD pathway for induction of specific degradation of the viral envelope E protein using novel recombinant fusion proteins called degradins. These molecules are formed by an N terminal moiety with target specific binding activity and a SEL1L moiety, which effectively targets the protein to ERAD.

Results: The screening of a VHH based phage-display library identified DENV2 and ZIKV specific antibodies which recognize E protein within the ER, both as secretory and membrane bound forms targeting them to proteasomal degradation following retro-translocation to the cytosol. These molecules were observed to significantly reduce the production of DENV2, ZIKV and WNV pseudoviral particles.

Conclusion: This technique represents an effective mechanism to knock-out viral proteins within the secretory pathway with high specificity and selectivity.
Cefepime resistance gram negative bacteria among clinical isolates from Khartoum State hospitals 2017.

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Purpose: The Gram negative bacteria is leading cause of Varity of infectious e.g. Urinary Tract Infection, Gastro Intestinal Tract infections, dysentery, wound infections, septicemia, bacteremia, and meningitis. majors organisms contributed for these infections, are E.coli, Klebsiella spp., Proteus, Salmonella, Shigella,Enterobacter and Citrobacter. this study aimed to detect cefpim resistant pattern

Methods & Materials: Across sectional study was conducted in Khartoum state hospitals during the period from July to September 2017. 200 clinical isolates of gram negative were collected and identified base on standard microbiological methods, cefepime susceptibility testing was done for detection of cefepime resistant using disc diffusion method.

Results: 200 clinical isolate samples were involved in this study 80 (40%)were male while 120( 60%) were female, common clinical isolates identified as Escherichia coli 114 (57%), proteus 39 (19.5%) klebsiella 33 (16.5%), and pseudomonas 14 (7%). cefepime resistant isolates were 53(26.5%), cefepime resistant bacteria commonly detected were pseudomonas aeruginosa (57.1%) followed by klebsiella pneumonia (36.4%), less common by proteus (23.7%) and rarely we detect E.coli(21%).

Conclusion: Most of Gram negative bacteria resist cefepime, Pseudomonas aeruginosa were found the most commonly isolate cefepime resistant, old patient isolates show more resistant than younger.

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Purpose: This study was conducted to estimate the frequency of MecA and van A, B genes in Staphylococcus aureus among children. Different clinical samples were collected from 81 child's age range from (1-15) years old that was diagnosed as Staphylococcus aureus infections in Khartoum hospitals during period from October 2017 to December 2017.

Methods & Materials: Out from 81 there were 50 Staphylococcus aureus which had been confirmed phenotypically and genotypically 16s gene Using PCR technique.

Results: Methicillin resistant 94% and Vancomycin resistant 44%. The isolated Staphylococcus aureus further the DNA was extracted from. Polymerase chain reaction (PCR) techniques for amplification of four gene 16s, MecA and van A and B. The (PCR) result 28/50 revealed that 56% were positive for Mec A and none for van A and B.

Conclusion: The high frequency of circulating MecA gene highlights the need for policies to overcome the Staphylococcus aureus problem in clinical settings.
Antibacterial Activity of Fractions (Chloroform, Ethyl Acetate and Methanol) Red Betel Leaf Ethanol Extract (*Piper crocatum*) against *Streptococcus pyogenes* Bacteria

**Purpose:** This study was aimed to find out an antibacterial activity of chloroform, ethyl acetate, and methanol fraction from red betel leaf ethanol extract (*Piper crocatum*) in inhibiting or killing *Streptococcus pyogenes* bacteria. In addition, it also aims to identify the group of compounds from the most active fraction.

**Methods & Materials:** Making extraction was done by maceration using ethanol 70% (ratio 1: 5). Fractionation was done by column vacuum chromatography method. The eluent used are chloroform, ethyl acetate and methanol. The antibacterial treatment test was performed by microdilution method with dilution series of 10%, 5%, 2.5%, 1.25% and 0.625%. The identification of the group of compounds using thin layer chromatography (TLC) with silica gel stationary phase and the mobile phase of ethyl acetate: methanol (3: 1).

**Results:** The result of this research is most of the active fraction is methanol fraction. Methanol fraction has antibacterial activity with minimum inhibitory concentration (MIC) at 5% and minimum bactericidal concentration (MBC) at 10% against *Streptococcus pyogenes* bacteria. The result of identification of methanol fraction by TLC method was known fraction contains flavonoid compound which functions as antibacterial.

**Conclusion:** Methanol fraction has most active antibacterial activity compared to chloroform and ethyl acetate fractions. Group of compounds contained in methanol fraction is flavonoids.
Blood Stream Infections by Carbapenem Resistant Bacteria: Critical Perspectives from Indian Tertiary Care Hospitals

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Purpose: Emergence of Multi-Drug Resistance (MDR) Gram Negative Bacilli (GNB) is increasing at a faster pace, and carbapenems are the drug of choice for treatment. But rampant use of these agents has given rise to Carbapenem-Resistant Enterobacteriaceae (CRE). CRE causes wide range of infections, with Blood Stream Infection (BSI) associated with highest mortality. Thereby leaving colistin as the only available option for treatment. Our study was aimed, to measure the occurrence of CRE BSI in our institute.

Methods & Materials: A prospective observational study was performed over six months (July-December 2017). Blood culture was performed by BacT/ALERT automated system followed by identification of isolates and susceptibility testing by MALDI-TOF (Vitek-MS) and VITEK 2 system (bioMerieux, Inc., Durham, NC). The clinical characters, demography and resistance profiles of patients with culture proven CRE BSI were studied.

Results: Among the 53 patients, the mean (SD) age was 48.94 ± 20.66 years, with a male preponderance (M: F=2.5:1). On admission, 62.3% patients received antibiotics. Secondary bacteremia and ICU admission seen in 88.7% and 71.7% respectively. Median (IQR) time to bacteremia (since admission), stay in ICU and hospital was 10(11.5), 7(11.5) and 19(18.5) days respectively. Diabetes mellitus (23) was the most common comorbid condition. Among the isolates, Klebsiella pneumoniae was the most common (26) followed by Acinetobacter baumannii complex (14). Lower respiratory tract (24.5%) was found to be the most common source followed by hepatobiliary tract (17%). Median (IQR) for Pitt’s bacteremia (<24 hr of bacteremia) and SOFA score (on admission) was 4(4) and 7(8) respectively. About 96.2 % of the isolates were sensitive to colistin and 47.2% to tigecycline. As per sensitivity, combination of drugs were started but Colistin was given in 43.4% cases. Adverse event was seen in 73.9% patients received colistin. Favorable outcome was not seen in 41.5% patients.

Conclusion: CRE causing BSI is a significant threat to public health, warranting increased efforts towards strategies focusing on prevention, detection and infection control practices. Severe underlying conditions, use of invasive procedures and antibiotic selection pressure may predispose the patient to acquire CRE infection. Efficient management of underlying condition with optimal antibiotic may better the outcome.
Serotypes and Antimicrobial Resistance of *Salmonella enterica* Isolated from Clinical Samples of Diseased Pigs

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**Purpose:** In recent decades, *S. enterica* has been found to show high rates of resistance to the conventional antimicrobials used in human and animal medicine, with serious risks for public health. The monitoring of *S. enterica* in livestock may help decrease or prevent the emergence of multidrug-resistant strains and improve the understanding of *Salmonella* infection. For this reason, the aim of this study was to evaluate the serotype distribution and antimicrobial resistance rates of *S. enterica* isolated from diseased pigs in Thailand.

**Methods & Materials:** One hundred and forty-four *S. enterica* isolates were recovered from clinical samples of diseased pigs obtained from veterinary diagnostic laboratory, Kasetsart University between 2016 and 2017. Antimicrobial susceptibility tests were done using Kirby-Bauer disk diffusion method with a panel of 14 antimicrobials.

**Results:** The serotypes found were *S. Choleraesuis* (67%), monophasic *S. Typhimurium* (19%), *S. Typhimurium* (9%) and *S. Rissen* (6%). All *S. enterica* isolates were resistant to ampicillin, amoxicillin and tetracycline. Moreover, the isolates were most often resistant to cefotaxime (89.6%), followed by ceftriaxone (87.5%), chloramphenicol (83.0%), gentamicin (79.2%), nalidixic acid (73.0%), ceftazidime (71.5%), sulfa-trimethoprim (14.0%), colistin (14.0%) and ciprofloxacin (11.0%). However, all tested isolates were susceptible to amoxicillin/clavulanic acid and fosfomycin.

**Conclusion:** Our results revealed that the resistance to penicillin, amoxicillin and tetracycline was significant and thus the use of these antimicrobials for the treatment of infection caused by this bacterium is not appropriated. On the other hand, the antimicrobial activity of amoxicillin/clavulanic acid and fosfomycin demonstrated to be sufficient against *S. enterica*. Our results suggested the significance of monitoring system for controlling the resistant *S. enterica* strains in order to select the most effective drugs for decreasing the resistance to currently used antimicrobials.
Setting Up an Integrated Antimicrobial Resistance Surveillance System

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Purpose: This study reports Singapore’s ongoing efforts to integrate antimicrobial resistance (AMR) surveillance across human, animal, food and environment sectors based on best practices of established national integrated AMR surveillance systems, which may serve as a guide to other countries planning to do the same.

Methods & Materials: We identified potential modifications to Singapore’s system by applying lessons from the integrated AMR surveillance systems of six countries – Canada, Denmark, the Netherlands, Norway, Sweden and the USA – all recommended by the WHO Advisory Group on Integrated Surveillance of AMR. We reviewed all available reports in English online pertaining to these systems (as of 1 June 2018).

Results: Three were fully integrated and helmed by a single organization, while the others were split between the human and animal sectors. We noted that while a single organization is not needed for integrated AMR surveillance, competing priorities and separate funding sources could hamper objectives. Most target pathogens and antimicrobials were selected for clinical importance and transmissibility between animals, food, and humans; common target pathogens were Campylobacter, E. coli, Enterococcus and Salmonella. Data was collected via web-based programmes, or when samples were sent to designated laboratories for testing and characterization. Most surveillance systems, being in the EU, interpreted antimicrobial susceptibility testing (AST) results in animal and human isolates according to EUCAST epidemiological cut-offs and clinical breakpoints. To facilitate comparisons over time, levels of resistance for retrospective data were recalculated using current interpretive criteria. All systems conducted genotyping to detect the genetic background of certain resistance profiles, and the US NARMS system used whole genome sequencing, in particular, to track resistance genes over time. Data was analyzed and compared between sectors to detect the emergence of resistance genes and/or profiles, as well as to examine trends, which helped to inform regulatory policy and action.

Conclusion: We have adopted a multi-agency model targeting AMR surveillance at the common pathogens identified in this study. Currently, aggregate resistance data is collected in Singapore but we are exploring data from pharmacy prescription and laboratory information systems, and standardized AST interpretive criteria, to facilitate quantitative analysis and comparison across sectors over time.
Systemic antibiotic prescription rates and patterns in private sector in India: Evidence from medical audit data

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Purpose: India was one of the top consumers of antibiotics in 2015. However, critical evidence on antibiotic usage across therapeutic categories and disease conditions is missing. The objective of this study was to investigate systemic antibiotic (J01) prescription rates and pattern in India by analysing private sector medical audit data.

Methods & Materials: We analysed IMS Health (now IQVIA) medical audit data for the 12-month period from May, 2013 to April, 2014. We first coded the diagnosis provided in the medical audit data to disease codes based on the International Statistical Classification of Diseases and Related Health Problems, 10th Revision. Next, we coded antibiotics related information to the 3rd level of Anatomic Therapeutic Chemical (ATC) classification on the basis of the World Health Organisation’s Collaborating Centre (WHOCC) of Drug Statistics Methodology’s (ATC index-2016). Finally, we mapped the antibiotic prescriptions coded to the 3rd level ATC classification to the diagnosis based on ICD-10 classification. We report our results in standardized matrices of annual prescription rate per 1,000 persons, by age group, antibiotic class and disease condition.

Results: In India, approximately 519 million antibiotic prescriptions were dispensed in the private sector in 2013-2014, which translates into 412 prescriptions per 1,000 persons. Highest prescription rates were observed in the age group 0-4 years (636 prescriptions per 1,000 persons). The most commonly prescribed antibiotic class was cephalosporins (38.2%) followed by penicillins (22.8%), quinolones (16.3%) and macrolides (14.0%). Majority of these antibiotic prescriptions were for diseases of respiratory system (55%), followed by diseases of genitourinary system (10%), and for symptoms, signs and abnormal clinical findings (9%). Top disease conditions as per ICD-10 classification were acute upper respiratory infections (J06) (20.4%); unspecified acute lower respiratory infection (J22) (12.8%); disorders of urinary system (N39) (6.0%); cough (R05) (4.7%); acute nasopharyngitis (J00) (4.6%); acute pharyngitis (J02) (3.9%); and acute bronchitis (J20) (3.4%).

Conclusion: Our study has provided first national level estimates on antibiotic prescription rate and pattern in private sector. Overall antibiotic prescription rates in India are still much lower than US and Europe. However, prescription rates for beta-lactam antibiotics and quinolones are much higher, especially in children.
Monitoring for the Circulation of Antibiotic-Resistant *Salmonella* in Poultry and Wild Birds in Ukraine in 2017

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**Purpose:** The purpose of the study was to assess the circulation of antibiotic resistant *Salmonella* among poultry and wild birds in Ukraine to build a basis for predicting potential epizootics and developing preventive measures.

**Methods & Materials:** Isolation pathogens, as well as determination of the antibiotic resistance were conducted in 11 oblasts of Ukraine during 2017. In total, 1,469 samples collected from poultry, exotic, wild birds were studied using dense differential-diagnostic media, as well as nonselective and selective concentrated liquid culture media.

**Results:** We found 150 isolates belonging to 8 families, including *Enerobacteriaceae* (69.05%), in poultry, exotic domestic birds; 5 of them (3.3%) were classified as *Salmonella spp.* Other pathogens included: *Proteus spp.* – 19.30%; *E.coli* – 7.94%; *E. vulneris* – 3.97%; *Escherichia hermanii* – 0.79%; *Morganella spp.* – 3.97%; *Citrobacter diversus* – 5.56%; *Citrobacter freundii* – 6.35%; *Enterobacter spp.* – 10.32%; *Providencia spp.* – 3.17%; *Edwardsiella spp.* – 0.79%; *Serratia spp.* – 2.38%; *Kluyvera ascorbata* – 0.79%.

Fifty-eight cultures, including species of *Enerobacteriaceae* (62.0%), were isolated from wild birds, with only one culture referred to the *Salmonella spp* (1.72%). The following bacteria were isolated from wild birds: *Enterobacter spp.* – 21.0%; *Citrobacter spp.* – 18.5%; *Proteus spp.* – 17.5%; *Escherichia spp.* – 12.5%; *Serratia spp.* – 3.5%; *Morganella spp.* – 3.0%; and *Edwardsiella spp.* – 2.0%.

Five *Salmonella* isolates from poultry were found to be resistant to penicillines, tetracyclines, macrolides; in addition, two isolates to be also resistant to fluorophilonium, polymyxins. The culture isolated from a wild bird was resistant to macrolides, benzylpenicillin, tetracyclines. All six isolates from poultry and wild bird were sensitive to carbapenems.

**Conclusion:** We confirmed persistence of *Salmonella* among poultry in spite of extensive ant Salmonella program in Ukraine. Resistance to commonly used antibiotics indicates the need for control of their use in poultry. Isolation of *Salmonella* from wild birds is an evidence of their role as potential reservoirs; there are need to study mechanism of antibiotic resistance and conduct risk assessment of multidrug resistance in nature.
Evolution Of Antibiotic Resistance In *Neisseria gonorrhoeae* Strains Isolated On A Regional Laboratory Of Catalonia

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**Purpose:** To evaluate resistance of *Neisseria gonorrhoeae* in our region

**Methods & Materials:** We retrospectively reviewed the susceptibility to antibiotics of 305 strains of *Neisseria gonorrhoeae* isolated from January 2015 to June 2018 and compared it to the isolates from a previous period, from 2010 to 2014. Sensibility testing was performed using E-test to different antibiotics and penicillase paper strips for detection of beta-lactamase (BBL™ cefinase™ paper disc), and interpreted following EUCAST recommendations.

**Results:** During the study, 13% of the strains had a cefotaxime MIC (minimal inhibitory concentration) equal or higher than 0.125mg/L, similarly to the previous period, when 19% of the strains had a MIC equal or higher than 0.125mg/L. Moreover, 8.5% were resistant to cefotaxime, with a MIC higher than 0.125mg/L. Evaluating separately each year of our study period, cefotaxime resistance increased from 2.7% in 2015 to 14.2% in 2017. Similarly, resistance to cefixime increased from 8.2% in 2015 to 16.3% on the first half of 2018. Global resistance to penicillin was 18% during our study period, 13% due to production of beta-lactamase. Susceptibility to penicillin was only of 13%, compared with 26% in the previous period. Regarding azithromycin, 39% of our strains had a MIC higher than 0.25mg/L, similarly to the previous period (31%), with a decreasing resistance from 73% in 2016 to 20% in 2018. Combined resistance to cefotaxime and azithromycin was 5% globally, raising from 0% in 2016 to 8.3% in 2017. Our global resistance prevalence to quinolones was 67%, varying from 79% in 2016 to 59% in 2017, while, on the previous period, resistance to quinolones was 71%. Since 2015 to June 2018 only 2 strains were resistant to spectinomycin, 0.7% of all the strains. On the previous period, this percentage was 1%.

**Conclusion:** Beta-lactamic resistance has clearly increased during our study period, while azithromycin and quinolones resistance has decreased. It should be noticed than combined resistance to cefotaxime and azithromycin, the current treatment of gonorrheal infections, is in out of 5% of the strains.
Characterization of Colistin resistant Acinetobacter baumannii from clinical samples, India.

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Purpose: Acinetobacter baumannii is a major nosocomial opportunistic pathogen that causes severe clinical infections like bacteraemia, pneumonia, meningitis and urinary tract infection, most commonly in immune-compromised and critical-care patients. Management of MDR Acinetobacter spp. infections is a challenge for physicians. Its ability to survive in hospital milieu and persist for extended periods on surfaces makes it a frequent cause of healthcare-associated infections leading to multiple outbreaks. Polymyxin antibiotics, including colistin, are currently used as last-line drugs to treat MDR A. baumannii infections. Until recently resistance to colistin among A. baumannii has been well documented where resistance is mediated by loss of LPS production caused by point mutations in any of lipid A biosynthesis genes or genes involved in lipid A modification. This study was aimed to determine the prevalence and characterize colistin resistant (CR) A. baumannii strains collected from Safdarjung Hospital, New Delhi.

Methods & Materials: A total of 385 A. baumannii isolates from blood, pus, urine and endo-tracheal secretions were screened for colistin susceptibility using broth-micro-dilution method. Colistin resistant isolates were further analyzed for drug susceptibility following CLS guidelines. Resistant isolates were analysed for base mutations in lipid A biosynthesis genes (lpxA, lpxC and lpxD ), lipid A modification genes (pmrC, pmrA and pmrB) and carbapenem resistance genes determined by PFGE.

Results: Colistin resistance was observed in 7/385 (0.018%) isolates during September 2017 – June 2018. A(blaNDM and blaOXA) using gene specific primers. Clonal relatedness among test isolates was II isolates were carbapenem resistant and colistin MIC was in the range of 4 - ≥64 μg/ml. Predominant of CR isolates were from ICU, where prior colistin treatment was reported only in one patient. Molecular characterization performed for six isolates revealed lpxA gene to be mutated in one isolate, while multiple mutations were observed in chromosomally located pmrB and pmrC genes potentially contributing CR phenotype. All isolates were negative for mcr-genes. However, blaNDM-1, blaOXA-48 and blaOXA-23 genes were positively amplified from two, one and six isolates, respectively.

Conclusion: These results underline the occurrence of extensively drug resistant phenotype of A.baumannii being reported in hospital settings posing health care risks.
Antibiotic prescribing and susceptibility patterns among Internal Medicine Inpatients in Rwanda

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Purpose: Determine the current levels and trends in prescribing antibiotics to patients in internal medicine and obtain information on the common organisms isolated and their antimicrobial susceptibility patterns.

Methods & Materials: This study is a cross section prospective study. It was conducted in Kigali university teaching hospital and data were collected from patients were hospitalized in internal medicine department from January 2016 to July 2017. All patients above 16 years of age hospitalized in internal medicine department with confirmed bacterial infection and whose samples were collected for antimicrobial susceptibility analysis, were all included in this study.

Results: A total of 222 hospitalized patients in Internal Medicine ward during one year period (January to December 2015), their samples (blood, urine, sputum, CSF, pus and nasopharynx) were analyzed, of which 107 (48.19%) yielded significant growth. The mean age of this study population was 48 years with a ranging from 17-83 years. The female 56.31% were predominant. The majority of patients were from Kigali 58.56%. The E.coli (42.99%) and Klebsiella ssp. (31.77%) are the most common pathogens isolated. They have also high resistance rate to the commonly used antibiotics: amox/clav (87%), (98%), cefotaxime (67%), (100%), ceftriaxone (63%), (99%) and showed few resistance to imipenem (16%), (14%) respectively. We found that S. aureus was resistant to the mostly used antimicrobial drugs treating staphylococcal infections, including ampicillin, oxacillin, clindamycin 100%, 100%, 50% respectively.

Conclusion: The amplitude of AMR in the Kigali University Teaching Hospital is very alarming and challenging to the success of treatment of common infections with most available antibiotics. The MDR E.coli, Klebsiella ssp. and S. aureus are prevalent in our setting and have shown high resistance rate to the commonly prescribed antimicrobial agents including cefotaxime, ceftriaxone and amoxicillin-clavuluronic acid but we have observed a low resistance rate to imipenem and clindamycin.
Purpose: The key objective of this research was to investigate the trends in consumption of major antibiotic classes, their prescription rates and patterns and resistance pattern across key pathogens in India.

Methods & Materials: We used IMS Health’s (now IQVIA) medicine sales audit and medical audit data for medicine consumption and prescription analysis respectively. We expressed our data in standardized matrices of standard units (SU) per 1000 population per year to report consumption estimates and annual prescription rate per 1,000 persons, by age group, antibiotic class and disease condition for prescription pattern. We used antimicrobial resistance data for selected pathogens available in published literature. Finally, to analyze relationship between antibiotic consumptions and AMR pattern, we conducted regression analysis on pooled AMR data of E. coli, K. pneumoniae and P. aeruginosa and antibiotic utilization.

Results: Our analysis suggest that per capita antibiotic consumption has increased from 21.8 SU/1000 population per year in 2014 to 23.0 SU/1000 population per year in 2015, an increase of ~5.5%. This is also reflected in, 519 million antibiotic prescriptions were dispensed in 2014, which translates into 412 prescriptions per 1,000 persons. Majority of prescriptions were for diseases of respiratory system (55%) and diseases of genitourinary system (10%). The top antibiotic classes for prescriptions were cephalosporin (38%), penicillin (23%), and quinolone (16%). However, in terms of antibiotic sales and revenue, top contributors were cephalosporin (sales, 28%; revenue 51%), penicillin (sales, 21%; revenue 21%), and quinolone (sales, 16%; revenue 8%). In addition, our pooled analysis suggest that per SU increase in selected antibiotics use increases antimicrobial resistance in E. coli by 1.17%, K. pneumoniae by 0.36% and P. aeruginosa by 1.00%.

Conclusion: Our study provides first reliable and holistic estimates of antibiotic use, prescription patterns, and relationship with AMR in India. Our study also provides a reference point to measure the impact of interventions directed towards reducing antibiotic use.
Reciprocal Relationships In Antibiotic Resistance Of *Salmonella* Spp. Carried By Wild Birds And Fish In The Danube Delta

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**Purpose:** *Salmonella* spp represents one of the most important pathogens for numerous animal species also holding a strong zoonotic potential, found in most variable habitats, including touristic areas such as the Danube Delta. We hypothesized that: a) domestic animals/wild rodents will represent the major source for fecal pollution of the area and therefore b) the antibiotic resistance of the *Salmonella* spp. in other wild inhabitants will be high, thus posing an increased public health risk.

**Methods & Materials:** Samples (n=52) were obtained in the summer season from Letea (45°20′24″N 29°32′13″E) and Sfantu Gheorghe (44°53′47.0″N 29°35′38.0″E), two tourist sites in the Danube Delta Biosphere Reserve, from birds (*Calidris feruginea, Calidris minut a, Charadius dubius, Parus major, Parus caeruleus, Fringilla coelebs, Phyrulla phyrulla, Paser montanus, Carduelis carduelis, Pica pica*) and fish (*Tinca tinca, Perca fluviatilis, Silurus glanis, Cyprinus carpio, Esox lucius*) and were processed by bacteriological methods (Mac Conkey, Oxoid, API 20E test, bioMerieux). The antibiotic sensitivity patterns (Kirby Bauer) to penicillin, ampicillin, kanamycin, amikacin, tetracycline, oxytetracycline, enrofloxacin, erythromycin, marbofloxacin, ciprofloxacina and chloramphenicol were adapted to CLSI standards. MAR (multiple antibiotic resistance) index for each strain as well as mean values were calculated and analyzed by Statistica program. PCR was carried out to identify invA (*Salmonella* specific), rfbJ (group B) and wzxC1 (group C) genes encoding antibiotic resistance.

**Results:** Salmonellae were present in 52.12% of the samples. The results indicated a high MAR index in birds (0.63-1.00, x=0.84), over that recorded in fish (0.36-0.91, x=0.65). *Salmonella* spp. was 100% resistant to penicillin, ampicillin, kanamycin, amikacin, tetracycline, oxytetracycline, enrofloxacin, erythromycin, marbofloxacin, ciprofloxacina and chloramphenicol were adapted to CLSI standards. MAR (multiple antibiotic resistance) index for each strain as well as mean values were calculated and analyzed by Statistica program. PCR was carried out to identify invA (*Salmonella* specific), rfbJ (group B) and wzxC1 (group C) genes encoding antibiotic resistance.

**Conclusion:** The results confirmed that b) the antibiotic resistance of the isolates was high, posing a high health risk to the ecosystem but not a) the exclusive domestic animal/wild rodent origin of the bacteria. Supported by PNII/61-2012
Therapeutic Efficacy of Propolis Ethanolic Extract, Topical and Oral Antifungal Agents against *Epidermophyton floccosum*, *Microsporum canis* and *Trichophyton rubrum*

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**Purpose:** *Epidermophyton floccosum*, *Microsporum canis* and *Trichophyton rubrum* are among common agents for ringworm infections. There are several reports about development of resistance of dermatophytes to some antifungal agents. The aim of this study was to investigate and compare the therapeutic efficacy of propolis ethanolic extract, oral griseofulvin, ketoconazole and clotrimazole ointment in treatment of dermatophytic lesions in eighteen guinea pigs infected with *E. floccosum*, *M. canis* and *T. rubrum*.

**Methods & Materials:** After establishment of infections in the test animals, the antifungal agents were administrated. The infected guinea pigs were divided to two groups. The first group were treated with oral griseofulvin and the second one, with the topical agents. Oral solution of griseofulvin was administrated in a single dose of 15 mg/kg per day for 4 to 6 weeks. Ethanolic extract of propolis, ketoconazole and clotrimazole ointment were applied topically twice a day for 4 to 6 weeks. The lesions diameter was measured and the results were recorded daily.

**Results:** In case of griseofulvin, eight infected guinea pigs were treated completely in the fourth week and one after sixth week. In the case of Ketoconazole, seven infected guinea pigs were completely treated after the fifth week, while, two cases were not treated even after sixth week. The therapeutic effect of clotrimazole was better than ketoconazole. One of the guinea pigs infected with *M. canis* was not cured after applying of ketoconazole and clotrimazole. The ethanolic extract of propolis could cured the infections after fifth week. Totally, *T. rubrum* had better response to antifungal agents than *M. canis* and *E. floccosum*.

**Conclusion:** Propolis treatment results are comparable with chemical antifungal agents and it seems that, can be used for management of dermatophytic infections.
Variations of Antibiotic Resistance Patterns In *Vibrio Spp.* Isolated From Wild Birds Captured In The Danube Delta

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**Purpose:** *Vibrio cholerae*, *V. vulnificus*, and *V. parahaemolyticus* represent bacteria with high zoonotic and environmental impact, found in aquatic environments, pathogenicity of which seems to increase with major climate changes. We hypothesized that in areas such as the Danube Delta Biosphere Reserve, due to river flow and temperature fluctuations and also intense tourism and heavy wildlife load, the antibiotic resistance index will continuously increase, amplifying the public health risk.

**Methods & Materials:** Samples were obtained during the two year observation period (2013-2014), in the fall season, from birds (*Parus major, Carduelis carduelis, Pica pica, Hirundo rustica, Hippolais icterina, Coracias garrulus, Sylvia curruca, Sylvia atricapilla, Ardea ralloides, Ficedula parva, Nycticorax nycticorax, Emberiza schoeniclus, Erithacus rubecula, Acipiter nissus, Paser domesticus, Turdus merula*) captured around Sfantu Gheorghe tourist site in the Danube Delta, and were processed by TCBS Cholera medium cultivation (Oxoid), Rapid O*One NF Plus* (ThermoFischer Scientific) identification and slide agglutination test using *Vibrio cholerae* polyvalent agglutination sera (Oxoid). The antibiotic sensitivity patterns (Kirby Bauer) to marbofloxacine, eritromycine, amikacyne, ampicilline, penicilline, enrofloxacin, ciprofloxacine, streptomycin, oxytetracycline were adapted to CLSI standards. MAR (multiple antibiotic resistance) (Krumperman, 1983) index for each *Vibrio* species as well as mean values were calculated and analyzed by Statistica program.

**Results:** The results indicated an equal distribution of total numbers of *Vibrio* strains by year (17 in both 2013 and 2014). Sedentary species *C. carduelis* (n=6) and *Parus major* (n=4), represented in most cases the hosts for *V. cholerae* strains, while *Paser domesticus* was the most frequent carrier for *V. vulnificus* (n=5) and *V. parahaemolyticus* (1 of 1). The diversity of bird carriers for *Vibrio spp.* decreased in time. The highest but decreasing incidence was recorded for *Vibrio cholerae* (71 and 59%, respectively), followed by *V. vulnificus* (23 and 35%) and *V. parahaemolyticus* (6% in both years). Opposite, the MAR index significantly (*t=0.0007, p<0.001*) increased in *Vibrio cholerae*, while decreasing in *V. vulnificus* (0.36 to 0.24 and 0.33 to 0.22, respectively).

**Conclusion:** Our hypothesis was only partially confirmed, the MAR index and implicitly the public health risk showing a *Vibrio* species-specific rather than continuously increasing pattern. Supported by PNII/61-2012
Purpose: Urinary tract infection (UTI) have generally been described as the presence of microbial pathogens in the urinary tract with related symptoms. UTI is one of the most common bacterial infections in humans and a major cause of morbidity. The pathogens traditionally associated with UTI and their antibiotic sensitivity patterns are changing from time to time and across different environment. Knowledge of the antibiotic resistance patterns of uropathogens in specific geographical locations is an important factor for choosing an appropriate empirical antimicrobial treatment.

Methods & Materials: This study therefore evaluates the causative organisms present in urine specimen and their antibiotic susceptibility profile among patients suspected for UTI attending the general outpatient department clinic of the University of Port Harcourt Teaching Hospital, Nigeria. One hundred and fifty (150) mid-stream urine samples were collected from patients suspected of having a UTI and subjected to macroscopic assessment, isolation, characterization as well as resistance-susceptibility test of isolates using standard conventional techniques.

Results: Results showed that UTI was frequently encountered in females 39 (26 %) than males 21 (14 %) while 60 % of the samples yielded no growth after 48 hours incubation. The most common pathogens isolated were Staphylococcus aureus (47.19 %), followed by Escherichia coli (20.22 %), Klebsiella pneumoniae (15.73 %), Candida albicans (10.11 %), Pseudomonas aeruginosa (5.62%) and Proteus mirabilis (1.12 %). Staphylococcus aureus isolates were highly susceptible to Amoxicillin/Clavulanate (88.10 %) with lower susceptibility to Ofloxacin (52 %), Cloxacillin (50 %).

Conclusion: This information will directly affect selection of empirical therapy for UTI and emphasizes the need for choosing an appropriate antimicrobial treatment in specific geographical locations.
Increasing Multidrug and Fluoroquinolone Resistance among *Salmonella* Typhi from Sporadic Outbreaks in Kenya.

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**Purpose:** Background: Typhoid fever (TF) caused by *Salmonella* Typhi remains a major public health problem in Kenya. A systematic surveillance in two slum areas in Nairobi, revealed a crude incidence of TF of 247 cases per 100,000 person-years of observation (pyo), with highest rates in children 5–9 years old (596 per 100,000 pyo). Currently over a third of *S.* Typhi isolates are multidrug-resistant (MDR), and show reduced susceptibility to Fluoroquinolones; the drugs of choice for treatment of MDR cases. The situation is worrying especially for resource-limited settings where the few remaining effective antimicrobials are either unavailable or too expensive to be afforded by the general public.

**Objectives:** To determine the epidemiology and trends in Antimicrobial Resistance patterns among *S.* Typhi isolated from patients acquiring treatment in four clinics in Nairobi in the last 5 years

**Methods & Materials: Methods:** We assessed the susceptibility to commonly available antimicrobials of 225 *S.* Typhi isolates from 5 years of study (2009-2014) from sporadic outbreaks in clinics around Nairobi.

**Results:** Results: S. Typhi outbreaks were due to a single haplotype H58, which is the main cause of epidemics in South East Asia. Over last 5 years only 17.9% were fully sensitive. The majority (60.5%) were multiply resistant to commonly available drugs - Ampicillin, Chloramphenicol, Tetracycline (Minimum Inhibition Concentration (MIC) > 256µg/ml) and Co-trimoxazole (MIC > 32µg/ml). Nalidixic resistance was observed in 10% in 2009 to 18% in 2014 of isolates while resistance to Ciprofloxacin susceptibility increased from 5% to 10% in 2014.

**Conclusion:** Conclusion: The rate of increase in MDR over the last 5 years is worrying as more *S.* Typhi have become less susceptible to Fluoroquinolones. Improved hygiene and sanitation and use of World Health Organization-recommended vaccines should be considered for effective management of MDR TF.
The prevalence of naturally occurring HCV resistance-associated variants amongst treatment-naïve hepatitis C patients

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Purpose: Direct-acting antiviral agents (DAAs) have transformed hepatitis C virus (HCV) therapy. Yet in this new era, little is known about the prevalence of naturally occurring resistance mutations in HCV patients. Selection of these drug resistance associated variants (RAVs) could potentially cause reduction or failure of the DAA’s efficacy in HCV treatment. This study aimed to determine the prevalence and frequency of pre-existing drug resistance mutations in the viral quasispecies among DAA treatment-naïve patients in Singapore.

Methods & Materials: Next-generation whole-genome sequencing was performed on 39 clinical samples, including 21 genotype 1 (subtypes 1a [n=11], 1b [n=9] & 1c [n=1]) and 18 genotype 3 (subtypes 3a [n=17] & 3b [n=1]) using Illumina MiSeq platform. Sequence reads were mapped to HCV genome references and single nucleotide polymorphisms (SNPs) determined based on known RAVs in the NS3, NS5A and NS5B regions using Geneious v10.2.3 with minimum variant frequency threshold of 0.1% at positions with at least 1000X coverage.

Results: Overall, 23.1% (n=9/39) of patients harboured currently known mutations that confer resistance at clinically relevant frequencies (>10%). The intra-host population frequency of these RAVs ranging from 16.6 to 98.8% to either NS5A (M28V, A30T, L31M, H58L and Y93H) or NS5B (L159F and S556N) polymerase inhibitors. Low frequency mutations were also observed and of 37 patients with the NS3 region successfully amplified, 25 (62.5%) carried at least 1 to 5 variants out of 12 RAVs at frequencies >0.1%. Similarly, for the NS5A region, 16 (50%) out of 32 patients showed 1 or 2 variants of 9 RAVs observed and in the NS5B region, 19 (59.3%) out of 32 patients showed the presence of 1 to 6 variants of 21 RAVs.

Conclusion: Mutations conferring DAA resistance were detected in protease and polymerase regions of HCV genotypes 1 and 3 from DAA-naïve patients screened in Singapore. While many RAVs were predominant in the viral population some low-level mutations were also observed. The significance of the RAVs identified here and their impact on the treatment options remains to be determined. Despite this, our work provides a baseline of RAVs prevalence to help establish guidelines for assessing the impact of DAA therapy.
Carbapenemases and efflux pumps in clinical Acinetobacter baumannii: biomolecular characterization of multi-drug resistant isolates from a hospital in Central Italy

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Purpose: Multi-drug resistant (MDR) Acinetobacter baumannii is increasingly responsible of hospital-acquired infections, mainly in intensive care units (ICUs). Aims of this study were to assess antibiotic resistance phenotypes and clonal relationships in A. baumannii isolates from hospital setting, and to evaluate circulating carbapenemases (blaKPC, blaGES, blaSHV, blaIMP, blaVIM, blaNDM, blaGIM, blaAmpC, blaOXA-23, blaOXA-24, blaOXA-51, blaOXA-58). Prevalence of multi-drug efflux pumps encoding genes (adeB, adeG, adeJ, abeS, craA, abeM) and their negative regulator (soxR) was also determined.

Methods & Materials: Clinical A. baumannii (n=24) were collected from "A.Cardarelli" hospital (95.8% from ICU), in Molise Region (Central Italy). The Minimum Inhibitory Concentration (MIC) was assessed for 20 antibiotics by BD Phoenix™, including carbapenems (imipenem and meropenem). Molecular typing was performed through Pulsed-Field Gel Electrophoresis (PFGE) with Apal and Ascl enzymes, while PCR assays were carried out to detect carbapenemases and multi-drug efflux pumps encoding genes.

Results: A. baumannii strains were resistant to almost all antibiotics, including carbapenems. The combined Apal/Ascl dendrogram analysis revealed four clusters and seven pulsotypes at 95% similarity (Simpson Index: D=84.0%). Among resistance genes, all strains harboured blaOXA-23, blaOXA-51 and blaAmpC, and 33.3% (n=8) blaVIM, while blaKPC, blaGES, blaSHV, blaIMP, blaNDM, blaGIM, blaOXA-24, and blaOXA-58 were never detected. On multi-drug resistance mediated by efflux pumps, all isolates were adeB, adeG, adeJ, soxR and abeS positive, while abeM and craA were not found.

Conclusion: The study findings highlight the circulation of MDR A. baumannii in hospital ICU, underlining the urgent need of strategies for limiting their spread. Molecular epidemiology showed that isolates are characterized by a remarkable resistance to carbapenems, explained by the highest prevalence of blaOXA-23 and blaOXA-51, in line with other Italian studies. Interestingly, detection of blaVIM suggest an important increase of this carbapenemase circulation, which is occasionally found in A. baumannii in Italy. The lack of other resistance genes confirm their very low occurrence, according to nationwide distribution. The value of D confirms the need of integrate other typing methods with PFGE. Further studies are required to evaluate clonal diversity through Sequence Types distribution and on the efflux pumps role in resistance mechanisms by transcriptional level analysis.
20.020
Epidemiology of Multi Drug Resistant Gram Negative Bacteria in Kathmandu, Nepal.

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Purpose: Multi drug resistant (MDR) bacterial infections are considered to be hospital-acquired in high income countries. Little data are available regarding the epidemiology of MDR bacteria in low and middle-income countries. Our study looked at the epidemiology of gram negative bacterial infections in Kathmandu, Nepal.

Methods & Materials: Kathmandu Model Hospital is a 125 bed tertiary care hospital, in Kathmandu, Nepal. All patients hospitalized with MDR bacterial infections over a 12 month period were retrospectively evaluated. MDR bacteria included those with extended spectrum beta lactamase (ESBL), carbapenem (CRE) or colistin resistance. Age matched controls of patients with clinical infection but negative cultures were selected during the same time period. Water surveillance was performed to identify a potential source of environmental exposure. 55 vials were filled from various drinking water sources in Kathmandu; including bottled water, home reservoirs, and community taps. Water samples were analyzed by centrifugation and conventional culture techniques.

Results: No significant differences in patient characteristics were noted between the groups (Table 1). Among the cases, 73% had E. coli, 15% had Acinetobacter sp (ACBC), and 12% had K. pneumoniae. The majority of infections in both groups were community acquired. 5 water samples from 4 locations were found to have ACBC.

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<td>Age years mean (range)</td>
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<td>54 (18-89)</td>
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<td>Sex</td>
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<tr>
<td>Male</td>
<td>13 (32%)</td>
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<td>Female</td>
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<td>Genitourinary</td>
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<td>Skin and Soft Tissue</td>
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<td>Abdominal</td>
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<td>Unknown source</td>
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<td>Surgery</td>
<td>9 (23%)</td>
<td>10 (25%)</td>
</tr>
<tr>
<td></td>
<td>Community Acquired</td>
<td>Hospital Acquired</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Gynecology</td>
<td>5 (13%)</td>
<td>3 (7%)</td>
</tr>
</tbody>
</table>

**Organism**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Community Acquired</th>
<th>Hospital Acquired</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>ACBC</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>No growth</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

**Conclusion:** Gram negative bacterial resistance is a prevalent concern in low and middle-income countries. Our study demonstrated gram negative bacteria in the drinking water in various locations throughout Kathmandu. This is a possible source of community acquired MDR bacterial infection.
Prevalence Of Antimicrobial Resistance In *Salmonella* Spp. Strains Isolated From Human In Kharkiv Region, Ukraine

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**Purpose:** To investigate the prevalence of antibiotic resistance in *Salmonella* strains isolated in epidemic foci of human Salmonella disease in Kharkiv region, Eastern Ukraine and to assess the changes in the antibiotic resistance rate in 2013-2016.

**Methods & Materials:** The samples were collected in foci of human Salmonellosis cases during 2014-2016. 3370 *Salmonella* strains were isolated according to The Clinical & Laboratory Standards Institute (CLSI) guidelines. Levels of antibiotic resistance were tested by agar disk diffusion method with 15 antimicrobial agents in 1570 *Salmonella* strains. The data was analyzed statistically using Microsoft Excel - 2016.

**Results:** We found an increase in the antibiotic resistance of *Salmonella* isolates, from 16.7 % in 2014 to 91.1 % in 2016. We observed resistance only to gentamicin in 2014, to ampicillin, doxycycline and gentamicin in 2015, to ampicillin, doxycycline, gentamicin, co-trimoxazole and tetracycline in 2016. Ampicillin resistance increased from 0% in 2014 to 42.1% in 2015 and 57.1% in 2016. Resistance to doxycycline varied from 0 % in 2014 to 16.8 % in 2015 and 8.9 % in 2016. For gentamicin proportion of resistant *Salmonella* strains ranged from 16.7 % in 2014 to 6.3 % in 2015 and 12.5 % in 2016. The isolates showed sensitivity to cephalosporins and carbapenems. Co-trimoxazole resistance level was higher than tetracycline resistance level (8.9 % and 3.6 % respectively) in 2016.

**Conclusion:** The resistance rate of *Salmonella* isolates was generally low for the most antibiotics in 2014 but prevalence of antimicrobial resistance in *Salmonella* strains was increasing. We observed the highest level of resistance for ampicillin and noted the widening of the spectrum of antibiotics to which resistance of *Salmonella* strains was developed. Because resistant *Salmonella* strains cause more severe cases of disease and increase the risk of death it is very important to monitor the resistance development in *Salmonella* strains. The results of this study show the value of surveillance of the antibiotic resistance of *Salmonella* for the development of the regional guidelines for the selection of the rational antibacterial treatment of Salmonellosis cases in humans.
Purpose: Streptococcus pneumoniae is one of the leading causes of death. Colonizers are main source for infections. In Jordan, children are majority as risk group. The aim is to determine pneumococcal carriage rate, resistance, serotype distribution and coverage of pneumococcal Conjugate Vaccines (PCVs) from 2-4 years old children attending DCCs in Irbid and Madaba, Jordan.

Methods & Materials: Nasopharyngeal swabs were collected from 329 children 2-4 years of age from Madaba (n=198) and Irbid (n=131) cities. Swabs were cultivated on Columbia blood agar base supplemented with 5% sheep blood and incubated for 18-24 hours at 37˚C with 5% CO₂. Suspected alpha hemolytic isolates were tested for optochin sensitivity and bile solubility. Isolates were analyzed for antimicrobial susceptibility by Vitek2 system (BioMérieux) and E test and serotyping by the Neufeld Quellung method.

Results: Total pneumococcal carriage in Irbid and Madaba was 29.0% and 37.4%, respectively. Coverage of PCV7, PCV10 and PCV13 in Irbid was 42.1%, 42.1% and 57.9%; where as in Madaba was 52.7%, 54.1% and 64.9%, respectively. Coverage of multi-resistant isolates by PCV7 and PCV13 in Irbid was 42.1% and 57.9% and for Madaba 76.5% and 94.1%, respectively. Resistance rates in Irbid and Madaba for the ages 2-3 years and 3-4 years was as follows: Penicillin (90.9%; 81.3%) for Irbid, and (91.1%; 89.7%) for Madaba; clarithromycin (72.7%; 81.3%) for Irbid and (64.4%, 75.9%) for Madaba; trimethoprim-sulfamethoxazole (100%; 100%) for Irbid and (100%, 93.1%) for Madaba. Multiresistance in Irbid and Madaba was 78.9% and 68.9% respectively. 81 isolates were macrolide resistant in both cities, where 48.3% and 51.7% were M-phenotype and cMLSₐ for Irbid and 42.3% and 55.8% for Madaba, respectively. Predominant serotypes were 19F (18.8%) and 23F (11.6%) in both cities.

Conclusion: There was high carriage of pneumococci in both cities. The resistance has reached an alarming rate for penicillin and trimethoprim-sulfamethoxazole in both cities. Localizing specific serotypes among specific areas is recommended for better control with the available PCVs.
Purpose: Gonorrhea, estimated 78 million people infected globally each year, was increasing in antimicrobial resistance. The global surveillance programme reported resistance rate to different antibiotics including ciprofloxacin (97%), azithromycin (81%), and cefixime and/or ceftriaxone (66%) from 2009 to 2014. Taiwan CDC reported over 4,000 cases each year and high resistance rate to ciprofloxacin (95%). An intramuscular injection of ceftriaxone was the first-choice therapy for uncomplicated infection. The aim of this retrospective study was to analyze the clinical and microbiological aspects of gonorrhea.

Methods & Materials: The data was from the records of medical charts and microbiology laboratory at Jen-Ai Hospital - Dali, a 602-bed regional teaching hospital in central Taiwan. This study enrolled patients with positive cultures for Neisseria gonorrhoeae from 1 January 2016 to 30 June 2018. The following information was collected: age, gender, clinical presentations, concurrent sexually transmitted diseases, specimens, antimicrobial susceptibility testing results, and antibiotic treatment.

Results: Most of 40 positive-culture specimens were urethral discharges from men, and one was vaginal discharge. The median age was 28 years (range: 16-56). The clinical manifestations of infected men were urethral discharge and dysuria, and only one person had scrotal pain. The woman had lower abdominal pain and fever. Two men had syphilis and one had HIV infection. The susceptibility rates of ciprofloxacin and extended-spectrum cephalosporins were 0% and 100% separately. One third of patients received azithromycin and another one third received minocycline together with ceftriaxone.

Conclusion: Neisseria gonorrhoeae was highly resistant to ciprofloxacin, but still quite susceptible to ceftriaxone. A small proportion of patients with gonorrhea had concurrent sexually transmitted diseases.
Non Sorbitol Fermenting Escherichia coli as Indicator Organism of Microbial Health Risks Associated with Integrated Urban Farming in Dar es Salaam, Tanzania

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Purpose: Escherichia coli such as E. coli O157:H7 a Non-Sorbitol Fermenting (NSF) E. coli is important human pathogen among other common zoonotic pathogens carried by animals especially cattle. They are discharged through their faeces into the environment. With the increasing practice of urban farming, livestock manure is used as organic fertiliser in either fish ponds or vegetable gardens. This practice increases the risk of transmission of such pathogens to humans. This study aimed at determining the occurrence, antimicrobial resistance profiles and genetic relatedness of NSF E. coli isolates from manure, vegetables and fish.

Methods & Materials: Microbiological standard methods were used to isolate and identify E. coli isolates from manure, vegetables and fish samples. Confirmed isolates on biochemical tests were tested for resistance against six antibiotics using the disc diffusion method. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) typing method was used to generate fingerprints and determine the genetic relatedness of the E. coli isolates.

Results: Out of 156 samples including manure, 53 vegetables and 16 fish, 36 (23.1%) samples were positive for NSF E. coli from where a total of 48 E. coli different isolates were recovered that were subjected to antimicrobial susceptibility testing and genetic relatedness. Of these isolates, 25 (52.1%) were resistant to at least one antimicrobial agent and 12 (48.0%) showed Multidrug Resistance. ERIC-PCR profiles of E. coli isolates from manure, vegetables and fish showed genetic diversity with genetic relatedness ranging from 74.5% - 100%. Nine phylogenetic clusters (I - IX) determined at 90% threshold level of genetic relatedness were identified among the isolates.

Conclusion: These results show potential microbial health risk urban integrated farming pose to humans and hence the need to monitor and improve husbandry practices in urban farming.
Assessment Of Multidrug Resistant Bacteria Removal After Environmental Ozone Treatment

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**Purpose:** Ozone (O\textsubscript{3}) is a powerful antimicrobial substance that could be used to disinfect human and veterinary hospitals and outpatient clinics, where multidrug resistant (MDR) bacteria represent a concern. The aim of this study was to assess the efficacy of an air O\textsubscript{3} treatment against MDR bacteria derived from pets.

**Methods & Materials:** MDR *Staphylococcus pseudintermedius*, *Enterococcus faecium* and *Enterobacter hormaechei*, isolated from infected pets, were used to perform decimal serial dilutions plated on Nutrient Agar plates (OXOID) (Total Plate Count). Two sets of plates inoculated with the same dilution were placed in two identical laboratories (18 m\textsuperscript{2} and 54 m\textsuperscript{3} each one). In lab 1 the plates were exposed to the air (negative control), in lab 2 to the air treated with O\textsubscript{3} using the Sany Water Plus device (Sanity System). After the exposure, the plates were incubated at 37°C for 24 hours. The experiment was conducted in triplicate for each strain.

The bacterial removal (%) was calculated as the difference between the negative control count (100%) and the count after O\textsubscript{3} exposure.

**Results:** O\textsubscript{3} reached a concentration of 1.09 ppm. The *S. pseudintermedius* removal was 98.6%, 91.8% and 99.5% in the 3 experimental repetitions with an average of 96.6%; the *E. faecium* removal was 98.5%, 99.5% and 99.9% with an average of 99.3% and the *E. hormaechei* removal was of 49.2%, 66.7% and 86% with an average of 67.3%.

**Conclusion:** The effectiveness of the O\textsubscript{3} treatment was assessed on two MDR gram-positive bacteria (*S. pseudintermedius* and *E. faecium*) and on a MDR gram-negative strain (*E. hormaechei*). The results showed a strong drop of the bacterial concentration with differences attributable to variations in the cell wall structures between Gram-positive and Gram-negative bacteria and to the particular features of resistance of *E. hormaechei*.

The use of O\textsubscript{3} for environmental sanitization can therefore represent a valid aid to strengthen the effects of the common sanitization and disinfection procedures, considering its ability to act on fine dust and on surfaces that are difficult to reach and considering that MDR is often associated with resistance to disinfectants.
Purpose: The aims of this prospective interventional study were to develop and implement a local antibiotic policy by applying antibiotic stewardship strategies at ICU setting and evaluating the adherence to the implemented policy.

Methods & Materials: All patients admitted to ICU of Ahmed Maher Teaching hospital, Cairo (August - October 2016) were included in this study. An antibiogram was developed based on culture sensitivity from isolates of urine, sputum, blood and swab, one year earlier. Local antibiotic policy was approved based on a harmony between the antibiogram and the updated international antimicrobial guidelines (e.g. Infectious Diseases Society of America (IDSA) and Sanford Guide to Antimicrobial Therapy). Daily interventions and recommendations for antibiotic selection, dose, duration and route of administration were documented by clinical pharmacists. Statistical analysis was performed using Excel® 2010.

Results: During three months’ interventional period, seventy-seven cases were reviewed, 55 % were females and the average age and weight were (64±12.66, 76.5±10.33), respectively. The most frequent co-morbidities were cardiovascular, renal, diabetes and hepatic diseases. The most commonly prevalent infections were community, hospital, aspiration pneumonias and line infections. Sixty drug-related problems were collected including: violation of protocol (68.3%), inappropriate dose (25%), inappropriate duration (3.3%) and inappropriate route (3.3%). Interventions recommended by clinical pharmacists were sixty including: implementing antibiotic policy (65.6 %), optimizing dose (23.3%), de-escalation (11.6%) optimizing duration (5%) and switching to oral route (3.3 %). Total number of accepted interventions was Fifty (83%) including: Implementing antibiotic policy (54%), optimizing dose (26%), de-escalation (10%) optimizing duration (6%) and switching to oral route (4%).

Conclusion: Developing and implementing the Egyptian antibiotic stewardship is an inevitable issue for enhancing rational antibiotic use and minimizing antibiotic resistance at the national level.
Multidrug Resistant (MDR) *Acinetobacter baumannii*: Rate of Occurrence from a Tertiary Hospital, Malaysia.

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**Purpose:** *Acinetobacter baumannii* (Ab) is a rapidly emerging nosocomial pathogen. In the last few decades, Ab has been documented not only as an important nosocomial pathogen, but to harbor multidrug resistance (MDR) properties. The ability of Ab to persist in the hospital environment, combined with its intrinsic resistance to many antimicrobial agents, contributes to the organism’s fitness and spread. This is worrisome, as it produced an increased rate of morbidity and mortality with significant financial burden worldwide. This study aimed to investigate the prevalence and occurrence rate of MDR *Acinetobacter baumannii* circulating in a tertiary hospital in Malaysia.

**Methods & Materials:** A total of 102 isolates were gathered from the Microbiology Laboratory, Hospital Sungai Buloh, Malaysia, from August 2017 to April 2018. Specimens were collected from patients admitted to an intensive care unit (ICU), coronary care unit (CCU), neonatal intensive care unit (NICU), high dependency ward (HDW), and general wards. Both sterile and non-sterile specimens including tracheal aspirate, bronchoalveolar lavage (BAL), blood, cerebrospinal fluid and others were received and processed accordingly. Standard biochemical methods were performed to confirm the identity of the isolates, with antimicrobial susceptibility test was performed using disk diffusion and Epsilometer test (E-test). The zone of inhibition and minimum inhibitory concentration were determined based on Clinical and Laboratory Standards Institute guideline. The antibiotics tested were imipenem (IMP), ceftazidime (CAZ), gentamicin (GN), amikacin (AK) and ciprofloxacin (CIP).

**Results:** *Acinetobacter baumannii* was most prevalent in HDW with an occurrence of 42.16%, followed by ICU (28.43%) and general wards (26.47%). The occurrence of isolate from CCU and NICU was 0.98% each. Majority of the isolates were obtained from tracheal aspirate (40.20%), followed by BAL (16.66%) and blood (15.70%). All isolates were resistant to imipenem, while 99.06% and 95.6% of the isolates were resistant to ceftazidime and ciprofloxacin respectively. Aminoglycosides resistance was also observed with 81.11% resistant to gentamicin and 63.73% towards amikacin.

**Conclusion:** Based on our preliminary study, multidrug resistant *Acinetobacter baumannii* is an alarming threat to the health system. In order to reduce its transmission, strict adherence to infection control measures with good clinical practice need to be enhanced.
Emergence of Carbapenem Resistance in Pseudomonas aeruginosa isolates from a specialized care facility for prolonged mechanical ventilation patients

W.-C. Lin; St. Joseph’s hospital, infection control team, Yunlin County/TW

Purpose: Pseudomonas aeruginosa is one of the leading gram-negative organisms associated with nosocomial infections (NI), especially in patients with mechanical ventilation. Among these P. aeruginosa, the carbapenem resistant strain is concerned.

The aim of this study was to investigate the situation of carbapenem resistant P. aeruginosa in respiratory care ward (RCW).

Methods & Materials: Retrospective data on all patients receiving prolonged mechanical ventilation (PMV) between January 2012 and December 2017 were collected at a regional hospital in Yunlin county, Taiwan.

PMV was defined as mechanical ventilation for≥6 h per day for ≥21 consecutive days. Admission criteria for the RCW is the patient needs PMV longer than 62 days with haemodynamic stability and stable oxygen requirements.

Patients in RCW were monitored for NI by the hospital infection control committee. Standard Centers for Disease Control and Prevention /National Nosocomial Infections Surveillance System definitions were used to identify NIs.

Results: Since 2012 to 2017, there were total 153 PMV patients hospitalized in RCW. The mean age was 79±10 years old. The major comorbidities were hypertensive cardiovascular diseases (76.4%), diabetes mellitus (33.8%) and stroke (30.1%).

The most common isolates in RCW during 2012 to 2017 were Pseudomonas aeruginosa, Proteus mirabilis, E. coli, Klebsiella pneumoniae and Acinetobacter baumannii.

In RCW, the nosocomial infection density of P. aeruginosa was 1.7 per 1000 patient days. Rapid raise of carbapenem resistance of P. aeruginosa in RCW was noted after 2014. The proportion of carbapenem resistance in P. aeruginosa was 10.2% in 2012 then up to 40% in 2017.

Conclusion: The incidence and prevalence of carbapenem resistant P. aeruginosa continue grow in RCW, which is an important emerging issue when caring these PMV patients in Taiwan.
The Clinical Profile of Native-Valve Infective Endocarditis In A Tertiary Hospital in the Philippines: A Twelve-Year Retrospective Study

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Purpose: Infective endocarditis (IE) is a disease with high mortality rate. Numerous studies across different countries were done and published regarding clinical profile of IE patients. However, there is a paucity of Philippine data regarding IE, thus the need for this study. This study aims to determine the clinical profile, etiologic pathogen and echocardiographic findings among patients with native-valve infective endocarditis (NVIE) admitted at the University of Santo Tomas Hospital (USTH) from 2005 to 2016.

Methods & Materials: This is a retrospective descriptive study. All adult patients diagnosed with NVIE from January 2005 to December 2016 were included in the study. Data from charts were reviewed and analyzed.

Results: The mean age of patients with NVIE was 45 ± 17 years, without gender predilection, with same frequencies between community-acquired and healthcare-associated cases. Hypertension was identified as the most common medical co-morbid illness. Mitral valve followed by the aortic valve was the most common valve affected. Most patients presented with vegetation and abscess with concomitant moderate to severe valvular regurgitation and pulmonary hypertension. The incidence of blood culture-positive and blood culture-negative NVIE was the same. Most patients did not have underlying cardiac conditions. However, among those with such, the most commonly related condition was rheumatic valvular heart disease followed by mitral valve prolapse. The most common microbiologic pathogen was Staphylococcus aureus, followed by streptococcal species, which was susceptible to the recommended antibiotic regimen (beta-lactam/beta-lactamase inhibitor plus gentamicin), except if with risk factors for methicillin-resistant Staphylococcus aureus (MRSA), where vancomycin was indicated. Prior antibiotic use contributed to high rates of culture-negative IE. Medical management alone when compared to medical-surgical management of IE yielded still elevated in-hospital mortality rate, thus geared to a more conservative (medical only) management of IE.

Conclusion: The clinical profile of NVIE patients was similar across various countries. Microbiologic pathogens at the USTH were similar to developed countries with Staphylococcus aureus as the most common pathogen. Current empiric antibiotic therapy must include methicillin-sensitive Staphylococcus aureus (MSSA) coverage among those without risk factors for MRSA. Medical management alone had same outcome with cases managed both medically and surgically.
Extended-spectrum beta-lactamases in Dubrovnik-Neretva county

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Purpose: The first ESBL positive Enterobacteriaceae were characterized in Croatia in 1998. However, there were no reports from Dubrovnik-Neretva County, sequestered area, located in South-East geographic region of Croatia. Recently, an increase of ESBL positive organisms was notified in the Public Health Institute of Dubrovnik-Neretva County and the isolates were sent to University Hospital Center Zagreb for analysis of resistance mechanisms.

Methods & Materials: Sixty-nine extended-spectrum β-lactamases-producing Enterobacteriacea strains were isolated in Public Health Institute of Dubrovnik-Neretva county during the period April 2015 - April 2016. There were 44 E.coli, 17 K.pneumoniae and 8 Enterobacter cloacae. The antibiotic susceptibility was determined by the broth microdilution method according to CLSI standards. The double disk synergy test (DDST) and the CLSI combined disk test with addition of clavulanic acid were performed to detect ESBLs. PCR was used to detect blaESBL genes. The isolates were genotyped by PFGE. WGS was done on four selected isolates.

Results: All 44 E.coli isolates showed resistance to amoxicillin, cefuroxime, ceftriaxone, ceftazidime and cefepime. High level resistance was noticed also for gentamicin (75%) and ciprofloxacin (97.8%). All 17 K. pneumoniae isolates showed resistance to amoxicillin, cefuroxime, ceftriaxone, ceftizoxime, ceftazidime, cefepime, gentamicin and ciprofloxacine. High level resistance is showed also to piperacillin-tazobaktam (76%). All E. cloacae isolates showed resistance to amoxicillin, cefuroxime, ceftriaxone, ceftizoxime, ceftazidime and cefepime. High level resistance is showed also to gentamicin (87%), ciprofloxacin (87%) and piperacillin-tazobaktam (75%). All isolates tested positive in DDST and combined disk test indicating the production of an ESBL. PCR revealed group 1 CTX-M in all isolates, with majority of them harbouring also TEM-1. WGS revealed the presence of resistance gene for non-β- lactam antibiotics: aminoglycosides, teracyclines, trimethoprim, suphonamides, fluoroquinolones and chloramphenicol in addition to blaCTX-M genes. The isolates showed high level of genetic variability and beloged all to different PFGE types. Five different plasmid-incompatibility groups were found: X, L/M, N, A/C and FIA.

Conclusion: The study demonstrated polyclonal spread of CTX-M producing Enterobacteriaceae in Dubrovnik-Neretva county. There was horizontal transmission of plasmids harbouring blaESBL genes between different stains and species. This is the first report of ESBLs in Dubrovnik-Neretva county.
Epidemiology of Diphtheria and Antimicrobial Resistance Among Diphtheria Cases, Bijapur district, Karnataka, India, 2012 to 2015.

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Purpose: India contributes to 80% of diphtheria cases. There were 177 deaths due to diphtheria in 2010. Many diphtheria outbreaks were reported from Bijapur district of Karnataka state, India. Analysis of burden and drug sensitivity pattern might help to identify risk groups and to provide guidelines for treatment of diphtheria cases. Our objectives were to describe the epidemiology of diphtheria and drug susceptibility among diphtheria cases in Bijapur district.

Methods & Materials: We did a cross-sectional study between 2012 and 2015. We defined a probable case as inflammation of upper respiratory tract with adherent membranes. We defined a confirmed case as a probable case that was laboratory confirmed by throat culture. We collected line list of probable and confirmed diphtheria cases, population details in Bijapur district, and antibiotic sensitivity of culture reports. We calculated attack rates and case fatality rate by taluks of Bijapur district. We calculated proportion of antibiotic resistance among lab confirmed cases.

Results: There were 229 probable cases and 26 confirmed cases of diphtheria. Attack rate was 110/ million and case fatality rate was 2% (5/255). Median age of males was 5 years (range: 3 months to 18 years) and females was 6 years (range: 1 year to 18 years). Highest attack rate (290/million) was in Bagewadi taluk, followed by Sindagi taluk (130/ million). Attack rate in Bijapur, Indi and Muddebihal were 80, 80 and 70 per million respectively. Incidence of diphtheria cases was 3/million in 2012, 15/million in 2013, 80/million in 2014 and 14/million in 2015. Penicillin resistance was found among 92% (24/26) of cases, cotrimoxazole resistance among 27% cases (7/26) and ampicillin resistance among 15 % cases (4/26). Multidrug resistance for penicillin and cotrimoxazole was found among 23% (6/26) of cases. Multidrug resistance to penicillin and ampicillin was found among 15 % (4/26) of cases. All cases were sensitive to azithromycin, erythromycin, doxycycline, clindamycin, ciprofloxacin, cefotaxime, gentamycin and tetracycline.

Conclusion: Diphtheria incidence increased between 2012 and 2014, but reduced after 2014 in Bijapur. Penicillin resistance was common. We recommend sensitising health workers about penicillin resistance and educating them not to use penicillin. We recommend estimating vaccine coverage and vaccine effectiveness.
The Recent Trend of MRSA Surveillance in Japanese Health Care Facilities

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Purpose: Methicillin-resistant Staphylococcus Aureus (MRSA) is an indicator for evaluation of AMR countermeasures. In Japan, Ministry of Health, Labour and Welfare have offered an incentive for health facilities which implemented an infection control team and other interventions from 2012. However, its impact has yet to be evaluated. As the prelude for evaluation, the objective of the present study is to clarify the trend in the prevalence of MRSA in Japan would be different between before and after intervention.

Methods & Materials: Annual surveillance data from JANIS (Japan Nosocomial Infections Surveillance) regarding Staphylococcus Aureus isolates from 2007 to 2016 were analyzed. We involved 391 Medical facilities mostly over 200 beds, that voluntarily participating JANIS and submitted at least two years’ data both before (2007-2011) and after intervention period (2012-2016). Data were analyzed by facility. We applied a logistic linear trend model to predict the proportion of MRSA (Model 1), and Poisson linear model to the absolute number of MRSA (Model 2) with time. In both models, we set two assumptions; model A is without and model B is with the change of trend in the year of intervention. Each model’s Akaike Information Criterion (AIC) value was compared to determine the better fitted one.

Results: Proportion of MRSA decreased from 2007 (median 0.62, IQR 0.52~0.72) to 2016 (median 0.42, IQR 0.34~0.53). Model B (with change in the trend) yielded AIC values lower in both models 1 and 2 (the median and IQR difference between Model 1-A and 1-B is 0.17, -1.49~5.38 and between Model 2-A and 2-B is 9.62, 0.52~35.1, respectively).

Coefficient parameter values which represent increase/decrease trend per year were negative (the median and IQR value estimated in Model 1-A: -0.073, -0.131~0.021; Model 1-B: -0.064, -0.17~0.048 before intervention, -0.081, -0.16~0.015 after intervention; Model 2-A: -7.4, -15.5~0.48; Model 2-B: -0.15, -19.1~16.2 before intervention, -11.4, -24.3~2.5 after intervention).

Conclusion: 10 years MRSA surveillance demonstrated an overall decreasing trend of both the proportion and the absolute number of MRSA in Japan. Models with change in the trend yielded lower AIC values, implying that the intervention since 2012 might have had an impact to vary the epidemiology of trend in MRSA.
Identification, Antimicrobial Susceptibility And Virulence Factors Of Enterococcus Species Isolated From Clinical Specimens At An Indian Tertiary Care Hospital

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Purpose: Enterococcus species have increasingly emerged as important pathogens in serious nosocomial and community acquired infections. The objective was to determine species identification, evaluate current susceptibility pattern and characterize various virulence determinants among clinical isolates of enterococci at an Indian hospital.

Methods & Materials: Consecutive isolates from clinical specimens (April to September 2017) were included. Species identification was performed by conventional biochemical tests. Antimicrobial susceptibility testing was as per CLSI guidelines, including detection of high-level gentamicin resistance (HLGR), high-level streptomycin resistance (HLSR) and glycopeptide resistant enterococci (GRE). All screen-positive GRE isolates were investigated by PCR for species confirmation and presence of vanA/vanB genes. Virulence genes asa1, esp, hyl, cyl, and gelE were investigated by molecular methods. Haemolysin and biofilm production were studied phenotypically.

Results: Of 71 strains isolated, 57 (80.3%), 9 (12.7%) and 5 (7.0%) were from urine, pus and blood respectively consisting of E. faecalis (45, 63.4%), E. faecium (20, 28.2%), E. durans (3, 4.2%), and E. avium, E. hirae, E. mundtii (1, 1.4%). Six (8.4%) were GRE (5 E. faecium, 1 E. faecalis), all vanA phenotypically and genotypically. HLGR and HLSR was observed in 41 (57.7%) and 23 (32.4%) isolates respectively, higher in E. faecium compared to E. faecalis (85.0% and 46.6% vs 40.0% and 28.8%). Resistance to other antibiotics was also higher in E. faecium. All were susceptible to daptomycin. Hemolysin activity was significantly higher in E. faecalis compared to E. faecium (55.5% vs 0%). Biofilm production was observed in 23 (32.4%) isolates. Virulence genes detected were asa1 (9, 45%), gel (9, 45%), esp (7, 35%), cyl (6, 30%) and hyl (1, 5%). Frequency of asa, gel, and cyl was higher in E. faecalis than E. faecium (7, 7, 5 vs 2, 2, 1), whereas esp and hyl was more in E. faecium compared to E. faecalis (5, 1 vs 0, 0).

Conclusion: Prevalence of a wide variety of Enterococcus species in clinical samples together with their variable antimicrobial susceptibility pattern and virulence traits emphasizes the need for their routine speciation and susceptibility testing which may help control the spread of multidrug resistant Enterococcus species.
Prevalence, Shiga-Toxin Producing Potential and Antimicrobial Resistance Patterns of *Escherichia coli* Isolated from Dairy Cow Farms in Transylvania, Romania

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**Purpose:** The prevalence and characteristics of *Escherichia coli* were assessed in dairy cow farms from Transylvanian counties, Romania.

**Methods & Materials:** A stratified random sample of 43 dairy farms from the specified geographical region, divided into four categories, depending on their size and range of production (100-2100 milking cow/farm, both conventional and organic farms) was considered for the following types of samples: milk, milking filters, feed and cattle feces. The samples were processed by standardized microbiological, serological and molecular methods aimed to identify *E. coli* and to detect O157:H7 and Shiga-toxin producing strains. All isolated were evaluated for antimicrobial resistance against 18 antimicrobial agents and the presence of selected antimicrobial resistance genes (tetA, tetB, tetC, tetD, tetE, tetG, and ampC).

**Results:** Overall, *E. coli* presence was detected in 67% of feces samples, 16% of milking filters and 0.8% milk samples, with significant variations of these values depending on the farm type. *E. coli* O157:H7 isolates were confirmed only in case of two units, while the presence of genes encoding Shiga like toxin (stx1 and stx2) was recorded for 15% of fecal strains. The great majority of *E. coli* strains (89%) exhibited resistance towards five or more antimicrobial agents, with positive results also for one or more target antimicrobial resistance gene(s) in different combinations. The most common multiresistant phenotype was the combination – tetracycline - streptomycin.

**Conclusion:** The presence of genes encoding Shigalike toxin (stx1 and stx2) and multidrug resistance highlights the risk associated with human exposure in terms of possible contamination of products – milk and dairy products provided by the bovine farms, imposing ensuring food hygiene throughout the chain of production process in order to minimize or eliminate the risk of contamination of the products provided by these farms.
Virulent and Multiple Antimicrobial Resistant profiles of Novel Atypical *Escherichia coli* O177 Strains In South Africa

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**Purpose:** Atypical enteropathogenic *E. coli* strains (aEPEC) belonging to the serotype O177 are generally not associated with human infections and the virulence and antibiotic resistance profiles of this pathogen is poorly understood. In this study we report the first characterisation of aEPEC *E. coli* O177 strains from South African cattle.

**Methods & Materials:** A total of 780 samples collected from commercial feedlot and dairy cattle were analysed and used for selective isolation of atypical *E. coli* O177 strains based on amplifications of the *rmlB* and *wzy* using multiplex PCR analysis. Confirmed *E. coli* O177 isolates were screened for *bfpA*, *eaeA*, *hlyA*, *stx₁* and *stx₂* and *stx₂* variant genes, bacterial susceptibility test and multidrug resistant PCR genes.

**Results:** A total of 780 samples were analysed and 1272 presumptive isolates were obtained. Of these, 915 *E. coli* and 376 *E. coli* O177 were confirmed using *rmlB* and *wzy* multiplex PCR analysis. The *E. coli* O177 isolates were screened for the presence of *bfpA*, *eaeA*, *hlyA*, *stx₁* and *stx₂* and *stx₂* variant genes. None of the *E. coli* O177 isolates possessed *bfpA* gene, thus belong to aEPEC. Large proportions of the isolates harboured the *hlyA* (74%) and *stx₂* (55.5%) genes. *Stx₂* subtypes *stx₂a* and *stx₂c* were detected in 8.1% and 1.9% of the isolates respectively while 47.6% and 35.9% isolates harboured the *stx₁* and *eaeA* genes. In addition, 32.7% of the isolates harboured *stx₁* and *stx₂*, while 30.1% possessed *stx₁*, *stx₂* and *hlyA*. Small proportion of the solates possessed all the four typical STEC virulence genes (*stx₁/stx₂/hly/eaeA*) while large proportions of the *E. coli* O177 isolates were multidrug resistant and harboured *aadA*, *streA* and *streB* resistant genes at percentages of 79.5%, 52.1% and 56.2% respectively. In addition, 50.7% and 42.5% of the isolates were positive for the *erm* and *tetA* genes respectively.

**Conclusion:** To the best of our knowledge, this is the first study to provide data on the occurrence of this novel aEPEC O177 strain in South Africa and these findings indicate the public health complications these isolates may pose when present in the food chain.
Antibiotic Use In Periurban Small Holder Dairy Farms In India: Prevalence and Assessment Of Risk Factors

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Purpose: Non-prescribed use of antibiotics (NPAb) in dairy farms is long reported as a prevalent practice. It is perceived as a serious threat to antibiotic effectiveness in animals and humans and an inevitable challenge to the agroecology and sustainable production of ‘healthy’ dairy produce. However, the practices have not been adequately investigated. Smallholder dairy farms in peri-urban areas are a significant contributor to this production. This study investigated the non-prescribed use of antibiotics by these farmers in India and determinants thereof.

Methods & Materials: Using a multi-stage random cluster sampling, we selected smallholder dairy farms (≤10 cows) in peri-urban ecosystems of five cities of India. Dairy farmer questionnaire and observation checklist was used to capture data on different variables. Milk samples were collected and investigated for antibiotic residues. NPU was defined as self-reported use of the allopathic medicine within the past 1 year for any of pre-specified 10 animal conditions without advice from a qualified veterinarian and/or detection of antibiotic residues from any of the milk samples.

Results: Total 510 farms were enrolled. The rates of NPU in the study cities were as follows: Bengaluru, 40.2% (30.5-49.9); Bhubaneswar, 35.3% (25.9-44.7); Guwahati, 43.1% (33.4-52.9); Ludhiana, 53.9% (44.1-63.8); Udaipur, 76.5% (68.1-84.8). Antibiotic residues were detected in 14.3% (11.3-17.4) of the total farm. The most commonly used antibiotics were tetracyclines [10.0% (7.6-13.0)], fluoroquinolones [2.8% (1.5-4.6)] and sulphonamides [1.8% (0.8-3.3%)]. The proportion of self-administration was significantly different between NPU (40.6%) and non-NPU farms (27.7%). NPU was significantly associated with age of the farm >5 years, self-administration of the antibiotics by the farmer, ease of administration, popularity of the drug.

Conclusion: NPU was widespread across all the cities but rates vary from city-to-city. Antibiotic residues indicated that about 1 in every 7 farms were currently using antibiotics and for common conditions. Rates were likely to be higher in cities that were more likely to use intensified dairy farming. Such widespread use is likely to fuel AMR and reflects unsustainable farming practices.
Combating Antimicrobial Resistance - Utility of Antimicrobial Combination Therapy and / or β–Lactamase Inhibitors

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Purpose: The range of antimicrobial agents that can be used to treat bacterial infections is becoming limited with the constant increase in antimicrobial resistance (AMR). Several genetic factors underlie AMR, including β-lactamase-encoding genes such as blaCTXM-15 that confers resistance to third-generation cephalosporins, and blaOXA-48, blaNDM-1, and blaKPC-2 that confer resistance to carbapenems. Remaining treatment approaches for such resistant infections include antimicrobial combination therapy and the use of β-lactamase inhibitors. This study assesses the molecular effects of such treatment approaches on antimicrobial resistant Enterobacteriaceae clinical isolates in vitro and in vivo.

Methods & Materials: Nine clinical Enterobacteriaceae isolates were included in the study. One harboring blaCTXM-15, one harboring blaOXA-48, one harboring blaKPC-2, two harboring blaNDM-1 and blaCTXM-15, and four harboring blaOXA-48 and blaCTXM-15. Minimal inhibitory concentrations were determined for carbapenems with avibactam, Ca-EDTA, and relebactam. Synergism between antibiotic combinations was determined by in vitro and in vivo studies when using colistin with several antibiotics. In vitro and in vivo gene expression levels were done on these combinations with and without inhibitors.

Results: Antimicrobial synergism was mostly detected between colistin and meropenem, fosfomycin, or tigecycline. The use of meropenem, imipenem, and ertapenem with the selected β-lactamase inhibitors restored isolate susceptibility in 100%, 87.5%, and 25% of the cases. Survival studies revealed a significant high survival rate in mice receiving antimicrobial combination therapy with β-lactamase inhibitors as compared to the controls. Overall gene expression levels of resistance genes were variable depending on treatment.

Conclusion: Combination therapy along with β-lactamase inhibitors proved to be highly efficient in determined in vitro and in vivo survival studies. The threat of antibiotic resistant bacterial infections remains viable; however, different approaches to therapy are available.
Evaluation of Resistance to Levofloxacin in Tuberculosis Treatment in a Hospital in Japan

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Purpose: In Japan, levofloxacin (LVFX) was launched in 1993 and approved as an antituberculosis drug in 2016. It is prescribed as second-line treatment for patients who have higher risks of developing drug resistance and adverse effects. Meanwhile, LVFX has been frequently administered for community-acquired pneumonia and urinary tract infection in Japan. However, *Escherichia coli* and *Streptococcus pneumoniae* developing resistance to LVFX has been a major issue; in fact, the resistance rates in our hospital are 5.0% and 37.6%, respectively. Despite these circumstances, no study has been confirmed. We investigated the usage and drug susceptibility of LVFX to lung tuberculosis in our hospital.

Methods & Materials: We retrospectively identified 83 consecutive patients with lung tuberculosis who were admitted to our hospital between April 2014 and March 2018 (age, 74±21 years; sex, male/female 43/40; first treatment, 74 cases). We analyzed the examination outcomes regarding drug resistance of isoniazid (INH), rifampin (RFP), streptomycin (SM), and LVFX in the medical records.

Results: LVFX was administered in 33 cases (45%) (74±21 years of age) as initial treatment and in 7 cases (100%) (73±22 years of age) as retreatment. The reasons for administering LVFX were adverse reactions in 13 cases (37%) and resistance to antitubercuotic drugs in 2 cases (6%). The drug resistance rate was 5% for INH, 3% for RFP, 0% for SM, and 0% for LVFX. One case was of multidrug resistance. No adverse effects were reported, and the bacteriological negative conversion rate was 100%. Only one patient was treated with LVFX alone for 7 days before admission, but no resistance developed.

Conclusion: LVFX was effective as tuberculosis treatment in cases wherein standard regimens could not be administered, in terms of no adverse effects and bacteriological negative conversion rates. The usage rate of LVFX in our hospital was more than 30% in lung tuberculosis treatment. However, we have never experienced its resistance as of 2018. Our study showed that LVFX is an essential drug if effective drugs are limited.
Emergence of metallo-beta-lactamases in Dubrovnik-Neretva County, Croatia

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Purpose: Carbapenemases involved in acquired resistance to carbapenems in Enterobacteriaceae belong to Ambler class A serin β-lactamases (KPC, GES, SME, IMI, NMC), class B metallo-β-lactamases (MBL) of the IMP, VIM or NDM family and OXA-48-like β-lactamases belonging to the class D. Recently carbapenem-resistant Enterobacter cloacae isolates were reported for the first time in Dubrovnik-neretva county in Croatia. The aim of this study was to characterize the carbapenem resistance mechanisms and to genotype the isolates.

Methods & Materials: In total four carbapenem resistant E. cloacae strains were isolated in Public Health Institute of Dubrovnik neretva county. The antibiotic susceptibility was determined by the broth microdilution method according to CLSI standards. The double disk synergy test (DDST) and the CLSI combined disk test with addition of clavulanic acid were performed to detect ESBLs. Chromosomal or plasmid-mediated AmpC β-lactamases were detected by combined disk test using cephalosporin disks with 3-aminophenylboronic acid (PBA. A modified Hodge test (MHT) and the carbapenem-inactivation method (CIM) were used to screen for the presence of carbapenemases. Carbapenemase, other β-lactamase and fluoroquinolone resistance genes were detected by PCR and sequencing. The transferability of meropenem resistance was tested by broth mating method. Plasmids were characterized by PCR-based replicon typing. The isolates were genotyped by PFGE.

Results: The isolates were resistant to penicillins, narrow and expanded-spectrum cephalosporins, all three carbapenems (imipenem, meropenem and ertapenem) gentamicin and ciprofloxacin but susceptible to colistin. Hodge test, CIM test and inhibitor based tests with EDTA, PBA and EDTAPCR revealed blaVIM, blaCTX-M and blaTEM genes. Sequencing of representative amplicons identified VIM-1, CTX-M-15 and TEM-1 β-lactamases. Plasmids encoding VIM-1 were found to belong to A/C incompatibility group. PFGE identified one pair of identical isolates and two isolates with distinct PFGE patterns.

Conclusion: Previous studies in Croatia revealed the spread of VIM-1 MBL among Enterobacteriaceae, particularly E. cloacae. In this study we demonstrated spread of MBL positive E. cloacae to Dubrovnik neretva county, a sequestered geographic area in Southest part of Croatia which was free of carbapenemases until two years ago. Similarly as in the previous study VIM-1 was carried by A/C plasmid and the isolates coharboured ESBLs belonging to group 1 CTX-M β-lactamases.
Purpose: This study was conducted to determine the prevalence and risk factors for colonization with fluoroquinolone (FQ)-resistant E. coli in hospitalized patients in Ado-Ekiti.

Methods & Materials: Rectal swabs were obtained from patients in hospitals in the study area and FQ-resistant E. coli were isolated and identified by means of Nalidixic acid multi-disk and a 1-step screening procedure. Species identification and FQ resistance were confirmed by automated testing (Vitek, bioMerieux, USA). Individual colonies were subjected to pulse-field gel electrophoresis (PAGE) to determine macro-restriction polymorphism after digestion of chromosomal DNA.

Results: FQ-resistant E. coli was detected in the stool sample of 37(62%) hospitalized patient. With multivariable analyses, the use of FQ before hospitalization was the only independent risk factor for FQ-resistant E. coli carriage and was consistent for FQ exposures for the 3-12 months of study. Pulsed-field gel electrophoresis of FQ-resistant E. coli identified conal spread of 1(one) strain among 18 patients. Loss (9 patients) or acquisition (10 residents) of FQ-resistant E. coli was documented and was associated with de novo colonization with genetically distinct strains.

Conclusion: It was concluded that FQ-resistant E. coli carriage was associated with clonal spread. The differential effects of individual fluoroquinolone on antimicrobial drug resistance are an important area for future study, as hospitals manipulate their formularies with regard to use of individual fluoroquinolone, often for economic reasons.
Is There a Link Between Antimicrobial Use and the Prevalence of MRSA and ESBL-Producing Escherichia coli on Austrian Dairy Farms?

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Purpose: The aim of the present study was to determine the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) and ESBL-producing Escherichia coli (ESBL-EC) on Austrian dairy farms, and to compare this to the level of antimicrobial use (AMU) on these farms.

Methods & Materials: Farms included were participating in a larger observational study of AMU on 250 farms, quantified by Defined Daily Doses for veterinary medicine (DDD_vet). Farmers were recruited using convenience sampling. Here, 50 farms (25 of the highest antimicrobial users and 25 of the lowest users) located in the Austrian federal state of Styria were included in an additional analysis of antimicrobial resistance (AMR). Each farm was visited once in July 2017 and the following environmental samples were collected: boot swabs from the alleyways of freestalls or the slurry passage in tie-stalls, pooled faecal samples from calf, and youngstock, pens (1-5 head per sample), and dust samples collected on dry gauze from 3-5 areas of the main cowshed. Samples were then cultured on selective media specific for MRSA or ESBL-EC detection.

Results: MRSA in the dust samples was only isolated from one farm (2% of farms), whereas ESBL-producing E.coli were detected in at least one faecal sample on 13 of 50 farms (26%). The Mann-Whitney U test determined no statistically significant difference between the level of AMU (calculated as total DDD_vet) on farms where ESBL-producing E.coli were detected, compared to those where all faecal samples were negative (U-score 189.0, asymptotic 2-tailed p=0.255). Similarly, no difference was noted when the level of use of third and fourth generation cephalosporins only was compared with the presence of ESBL-producing E.coli (U-score 206.5, asymptotic 2-tailed p=0.447). Due to the extremely low prevalence of MRSA in this farm population, statistical analyses would not be meaningful and were not done.

Conclusion: MRSA was rarely detected, whereas ESBL-producing E.coli were isolated in around a quarter of 50 Austrian dairy farms. At this relatively low level of AMU in a small sample of Austrian dairy farms, no relationship between AMU and the presence of ESBL-EC could be demonstrated.
Characterizing Antibiotic-Resistant Pneumococci in the Nasopharynx of Healthy South African Infants using Shotgun Sequencing and Conventional Typing

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Purpose: The increased prevalence of antibiotic-resistant *Streptococcus pneumoniae* is of public health concern. *S. pneumoniae* is one of the leading causes of death in infants. We longitudinally investigated antibiotic-resistant *S. pneumoniae* in the nasopharynx of healthy infants in the Drakenstein Child Health Study, using conventional and shotgun sequencing methods.

Methods & Materials: Nasopharyngeal (NP) swabs were collected fortnightly from birth through the first year of life, from 137 infants. Infants received 3 doses of 13-valent pneumococcal conjugate vaccine (PCV13). *S. pneumoniae* isolates were serotyped using sequencing and Quellung. Antibiotic susceptibility profiles were determined using disc diffusion and E-test. Metagenomic shotgun sequencing was performed on a subset of 200 NP samples from 23 infants, selected on the basis of changing serotype or antibiogram over time.

Results: *S. pneumoniae* was isolated from 54% (1809/3331) NP swabs. After correcting for repeated acquisition of the same serotype with a unique antibiogram (33%; 591/1809), non-susceptibility to penicillin G, erythromycin, and cotrimoxazole was found in 26% (125/591), 20% (120/591), and 42% (250/591) of the isolates respectively. Multidrug resistance (MDR) was observed in 11% (67/591) of the *S. pneumoniae* with vaccine types 9V (n= 5), 19F (n= 5), and non-vaccine type 15B/C (n= 9), being predominant serotypes. We found a 68% (136/200) concordance between shotgun sequencing and conventional serotyping, with co-colonization by multiple pneumococcal serotypes identified in 23 samples by shotgun sequencing. We detected 26 different sequence types (including 4 novel ST), predominantly ST8687 and ST2068 (ST2068 not previously described in Africa) and 31 different antibiotic resistance genes by shotgun sequencing.

Conclusion: MDR was noted in a small proportion of isolates. Shotgun sequencing is a valuable technique for detailed evaluation of the pneumococcal component of the NP microbiome.
Combating Antimicrobial Resistance by Utilizing Novel Antibiotics from Soil and Marine Microorganisms in Lebanon

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Purpose: Antimicrobial resistance (AMR) is emerging at an alarming rate as mortality due to resistant pathogens could rise to 10 million per year by 2050. Since AMR is against all clinically utilized antibiotics, finding novel antimicrobials with unexploited targets remains the main goal worldwide. Soil microorganisms produce natural products as a significant number of drugs in clinical use are derived from these metabolites. Actinomycetes and Myxobacteria are soil dwelling microorganisms that produce secondary metabolites to be screened for antibacterial activity. More than 80% of clinically utilized antibiotics are either natural products or natural product-derived molecules such as vancomycin, teicoplanin, daptomycin, and tetracycline. This study aims to isolate and identify novel antimicrobials from Actinomycetes and Myxobacteria.

Methods & Materials: Soil samples were collected from several areas in Lebanon. Samples were serially diluted for Actinomycetes isolation and boiled for Myxobacteria extraction, then plated on suitable media. Colonies obtained were purified and subjected to genomic DNA extraction then 16s rRNA analysis. Novel isolates were tested for their antimicrobial activity against Gram-positive \textit{Bacillus subtilis} (ATCC 6051), \textit{Staphylococcus aureus} (ATCC 29213, Newman, N315), \textit{Enterococcus faecalis} (ATCC 19433), and \textit{Enterococcus faecium} (DSMZ 17050), and Gram-negative \textit{Escherichia coli} (ATCC 9637), \textit{Klebsiella pneumoniae} (DSMZ), \textit{Pseudomonas aeruginosa} (ATCC 27853, MEXAB), and \textit{Acinetobacter baumannii} (ATCC 15308).

Results: Strain isolation and cultivation yielded a number of novel isolates whose extracts demonstrated strong antibacterial activity against pathogens including MRSA, VRE, and \textit{Escherichia coli} (ATCC 9637).

Conclusion: Our efforts now focus on purifying these compounds, elucidate their structures and study their mode of action.
Genetic Determinants Supporting The Multi-Drug Resistance of Acinetobacter spp. in Georgia


Purpose: Emergence and spread of multidrug- resistant (MDR) bacteria have become a serious challenge for health care workers around the globe. Acinetobacter baumannii represents one of the most widely distributed nosocomial pathogens quickly acquiring resistance to various groups of antibiotics, making treatment a challenging process. Plasmid-encoded genes often contribute to the steadily increasing MDR phenotype. As the molecular mechanisms of antimicrobial resistance of Acinetobacter spp. in Georgia are not yet fully understood, we have examined genes potentially supporting multi drug resistance of Acinetobacter spp. in the Country.

Methods & Materials: Bacterial isolates, recovered from the samples of hospitalized patients, were collected at various laboratory sites of Georgia. Identity and resistance patterns were detected by the automated system Vitek 2 and manual methods, such as disc diffusion and E-test. Antimicrobial resistance genes were identified by PCR and sequence analysis.

Results: Twenty eight isolates of Acinetobacter baumannii complex were collected between July 2017 and February 2018. It was found that all isolates were resistant to 3rd generation cephalosporins and nitrofurantoin, but sensitive to colistin and minocycline. In addition, 86% (n=24), 75% (n=21) and 61% (n=17) of Acinetobacter strains displayed resistance to carbapenems and fluoroquinolones, aminoglycosides and Trimthromprim/Sulfamethoxazole, respectively. The only gene detected by PCR-based analysis was the New Delhi metallo-beta-lactamase (NDM) presenting in four isolates with intermediate resistance to imipenem and meropenem. In addition, the preliminary whole genome sequence analysis of one MDR isolate revealed the presence beta-lactamase genes blaOXA-72, blaOXA-90, blaOXA-24, as well as blaCTX-M-115 and an ADC-type class C β-lactamase. Point mutations contributing to fluoroquinolone resistance in gyrA and parC were also identified.

Conclusion: This is the first sequence-based report describing multiple genetic elements supporting multi-drug resistance of Acinetobacter species in the Country of Georgia. Detection of various transmissible plasmid-associated genes in Acinetobacter is indicative of a potential horizontal spread contributing to the evolution of extensively drug resistant “superbugs” in Georgia.
Is Cefmetazole Effective Like Carbapenems To Treat ESBL-producing Bacterial Infections in vivo?

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**Purpose:** Carbapenems and cefmetazole have been shown to be effective against the hydrolyzing activity of ESBL in vitro. Because spectrum of cefmetazole is narrower than that of carbapenems, usage of cefmetazole is more recommended to avoid increase of multi-drug resistant strains. However, not many studies have compared effectiveness of carbapenems and cefmetazole clinically in vivo. Hence, we conducted a retrospective study with patients if cefmetazole is similarly effective like carbapenems against ESBL-producing bacteria.

**Methods & Materials:** One hundred and twenty-eight patients who admitted our hospital from 2008 to 2016 enrolled this study. Fifty-three cases had urinary tract infections, 30, 19, 9, 17 cases had infections at respiratory tract, blood stream, bile duct, or other organs, respectively. Identified ESBL strains were *E.coli* (N=93), *Klebsiella spp* (13), *Proteus mirabilis* (21), and polymicrobial infection (1). On their admission, all the patients had bacterial cultures, but ESBL-producing bacteria had not been identified yet at this point. By their physicians’ decision, all the patients received one antimicrobial empirically; carbapenem, cefmetazole, or other antimicrobials. All antimicrobials were administered intravenously for at least three days. After identification of ESBL, their medical records were checked retrospectively. If there are (1) improvement of clinical symptoms and/or (2) de-escalation or finished antimicrobial treatment, we concluded “clinically effective”. If the patient was dead or symptoms got worse, we concluded “not-effective”. Diagnosis of ESBL was performed using Siemens’ Microscan NegTM under the standard of Clinical and Laboratory Standards Institute. The institutional Review Board approved this research protocol.

**Results:** Among 128 patients, 100 of them showed clinically effective, 28 were not. Carbapenem group showed clinically effective in 55 out of 62 cases, while cefmetazole group showed 27 out of 35 cases (p=0.151). No significant differences in the 30-day mortality rates were observed between the groups. However, multiple logistic regression analysis revealed the use of carbapenems was significantly associated with the efficacy of treatment (odds ratio, 3.73; 95% confidence interval, 1.34–10.35; P = 0.012).

**Conclusion:** Carbapenems were considered more effective than cefmetazole. However use of carbapenems should be limited properly because frequent usage induces resistant strains. More study is needed with consideration of antimicrobial dosage and bacterial genetic background.
Determinants for relapse and development of drug resistance among Pulmonary Tuberculosis cases in Punjab, Pakistan

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\textbf{Purpose:} Pakistan is ranked 5\textsuperscript{th} in the world among 22 high burden tuberculosis countries. For the successful Anti-tubercular treatment the relapse rate, is an important indicator for the success of any treatment regimen. A study was planned to examine the predictors of relapse among a cohort of sputum smear-positive pulmonary tuberculosis (PTB) patients and the frequency of drug resistance among them.

\textbf{Methods & Materials:} A total of 200 relapsed cases of TB patients were selected. Two sputum specimens from each subject were collected for Ziehel Neelsen (ZN) smear microscopy, GeneXpert and culture for MTB detection on Lowenstein-Jensen media. Those found positive on culture were then be subjected for drug susceptibility testing for first line and second line drugs by standard drug proportion method on L-J medium. A semi-structured questionnaire was used to collect the information. Data was analyzed using the SPSS.

\textbf{Results:} The major factors for the relapse were found to be addiction n=100 (50%). Next was non-adherence to previous treatment 105 (52.5%). Regarding Co-morbidities mostly the relapsed cases were diabetic 45(22.5%). The Multi-Drug Resistance is found to be positive among 57(28\%) cases of relapse. Also XDR-TB is reported in the relapsed cases but the frequency was quite low.

\textbf{Conclusion:} Tuberculosis remains to be a major disease in the third world countries and its successful treatment is very necessary for the complete eradication of tubercle bacilli. Among factors associated with the relapse of TB, addiction, employment, addiction were significantly different between males and females. We recommend further studies with larger sample sizes to be conducted for further evaluation and reaching a firm conclusion. Such studies would definitely provide key solutions for TB control agencies in the future.
Occurrence, Genetic Diversities and Antibiotic Resistance Profiles of *Salmonella* Species Isolated from Chickens in Mafikeng, North-West Province, South Africa

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**Purpose:** *Salmonella* belongs to the group of the enteric pathogens commonly found on foods, poultry feed and products, which have the ability to colonize hosts thus resulting in illness and/or death. *Salmonella* spp. has been reported as a normal flora in the gut of chickens, however, little or none has been reported on the diversity of this enteric pathogen in hosts found in Mafikeng, North West Province. Hence, this research investigated the occurrence, diversities and antibiotic sensitivity of the non-typhoidal *Salmonella* spp. in chicken found in Mafikeng.

**Methods & Materials:** Swabs of the gut of chickens comprising Layers, Broilers and Indigenous chickens were aseptically collected and processed in the laboratory using standing culturing techniques. Microscopic morphology (gram reaction) of purified colonies and biochemical characteristics were determined. Presumptive *Salmonella* isolates were characterized using the amplification of 16S rRNA and the invA gene. *Salmonella enteritidis* (ATCC:13076™), *Salmonella typhimurium* (ATCC:14028™) and *E. coli* (ATCC:259622™) served as positive and negative controls respectively. Amplicons were sequenced, blasted and accession numbers were deposited in the NCBI Genebank database. Diversities of identified *Salmonella* isolates were investigated through the construction of a phylogenetic tree. Antibiotic sensitivity of isolate was tested against 11 groups of antibiotics (Ampicillin, Oxy-tetracycline, Ciprofloxacin, Streptomycin, Sulphonamide, Chloramphenicol, Erythromycin, Norfloxacin, Cephalothrin and Nalidixic).

**Results:** The percent occurrence and diversity of *Salmonella* in chickens at Mafikeng ranged from 67% (*Salmonella enterica*) to 1.81% (*Salmonella weltevreden, Salmonella chingola, Salmonella houten and Salmonella Bareily). *Salmonella bongori* (10.09%), *Salmonella* spp. (9.09%), *Salmonella Typhimurium* (12.72%) while (1.81%). Fifty-six percent of isolates were resistant to ampicillin, erythromycin (100%), oxy-tetracycline (69%), streptomycin (95%) and 50% Ciprofloxacin (31%), norfloxacin (49%), nalidixic (47%), trimethoprim-sulphamethaxole (78%). Multiple antibiotic resistance (MAR) of isolates showed a multi-resistance patterns to not less than nine antibiotics (octa-resistance) commonly used in the province. MAR index ranged from 0.27-0.81 with a highest MAR in samples from the indigenous chickens. Further studies on the antibiotic resistance genes in *Salmonella* strains is important.

**Conclusion:** Almost 84% of isolates posses the invA genes indicating the ability of the pathogens could invade the gastrointestinal tracts of hosts thereby posing a threat to food security, safety and public health.
Antimicrobial Resistance Local Data in Sepsis

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Purpose: Antimicrobial resistance (AMR) is one of the life-threatening world problems which should be noticed and controlled seriously. One of the major causes of the increasing AMR is the inappropriate use of antimicrobial where antimicrobial choices should be based on local data pattern to select the proper antibiotic. Sepsis causes high mortality and needs appropriate antibiotic while antimicrobial treatment should be started as soon as sepsis is suspected to prevent the development of further complications and progression. The aim of the study is to find the AMR prevalence, the causal bacteria in sepsis adult can be used as antibiotic guidelines for sepsis.

Methods & Materials: Retrospective study of all of the sepsis patient from medical report data and hospital information system (HIS) in 2016, which in total are 962 cases in Adam Malik tertiary Hospital in Medan, Indonesia. Diagnosis of sepsis was by using the surviving sepsis campaign (SSC) 2012. 91 cases (paediatrics) and 314 cases (no correlation with SSC criteria) were excluded. Finally, 535 sepsis patient data were evaluated and analyzed with STATA version 12.2

Results: The most aetiology diagnosis of sepsis were: pneumonia (70,84%), urinary tract infection (10,28%), intra-abdominal infection (4,11%), and skin soft tissue infection (3,93%). The gram-negative bacteria was the most common bacteria from the culture both in ICU (73,7%) and non-ICU (72,7%), which were Acinetobacter baumanii (26,6%), Klebsiella pneumonia (22,8%), and Escherichia coli (18,8%). The gram-positive bacteria were Staphylococcus sp. (54,2%), Enterococcus sp. (33,6%), and Streptococcus sp. (8,4%). AMR for sepsis patient in non-ICU for gram-negative bacteria were amikasin (19,1%), meropenem (30,6%), and Cefoperazone sulbactam (33,5%) while for gram-positive bacteria was only vancomycin with the resistance level below 20% (18,3%). In the ICU patients, the AMR prevalence was higher than the non-ICU.

Conclusion: AMR in sepsis patients is very high in Indonesia. It needs local data as guidelines for empirical treatment that can reduce the inappropriate use of antibiotic in an effort to control the AMR from spreading and increasing.
Efficient Isolation Of Therapeutic Erythromycin-Resistant Campylobacter.

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Purpose: Campylobacter is one of the most common causes of human bacterial gastroenteritis in the world. C. jejuni and C. coli are the species most commonly associated with the disease. During the past decades, Campylobacter has become increasingly resistant to both macrolides and fluoroquinolones. Resistance to these classes of antibiotics in Campylobacter is a serious threat to public health. Since Resistant Campylobacter spp. to fluoroquinolones have emerged in Japan, the most recommended drug is erythromycin (macrolide) when an antibiotic chemotherapy is needed for treatment of patients with Campylobacter infection.

In contrast to significantly high rate of fluoroquinolone resistance, Japan has reported a low and stable rate of macrolide resistance in C. jejuni and C. coli isolated from humans. However, the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM) has revealed a general trend of rising erythromycin resistance in Campylobacter.

Our final goal is to show the correlation among erythromycin-resistant Campylobacter isolates from patients with enteritis, food-producing animals and foods (such as chicken meats) in the same geographic area in Japan. As the first step in the project, the aim of this study is to develop a selective medium for the isolation of erythromycin-resistant Campylobacter from materials.

Methods & Materials: The medium contains erythromycin as the selective agent in Preston Agar with 5% defibrinated sheep blood. Seventeen Campylobacter strains with erythromycin MICs (Minimum Inhibitory Concentrations) of 0.25 to 512 μg/ml were used as erythromycin-resistant or erythromycin-susceptible reference strains to examine the selectivity of this medium in this study. MICs were determined by Etest (bioMérieux). The strains were grown on the agar plates at 37° or 42°C under microaerobic conditions.

Results: Among the reference strains, all the erythromycin-resistant Campylobacter strains grew well on the medium but the growth of the erythromycin-susceptible strains were significantly inhibited. Seventy two clinical isolates were examined and five strains were grew on the medium. Erythromycin MICs of the three of them were more than 256 μg/ml.

Conclusion: The results suggested that the medium allow the successful selection of erythromycin-resistant Campylobacter strains. The medium will useful efficient isolation of erythromycin-resistant Campylobacter.

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Antimicrobial Resistance against Critical Antibiotics in the Environment of Intensive and Organic Turkey Farms

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Purpose: The high levels of antimicrobial resistance (AMR) against critical antibiotics in poultry environment are a cause for public health concern for the impact on human health. Intensive rearing conditions for turkeys seem to be associated with increased need for antimicrobial use. As antimicrobial treatments are mostly administered to turkeys via drinking water, we aim to quantify and compare AMR levels in indicator bacteria from turkeys' litter/faeces and drinking water.

Methods & Materials: AMR levels in indicator bacteria (E. coli) isolated from litter and from the drinking water pipeline were measured in 28 intensive turkey farms and in 4 organic turkey farms where antimicrobial use is restricted. Subsequently, a selection of 10 farms was categorized in 3 AMR groups: farms with 1) <50%(n=3), 2) 50-60%(n=5), or 3) >60%(n=2) resistance to antibiotics. In all farms, 20 animals were bled for quantification of natural immunity parameters like serum bactericidal activity and lysozyme.

Results: AMR for a panel of 14 antibiotics was high both in turkey faeces and drinking water, particularly for critical antibiotics, e.g. 80% of isolates was resistant to Ampicillin, Ciprofloxacin and/or Streptomycin. Observed agreement between AMR in water and in litter ranged between 36% and 100% depending on the antibiotics in question. In organic farms, AMR prevalence was lower, but turkeys' immune response was significantly higher (P<0.001) than in intensive farms. No significant associations were found between immunity and AMR groups.

Conclusion: AMR in intensive turkey production is higher than in organic production, but organic turkeys have higher levels of natural immunity. Moreover, an association between AMR in drinking water and litter exists for some antibiotics.
Purpose: The aim of this work is to analyze and compare the requirements for the transport Infectious Substances Category A in the ADR (European Agreement Concerning the International Carriage of Dangerous Goods by Road) and the norm CWA 15793:2011 Laboratory Biorisk Management with the objective of identifying the differences and limitations that may create a risk in the transport of infectious substances’ transportation.

Methods & Materials: The international Dangerous Goods Regulations (ADR, IATA-DGR) Laboratory biosecurity guidance WHO and the CWA 15793:2011 were analyzed and compared.

Results: Dangerous Goods Regulation is, in general, not well known. The laboratories, companies or centers must have an internal control system to monitor and control the risk. However, most of the laboratories do not have a good control over their own infectious substances category A, during shipment. The main reason is for not considering the protection plan or safety provisions written and agreed among the participants for the transportation. Companies and laboratories have to include among their personnel a Security Advisor to supervise the manipulation, packaging and transportation of infectious substances. Moreover, the centers or facilities must develop a security plan with the, among others, the following elements:

a) Specific allocation of responsibilities on security to qualified personnel.

b) Supervise all the operations involving infectious substances and assess the security risks, including transportation, stops in the road, the intermediates temporary storages.

c) Identify the measures to reduce security risks, considering the responsibilities and duties of all the participants in any operating involving infectious substances.

d) Effective and up to date procedures for reporting and dealing with security threats, breaks or incidents.

e) Procedures for the evaluation and testing of security plans and for periodic review and plan update.

Conclusion: The differences between the two norms/regulations (ADR and CWA) may result in unacceptable break of security during transportation. The Security Advisor is the person that has to provide the measures, protocols and monitoring to assure the minimization of risks in the management and transportation of infectious substances.
Bioterrorism: Toxins as Potential Biological Weapons - An Emerging Global Health Threat

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Purpose: Intentional bioterrorism attacks have been recorded throughout history, and research in medical defense against intentional biological threats has become a growing priority. Biological warfare is defined as the intentional use of living organisms such as bacteria, viruses and fungi with the intent to cause disease, death, or environmental damage. Toxins represent a specific new category of possible bio-terror agents owing their high toxicity, ease of production and ease of dissemination. Most of bacterial toxins are large proteins that affect either the nervous system (neurotoxins) or damage cell membranes. Some of these compounds are used for military purposes, other are less known and used but have the potential for use in terrorist-designed biological weapons.

Methods & Materials: Toxins are and heterogeneous groups of compounds, that share commonalities both with biological and chemical agents, produced by bacteria, plants and animals; most of the toxins specifically affect the mammalian nervous system, developing severe adverse health effects. The detection of biological agents is often very difficult as the illness can take anywhere from several hours to week, depending of the agent. There is not specific antidote or effective treatment from most of the biotoxins. Different technologies for toxin detection have been established, but hardly any universally agreed reference methods or reference materials are available. Understanding the mechanism of structure, action and toxicity represent the starting approach to develop adequate and timely medical countermeasures. The authors would like to analyze and reviewing these toxins, discussing the structure, symptoms, toxicity and treatment, along with their potential malevolent use.

Results: The aim is to provide specific information to enhance specific medical preparedness and response, to enable further understanding of these toxins and their potential role as biological weapons.

Conclusion: Biotoxins are highly toxic and can be used as potential bioweapons for military or terrorist scope, representing a problem of national or international concern in public health and homeland security. Understanding the global profile of these toxins is crucial for health and risk assessment and to develop effective medical countermeasures, in order to minimize the adverse health effects and prevent fatalities.
Assessment of Municipal Opened Landfill And Its Impact On Environmental And Human Health In Central Thailand

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**Purpose:** Non-sanitary landfill or open dumping stored both non-hazardous and hazardous waste disposed from households, restaurants, agricultural activities, and industrial sectors that could contaminated to the environment. This study aimed to assess the environmental status and its impact of opened solid waste landfill located nearby agriculture and natural resources in municipal area of central Thailand.

**Methods & Materials:** Soil and water samples were collected from six locations of landfill and surrounding areas such as paddy field and water reservoir at two-month interval for six consecutive months. Heavy metal contamination and quality of soil and water including physical, chemical, biological parameters were analyzed by using standard methods. Agricultural activities of the land nearby the landfill and practice behavior of workers in the landfill have been observed.

**Results:** We found Salmonella spp. and *Clostridium perfringens* contaminated in soil samples collected from both inside and outside of landfill. Heavy metals such as Iron (Fe), Magnesium (Mg) and Aluminum (Al) were found in high level, additionally, contamination of Copper (Cu) in soil collected inside the landfill was presented. Water collected from leachate and natural resource was contaminated by coliform bacteria and Salmonella spp. Level of Fe in all water samples was high, however this amount was not above limited value. Inappropriate self-protective and non-hygienic behavior of workers in the landfill was also found.

**Conclusion:** Soil and water samples collected from the opened landfill were contaminated with bacteria. Occupational hazard, protective awareness and protective measure of people who exposed to the landfill such as garbage collectors and farmers should be concerned. Level of heavy metals in soil and water, especially in paddy fields around the landfill should be periodically monitored.
Factors of Rabies Maintenance in Dog Population in Kinshasa, Democratic Republic of Congo (DRC)

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Purpose: Rabies kills every year 61 000 humans in the world, mainly in Asia and Africa. In the Democratic Republic of Congo (DRC), rabies is endemic and the dog is the main vector of human rabies. From 1937 to 2017, around 1400 positive dog rabies cases were recorded across the country. The study aims to better understand the epidemiological factors of rabies maintenance in dogs in Kinshasa.

Methods & Materials: We conducted 4 studies. (i) A household survey in 22 study sites in 3 municipalities of Kinshasa suburb (Mont-Ngafula, Ngaliema and Lemba). It provided information about the dog density, roaming and vaccination coverage. Based on these factors, a risk scale of rabies transmission was established. (ii) Owned dogs (n=16) were tracked during 24 hours using GPS collars. The probability of contact with other dogs was calculated. (iii) The proportion of feral dogs was estimated by the street count method. (iv) Finally, the serological status of dogs that were reported vaccinated was checked. Generalized linear models were used for statistical analysis.

Results: We recorded 922 dogs in 504 surveyed households. 60% (2-100%) of these dogs were free roaming. Feral dogs were estimated to be less than 2% of the free roaming dogs. Dog density was estimated to be 47 dogs/km². The mean distance covered by tracked dogs was 0.718 km (0.046-2.341 km) per day and each dog had a chance to come in contact with 27 free roaming dogs. The vaccination coverage was 53% (24-81%). 91% of the dogs that were reported vaccinated (n=131) were seropositive, confirming owners’ reports. The proportion of protected dogs (serology ≥0.5 IU/ml) was not associated with the time since the last vaccination (p=0.4). In DRC, vaccination of dogs is exclusively funded by owners. This may explain why the coverage is low in young (<1 year old) and free roaming dogs. The estimated risk of rabies transmission ranged from moderate to high in 80% of study sites (n=22).

Conclusion: The risk of rabies transmission is high in the study area, given the high proportion of unvaccinated free roaming dogs and low vaccination coverage (<70%) mainly in young dogs. ARES-CUD funded this research.
Diagnosis Of Rift Valley Fever Virus At The National Public Health Laboratory: Nairobi, Kenya

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Purpose: Rift Valley fever virus (RVFV) is regarded as a high biodefense priority owing to its ability to cause infections in both animals and humans, and its potential for aerosol spread. Prompt laboratory diagnosis and characterization of the causative agent in outbreaks is key to disease control and other public health interventions. The National Public Health Laboratory (NPHL) plays a central role in outbreak response and either provides or coordinates specialized testing for outbreak-prone emerging and re-emerging pathogens including RVFV. Here we present data from the on-going RVFV outbreak in Kenya thus far.

Methods & Materials: Serum and plasma samples obtained from 102 suspected cases were sent to NPHL by field epidemiology teams from affected counties. Aliquots were tested for RVFV using reverse-transcriptase PCR (RT-PCR) and ELISA. Additionally, infection with dengue virus (DENV), chikungunya virus (CHIKV) and zika virus (ZIKV) was ruled out using PCR. Case demographic and clinical data was abstracted from the accompanying Integrated Case Management (ICM) forms used by the Kenyan ministry of health (MOH) for outbreaks.

Results: Majority of the cases were females (78.4%) and the median age was 34.7 (range; 14-72) years. RVFV IgM was detected in 43/102 (42.2%) of all suspected cases. By PCR, RVFV RNA was detected in 18/102 (17.7%). No samples tested positive for CHIKV, DENV and/or ZIKV. Fever and headache were the main clinical presentation among the cases (67% and 54%) respectively.

Conclusion: The NPHL plays a critical role in diagnoses and confirmation of the suspected condition during outbreaks in Kenya. The current laboratory findings confirm the presence and circulation of RVFV in the current outbreak in Kenya, and underscore the need for heightened surveillance in order to institute the appropriate control disease control measures.
Unraveling The Role Of Myotis myotis In The Ecology And Transmission Of Rabies-Related Lyssaviruses (RRLVs)

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Purpose: Insectivorous bats have been found to carry a variety of Rabies-Related Lyssaviruses (RRLVs) across Europe, among which European bat Lyssavirus 1 (EBLV-1) is the most widely distributed. Despite the majority of lethal infections are reported from serotine bats, serological data suggest that other species might have a role in the ecology of this pathogen. EBLV-1 has been associated with spillover events to humans, domestic and wildlife animals, causing a neurological disease clinically indistinguishable from classical rabies. Unraveling its ecology would then be crucial in predicting and mitigating associated risks for public health.

Methods & Materials: During 2012-2018, we investigated the role of Myotis myotis in the maintenance and transmission of EBLV-1, through the longitudinal screening of roosts for neutralizing antibodies and viral shedding. Five neighboring maternal colonies were investigated in Northern Italy, whose connectivity was assessed using genetic and capture-recapture studies.

Results: Antibodies neutralizing EBLV-1 were detected yearly, confirming that M. myotis are constantly exposed to the virus in the area and do survive the infection. In addition, longitudinal data revealed wide oscillations of antibodies during a single reproductive season, suggesting waning of antibodies and waves of infection. All colonies showed similar serological trends over consecutive years. Indeed, we found evidence for genetic panmixia at the nuclear and mitochondrial level and confirmed the movement of adult females between roosts.

Conclusion: Altogether, our results support the role of Myotis myotis as natural host for an EBLV1-like RRLV. While this bat species usually inhabits underground sites in the Mediterranean areas, M. myotis is a strictly house dweller species in northern areas, with all colonies investigated in this study being located in church roofs. Based on both serological and virological findings, we suggest that the risk for human exposure to the virus is limited, with viral excretion restricted to short predictable windows of time. We predict that this study will be useful to secure public health while maintaining a positive relationship between humans and these large vesper bats, whose critical role in the control of pest insects is well recognized in the area.
Purpose: Considering internal medicine point of view, brucellosis can cause a systemic infection involving many organ systems of the body but symptoms are not specific including fever, lassitude, malaise, headache, backache, arthralgia, and can go unrecognized so that the true incidence still unknown. Most of Rwandans especially in rural areas consume unpasteurized milk and other dairy products and are in contact with cattle and other domestic animals reservoir of this bacterium. This study determined the magnitude of human brucellosis in patients presenting with acute febrile illness at the selected primary health care facilities of the selected sites in Southern province Huye and Gisagara districts comparing low and high risk patients as defined in other published studies for human brucellosis.

Methods & Materials: This is a cross-sectional study among population at high risk of brucellosis infection compared to low risk population. We used both qualitative and quantitative strategies of data collection. The study population was comprised of patients presenting to health centers with fever and one of the following: headache, backache, arthralgia, joint swelling, skin rash, enlarged spleen or lymph nodes at the time of visit. Data were collected from September 2016 to March 2017 using questionnaire and drawing blood sample for RBPT which was used to test patients’ sera for brucella antibodies.

Results: Our study recruited 250 symptomatic patients presenting at health centers in Southern Province. The overall seroprevalence of brucellosis during study period is 2.8% with Gishubi health center having the highest seroprevalence of 5% and Rango having the lowest 1.4%. Of the above clinical features, recurrent fever was the only significantly associated with brucella seropositivity, p value<.001 and Most of the assessed risk factors were having an OR between 1.11-2.14 but with a wide confidence interval and a p value greater than .05.

Conclusion: Brucellosis exist in Rwanda Southern province but the prevalence is low resulting in lack of significant association between studied exposures and brucella seroprevalence. Recurrent fever is an important predictor of having brucella antibody in our study participants.
Purpose: Rotavirus and Cryptosporidium spp. are the major cause of diarrhea in the world and Mozambican children. The report of waterborne cryptosporidiosis and rotaviruses and the cross infection of both between humans and animals, suggests the existence of zoonotic transmission. In this context the aim of this study was to determine the occurrence of Rotavirus and Cryptosporidium spp. in children admitted to Hospitals in central Mozambique.

Methods & Materials: A total of 171 children up to 14 years old admitted with diarrhea at Central Hospital of Beira and Provincial Hospital of Quelimane, from June 2015 to March 2017, were enrolled. In total 165 fecal samples were collected and examined by ELISA method to detect Rotavirus and microscopic method (modified Ziehl-Neelsen) to search Cryptosporidium spp. Data were analysed by means of SPSS v.16, using Chi-square test.

Results: Overall 15.8% (26/165) of the samples were positive for Rotavirus and 6.6% (11/165) for Cryptosporidium spp. Children aged from 01 to 11 months were the most affected, 23.2% (13/56) and 12.5% (7/56) by both parasites, respectively. The most of children who drank untreated water were infected by Rotavirus, 17.3% (18/104) and Cryptosporidium spp., 8.7% (9/104), resulting in a significant association between the consumption of untreated water and the frequency of infection by Cryptosporidium spp. ($p = 0.044$). The majority of the children, 66.4% (109/164), had contact with animals and 20.1% (22/109) of them were Rotavirus positive. There was a significant association between the frequency of infection and the contact with animals ($p = 0.044$), at 5% significance level.

Conclusion: The frequency of Rotavirus and Cryptosporidium spp. was high. The association of the exposure of children to the consumption of untreated water and contact with animals to the infection by pathogens with zoonotic potential represents an alert for the implementation of researches which would elucidate the role of zoonosis in diarrhea outbreaks and consequently generate subsidies to direct policies towards its prevention and control in Mozambique.
Zoonotic Risk Related To Importation Of Live Animals

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Purpose: In the course of globalisation, trade in live animals occurs on a large scale, such as companion animals traveling with their owners, horses for sport, animals for zoological gardens, etc. The goal of this study was to make an inventory of animals imported and the possible zoonotic risk.

Methods & Materials: Using the importation data of the Netherlands Veterinary Authority we made an overview of the animals imported in 2017 and a list of the relevant zoonotic diseases per species as ruled by current EU legislation.

Results: 7,559 shipments of live animals (with 3,098 horses, 630 dogs, 192 cats, 518 pigs, 13,270,159 ornamental fish, 1,450,500 1-day old chicks, 723 rodents for research, 5,5 billion insects, 143,808 reptiles and amphibians, 1,641 birds and 806 wild and exotic mammals) coming from the countries outside the European Union were controlled in 2017 at the Border Inspection Point (BIP) at Schiphol airport, the Netherlands.

In order to prevent the incursion of zoonotic infections all animals undergo veterinary import control at first point of entry into the EU by an official veterinarian. This control consists of checking the health certificate, identification of the animals, a physical health check and control of wellbeing. No zoonotic diseases have been reported in legally imported animals in 2017.

Conclusion: The import control of legal trade offers good protection against incursion of zoonotic infections. However, there is always a possibility that imported animals are not detected because they are still in the incubation period and/or do not show evident clinical symptoms. Especially non-human primates, evolutionary close to humans, bats and rodents are the species with the largest potential zoonotic risk.

An additional potential risk for the introduction of infectious zoonotic diseases are exotic vectors coming with imported animals or goods. With climate change and global warming, it is possible that these vectors can survive in the EU in the future and transmit zoonotic diseases.

In 2017 at Schiphol airport handled 68,4 million passengers sometimes bringing live animals illegally, which can have a substantial risk of importing zoonotic infections, because of unknown health status.
Arachnid Envenomations: A Systematic Review of Envenomation Prevention Measures, Therapeutics, and Antivenom Accessibility

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Purpose: Members of the class Arachnida are eight-legged arthropods, and include spiders (Araneae) and scorpions (Scorpiones). Spider and scorpion injury can cause pain at the site, with systemic symptoms, including neurotoxicity, hemolysis, tissue necrosis, and autonomic dysfunction, arising after severe envenomations. In such scenarios, prompt administration of antivenom may substantially reduce morbidity and mortality, however, awareness of appropriate management strategies and access to antivenom amongst clinicians is generally lacking. Additionally, clinicians may misdiagnose envenomations due to the absence of bite history and knowledge of cardinal symptoms. With increased human migration and transcontinental shipment of produce from the tropics, the incidence of arachnid envenomations may increase in non-endemic areas. We aim to synthesize existing evidence around prevention and treatment of arachnid envenomations into a clinical resource, including provision of information on access to, and indications for, antivenom.

Methods & Materials: PubMed (NCBI) and MEDLINE (OVID) were searched for from inception to June 2018 using combinations of the search terms "spider", "scorpion", and "envenomation*". Iterative inclusion and exclusion of search terms was employed to maximize relevant article extraction. For the systematic review, we will include observational studies, case reports, case series, and cohort studies, as well as clinical trials reporting therapeutic outcomes, and antivenom safety, tolerability, and efficacy. Molecular epidemiology and purely mechanistic pathogenesis studies will be excluded. The GRADE approach will be used to assess quality of studies reporting therapeutic interventions. Evidence will be summarized using descriptive measures for each intervention type. Meta-analysis will be planned if sufficient efficacy measures for the same intervention are available.

Results: 412 MEDLINE articles and 788 PubMed articles were retrieved for title and abstract screening; after duplicate removal, 637 remained. Data will be grouped and summarized for ease of clinician use by prevention and therapeutic strategies including antivenom, and according to geographic location and species.

Conclusion: Increased transcontinental movement of people and tropical produce has facilitated importation of arachnids to non-endemic regions where clinicians lack familiarity with envenomation syndromes and appropriate therapeutics. Synthesizing the current evidence around therapeutic strategies including antivenom for medically significant arachnid envenomations can inform the development of appropriate treatment and prevention protocols.
Rabies neutralizing antibodies in terrestrial sylvatic animals from a region in Brazil endemic for two independent wildlife rabies variants

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Purpose: Rabies is a zoonotic viral disease that still presents importance in different regions of the World. Rabies lyssavirus (RBV) wildlife species has emergent importance in Brazil, in the Northeast region, two species of terrestrial mammals – marmosets and crab eating foxes were identified as reservoirs of independent RBV variants responsible for annual cases in these species, domestic animals and humans. The aim of this study was the detection of rabies neutralizing antibodies (RVNAs) in terrestrial sylvatic mammals from an endemic region for wildlife rabies.

Methods & Materials: Serum samples were collected from animals captured in peridomestic environments or kept as pets. RVNAs were detected using Rapid Fluorescent Focus Inhibition Test – RFFIT according to the WHO guidelines. A sample was considered positive when a complete neutralization was observed from the first dilution (1:5) and the cut-off value was established 0.11IU/mL.

Results: A total of 159 samples from 10 different species were tested. Out of these, 16 (10%) presented positive titers: 07 capuchin monkeys, 05 marmosets, 03 crab eating foxes and 01 raccoon. With the exception of the capuchin monkeys, all animals were free ranging and from regions presenting cases of RBV due to the two terrestrial wildlife variants from Brazil. The RVNAs observed indicate that the animals had previous contact with the virus. The ways in which animals can make contact with the virus in nature are unclear and can be a consequence of an insufficient amount of virus to produce the infection. The capuchin monkeys were kept as pets and were probably vaccinated during the vaccination campaigns for cats and dogs.

Conclusion: The detection of RVNAs demonstrates the possibility of contact with RBV without apparent disease and the response to the misuse of the vaccine aimed to cats and dogs. There is a lack of data regarding RVNAs in sylvatic animals from wildlife rabies endemic regions, and Brazil does not have wildlife vaccination campaigns. Samplings of the animals were made considering animal, environmental and human interactions characteristics. These results can be important for the adoption of more efficient measures for wildlife rabies surveillance and control, as the implementation of specific terrestrial wildlife vaccination.
Monitoring systems of Salmonella in Spain to assess a "One Health" approach towards a potential risk to humans from ingestion of contaminated pork meat

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Purpose: Salmonella are a ubiquitous genus of bacteria commonly found in the intestines of healthy birds and mammals that can cause food-borne illness in humans. It is frequently found in eggs and raw meat from pigs and poultry. Pork is the third most frequently contaminated meat, after fresh chicken and turkey. Salmonella is the agent more frequently involved in foodborne outbreaks in Spain.

Spain is a powerful global producer of swine, ranking first in swine census of the EU in 2015 (28.3 million heads). The production of pork in 2015 almost reached 4 million tonnes (3.8 millions), with more than 45 million heads being slaughtered. Spain is the world's fourth pork producer, after China, USA and Germany. Mainly an exporting country, it has become the EU's third largest exporter of swine, after Germany and Denmark. The Salmonella status in pigs is monitored through faeces sampling at the farm, or more generally, at the slaughterhouse, where also mesenteric lymph nodes can be tested.

When Salmonella is diagnosed in humans, very often the patient receives treatment without further investigation, unless there is an outbreak associated with the case which would then result in an investigation of the possible source of infection.

Methods & Materials: Here we review the monitoring systems in Spain of Salmonella in swine, pork and pork products and humans to assess.

First, we have reviewed the different directives and regulations that mandate the monitoring of salmonella in Spain, focusing on salmonellas of swine origin. Next, we have identified the official sources of Salmonella monitoring in Spain. Then, we have analysed the results of the different monitoring systems. Finally, we argue the differences between systems and potential ways to integrate surveillance across sectors if needed for the benefit of all parties.

Results: Results obtained allow us to evaluate the feasibility of a one-health perspective towards surveillance, identifying potential gaps and suggesting improvements towards a more integrated approach in this significant zoonosis disease.

Conclusion: Control of Salmonella must focus on preventive action and monitoring targeted. This work has been funded by the EU Project JRP6 - NOVA - FBZ1 - 1st Call.
Detection of Zika virus RNA and neutralizing antibodies in neotropical primates during the epidemic period for the disease in Brazil

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Purpose: Zika virus (ZIKV) is a mosquito-borne viral disease associated with fetal microcephaly and other central nervous system (CNS) symptomatology. It was first identified in a Rhesus macaque in Uganda and later in humans (Zika fever), in 2015 ZIKV was notified in Northeast Brazil related to CNS alterations and rapid spread. Considering that old-world primates are susceptible to ZIKV, samples from neotropical primates from Ceará state, Northeast of Brazil, were preliminary tested and found to be positive for ZIKV by qPCR. The aim of this study was to extend the sampling of primates by revisiting the localities with positive animals and to study the presence of immune response to the virus.

Methods & Materials: A total of 132 samples from six species of neotropical primates captured from June 2015 to December 2016 was tested, out of these, nine (six marmosets – Callithrix jacchus – and 03 capuchin monkeys – Sapajus libidinosus) were positive by quantitative RT-PCR assay. The genetic sequences showed high similarity with the strains circulating in Brazil. Neutralizing antibodies for ZIKV were detected by PRNT in two samples (PRNT₅₀ = 73,7 and 13,2).

Results: All the positive samples were obtained from peridomestic animals captured in proximity to humans and captured between the June of 2015 and February of 2016, from March to December of 2016 there were no positive samples. The temporal detection of ZIKV in the primates is in agreement with the reports and confirmations of congenital syndrome related to ZIKV in Ceará, where the majority of reports occurred from November/2015 to March/2016 with significant decrease after this period. The two animals that had neutralizing antibodies for ZIKV as indicated by PRNT were in direct or close contact with animals positive for qRT-PCR and these antibodies may constitute additional evidence for virus circulation.

Conclusion: These results demonstrate the occurrence of natural ZIKV infection in neotropical primates and the temporal detection suggests that virus detection in animals accompanied the epidemic period in humans. However since some positive animals were free raging, a continuous surveillance is necessary to exclude the possibility of virus circulation and transmission in wild environments.
20.064
Tularemia - possible increase and new risk factors

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Purpose: Tularemia is a zoonotic disease caused by the bacterium \textit{Francisella tularensis}. In Europe each year approximately 1200 human cases are reported. Four subspecies are currently known: \textit{tularensis} (the most virulent form), \textit{holarctica} (the most widespread form), \textit{mediasiatic}, and \textit{novicida}. In Austria \textit{Francisella tularensis} supsp. \textit{holarctica} is endemic in the eastern part of the country (Lower Austria and Burgenland), and is known to have a 5-year cycle. Zoonotic transmission from pet species in Europe has only been described in Norway due to a cat bite, as well as after an accidental exposure to the disease while spaying a cat. In 2014 first reports of clinically ill dogs were reported from Norway.

Methods & Materials: As hunting with dogs has a long tradition in Austria, and as there are endemic areas for the disease a first serological screening of 80 hunting dogs used in the hunt for European brown hares (\textit{Lepus europaeus}) was conducted.

Results: Of these 80 dogs 5 tested positive for tularemia (6.25%, CI 2.1% - 14%). One positive dog had shown some clinical symptoms, however this female dog also tested positive for \textit{Brucella canis}.

Conclusion: This result shows that dogs not only have contact to the pathogen, but also seroconvert. The occurrence of the disease is thought to increase in the next years due to our changing climate, and this year there is a new hotspot of the disease in Austria (i.e. Salzburg). These changes, as well as the result of this study highlight the need to raise the awareness level of the disease, its possible increase and new risk factors.
Evidence of Filovirus and Henipavirus In Bats and Bat Harvesters, India

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Purpose: Bats are reservoirs of several medically-important viruses. There has been little research on bat-borne viruses in India. The northeast state of Nagaland in India is a mountainous region where bushmeat is a key source of protein. Bats are hunted by several groups there and this provides an opportunity for cross-species transmission.

Methods & Materials: Our work examines sites with intense human-bat interfaces, such as traditional bat harvests in India. Here we collected 121 serum samples from three species of bats harvested from across the region and 85 human serum samples from individuals who participate in the traditional bat harvest. We also collected several tissue samples from the bats collected at the harvest. Sera were screened with a multiple serological assays for antibodies against medically henipaviruses and several members of the family filoviridae. Bat kidney, lung, and spleen tissues were pooled and tested with a pan-filovirus PCR and a pan-paramyxovirus PCR.

Results: All bat tissues were PCR-negative for filoviruses, but there were paramyxovirus positive samples. All three species of bats were seropositive for filoviruses and paramyxoviruses, while there were human sera samples that were serologically positive for filoviruses.

Conclusion: This study demonstrates that there likely has been exposure of humans to filoviruses.
Purpose: Nipah virus (NiV), a paramyxovirus, causes febrile encephalitis and severe respiratory disease in humans and animals. NiV outbreaks have been reported from Malaysia, Bangladesh, India and Singapore. The case positive in humans have been attributed to zoonotic transmission from pigs and bats, human-to-human transmission, and eating fruits or juices contaminated with bat secretions. At present no vaccines or drugs are available for those infected with NiV. Fruit bats of family Pteropodidae have been identified as the reservoir for NiV. Whole genome Nipah virus can be help in understanding the epidemiology, evolution and origin of this virus.

Methods & Materials: The first whole genome of NiV from pooled urine of Pteropus lylei bat (flying fox in the Pteropodidae family) was sequenced using Next generation sequencing in Thailand. The total read of MiSeq sequencer was 19.8 Gb, where sequences of Pteropus host genome were removed, and NiV was identified using virus sequences from NCBI database.

Results: The 4,735 sequences mapped to reference generated 18,236 nucleotides, and average depth of coverage was 41.67% for NiV from Bangladesh 2004 (GenBank accession number: AY988601.1). The genome shared greater than 99% identity (18084/18236 nucleotides) with NiV isolated from a patient in Bangladesh in 2004 (AY988601.1) and 92% identity with NiV Malaysia strain. The phylogenetic tree of whole NiV genome sequenced in Thailand also showed it was closely related to NiV from Bangladesh 2004 (AY988601.1).

Conclusion: This finding shows the potential risk of NiV outbreak in Thailand. The preparedness to prevent the potential transmission of NiV from bat to the community or livestock animals, is essential.
The Significance of the Entomological Surveillance in the Prevention of Vector-Borne Diseases

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Purpose: The aim of this presentation is to highlight the role of the entomological surveillance in the prevention of mosquito-borne diseases (MBD), using mainly, but not only, the long term mosquito-West Nile virus (WNV) monitoring in north-eastern Italy as a paradigmatic example. Going through the objectives of the entomological surveillance and using other targeted surveys we show how the results obtained were used to better target surveillance, to drive methods of mosquito control and risk communication and to prevent human transmission by modulating the screening of blood donors.

Methods & Materials: Since 2010 an entomological monitoring was set up using CDC-CO₂ traps and screening of mosquitoes by RT-PCR and sequencing for Flaviviruses. Other surveys included captures of mosquitoes every 2hrs for 24hrs, pre- and post-disinfestation captures, PCR blood meal analysis of fed Culex pipiens, retrospective analysis for other MBDs using stored mosquito DNA, and WNV complete genome.

Results: Over 1 million of mosquitoes were collected in 158 sites, with Cx.pipiens the most abundant (88%) and the only vector of WNV and USUV. Tahyna, Marisma and mosquito-only Flaviviruses were isolated for the first time. The retrospective analysis on zoonotic filariae indicated the presence and distribution of Dirofilaria immitis and D.repens. Cx.pipiens fed preferentially on birds, mainly blackbird, magpie, sparrow and collared dove. Cx.pipiens changed its host searching activity according to the season, showing a night/evening peak in early/late summer, respectively. The disinfestation in rural areas did not reduce Cx.pipiens density. The entomological monitoring proved to early detect the viral circulation, before human cases.

Conclusion: The entomological monitoring defined the risk areas for WNV. New viruses could also be detected. The blood-meal analyses indicated possible bird targets for surveillance. The determination of Cx.pipiens peak of activity defined the highest risk of human bite and WNV transmission. The control of the efficacy of disinfestations highlighted its poor efficacy in rural areas. The early detection of WNV in mosquitoes is now one of the triggers for the starting of WNV human blood screening, as stated by the National Integrated Surveillance and Control Plan for WNV and USUV, in a perfect One Health perspective.
The role of service dogs in the epidemiology of dirofilariosis

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Purpose: Climate change has affected mosquito abundance and their seasonal survival in many areas of Europe, greatly influencing the spread of vector-borne diseases. Dirofilariosis is an emerging zoonotic infection in which the human serves as an accidental host. The definitive hosts of Dirofilariosis are animals of the families Canidae, Felidae, and Viverridae, the intermediate hosts are mosquitoes of the family Culicidae. The most important source for human transmission are dogs. In recent years, the number of dogs used by Russian police and military services has been more than 100 000. Purpose is to study the dynamics of infection in service dogs and their role in the spread of dirofilariosis.

Methods & Materials: Study was conducted during 2000 -2018. We examined 1078 blood samples of service dogs from different territories of Russia by concentration method with 3% acetic acid. The species of microfilariae were determined by the structure of the head and the tail-end of worms.

Results: Within 2000-2008 years, the contamination of service dogs with dirofilariosis was 29.0%, for example in Veliky Novgorod this indicator reached up to 50%. Due to active therapeutic and preventive measures infestation decreased to 17.2% in 2009 -2017. The results of a survey of dogs arrived to the territory of Rostov-on-Don for the protection of socially significant objects in June 2018 showed the prevalence of infection of service dogs was 13.0%. Animals from territories of central Russia were infected in 16.6%, this indicator in dogs from the territories of the south of Russia was 9.1%. The infestation of service dogs with D. repens was 8.7%, D. immitis - 1.4%, mixed invasion was noted in 2.9% dogs.

Conclusion: Service dogs are important source for spread of dirofilariosis in various territories due to frequent change of places of their service and movement throughout the territory of Russia. Active preventive measures have led to a decrease in the incidence of contamination with dirofilariosis among service dogs. However, their infection with dirofilaria remains quite high.
Suspected Exposure to Filoviruses Among People Contacting Wildlife in Southwestern Uganda

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Purpose: The extent of filovirus exposure and the primary sources of ebolavirus spillover to high-risk communities remains poorly characterized. In the Bwindi region of Uganda, a hotspot of mammalian biodiversity in Africa, human livelihoods are intimately connected with wildlife, creating potential for exposure to filoviruses. Our objectives were to investigate previous exposure to filoviruses in people in the Bwindi region and characterize risk factors for past exposure, including livelihoods and interactions with wildlife.

Methods & Materials: Samples from 331 febrile patients presenting to healthcare facilities near the Bwindi Impenetrable Forest, Uganda, were tested using molecular (PCR) and serological assay detection techniques. Serum was tested by Western blot utilizing recombinant glycoprotein antigens for Ebola virus (EBOV), Sudan virus (SUDV), Bundibugyo virus (BDBV) and Marburg virus. Questionnaires were used to collect demographic information, travel and medical history, and data on interactions with domestic and wild animals.

Results: All patients were negative for active filovirus replication by PCR. However, several patient serum samples were reactive to SUDV (4.7%), EBOV (5.3%) and BDBV (8.9%) indicating likely previous exposure to these ebolaviruses. Direct contact with duikers was a significant risk factor associated with EBOV seropositivity (OR = 5.6; P = 0.026), while hunting primates (OR = 37.5; P < 0.001) and contact with/or consumption of cane rats (OR = 10.7; P = 0.006) were significant risk factors for SUDV seropositivity.

Conclusion: People in Southwestern Uganda have suspected previous exposure to filoviruses, particularly those with a history of wildlife contact. Identification of antibodies to ebolaviruses in humans in Bwindi likely represents previous infection with SUDV, EBOV, or BDBV or infection with serologically cross-reactive low or non-pathogenic undiscovered filoviruses that also share wildlife hosts. Our findings indicate that spillover of ebolaviruses in humans, and circulation of ebolaviruses, could be more common than previously reported. The results of this study inform ongoing surveillance efforts needed to improve our understanding of the role of wildlife in spillover of ebolaviruses, not only adding to investigations of bats as likely reservoir hosts, but also indicating that secondary spillover host species remain important sources of human infection.
Bovine tuberculosis in the Austrian alpine region

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Purpose: Mycobacterium caprae (M. caprae), a member of the Mycobacterium tuberculosis complex (MTBC), can infect a wide range of domestic animals, wild-life species and humans. M. caprae is the main causative agent for tuberculosis in cattle and red deer in the Alpine regions of Austria, Switzerland, Italy and Germany. It is not the major cause of human TB, which is caused by M. tuberculosis, but humans are susceptible and therefore, the zoonotic disease continues to have considerable public health implications. To estimate the prevalence of M. caprae in the Alps, 1 655 samples of free ranging red deer of different sexes and ages from Austria, Germany, Switzerland, and Italy were studied. Cattle samples from these regions were also tested.

Methods & Materials: Tissues from organs with or without visible lesions were analysed by Real-Time PCR and bacteriological culture. To resolve the genetic evolution of M. caprae in Austria and the Alpine region in more detail, whole genome sequencing and SNP analyses were performed. SNP analysis revealed a more precise subtyping, suggesting transmission pathways. With matrixlysis, our new established detection method, we were able to detect small amounts of mycobacteria in tissue and a higher percentage of positive samples with non-visible lesions than shown for bacterial culture, the gold standard in M. tuberculosis complex diagnostic.

Results: It was shown, that free ranging red deer is a maintenance host in hot-spot areas which are mainly located in Austria and Germany. Regions with prevalences up to 23% of infected red deer were defined. Interestingly, more than 2% of the positively detected animals showed no visible lesions but were positive by bacterial culture. Positive red deer isolates were subtyped by a RD4-based PCR allowing the genetic differentiation of M. caprae isolates into three subtypes termed “Allgäu”, “Karwendel” and “Lechtal”, according to their geographical origin.

Conclusion: The different RD4-subtypes are found spatially distributed and occur in cattle as well as in red deer, suggesting transmission between the two species. Since subtypes are genetically stable in both host species, it is hypothesized that the genetic variations might have developed in the past due to “within-host” replication.
**Coxiella burnetii Infection Presenting as Fever of Unknown Origin: A Retrospective Case Series from Bulgaria**

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**Purpose:** Fever of unknown origin (FUO) is a perplexing medical problem. The causes for FUO are more than 200 diseases. The aim of the study was to present human clinical cases of *Coxiella burnetii* infection debuting as FUO.

**Methods & Materials:** The following methods were conducted in the study: literature search, laboratory, imaging, and statistical methods. Criteria of David T. Durack and Alan C. Street were applied for FUO definition. For the etiological diagnosis indirect immunoenzyme assay (ELISA) for antibodies detection against *Coxiella burnetii* were used. *Coxiella burnetii* phase 1 IgA/IgG and *Coxiella burnetii* phase 2 IgG/IgM antibodies were detected in serum by indirect immunoenzyme assay (SERION ELISA classic, Virion/Serion, Würzburg, Germany).

**Results:** For the period of January 2008 to March 2015 nine patients with FUO caused by *Coxiella burnetii* were hospitalized at the Department of Infectious Diseases, Military Medical Academy, Sofia (Bulgaria). Male gender were predominant (male/female – 77.8%/22.2%), mean age was 48.78±14.52 years (range: 26–67), hospital stay was 9.78±2.95 days (range: 5–15), fever duration was 54.33±56.23 days (range: 21–180). Laboratory investigations estimated elevation of ESR 49.11±31.74mm/h (95% CI: 13.09–111.31), CRP 37.68±37.62mg/L (95% CI: 36.07–111.42) and Fibrinogen 5.69±1.59g/L (95% CI: 2.57–8.81). Mean values of liver enzymes were in reference range. Abdominal ultrasound and X-ray demonstrated 33.3% each of them Contribution to the final diagnosis. Transthoracic echocardiography found 22.2% Contribution. Serological methods presented 100% Contribution.

**Conclusion:** *Coxiella burnetii* infection was accepted as a final diagnosis based on the integrated information from the applied methods. Active search and establishment of this pathogen in case of FUO should avoid potential complications and consequences in case of untreated *Coxiella burnetii* infection.
Purpose: Hepatitis E virus infection (HEV infection) is an emerging zoonosis. It becomes increasingly important in developed countries and occurs as current health and veterinary problem. In pigs, HEV infection is subclinical, but swine are main reservoir of HEV. The aim of the study was to analyze the seroprevalence of HEV in industrial pig farms from Southern Bulgaria.

Methods & Materials: The study was conducted in two different Bulgarian districts (Yambol district and Sofia district). One hundred and eighty serum samples were tested, they belonged to three different age and technological swine groups: piglets (age: 1–3 months), fattening pigs (4–6 months) and sows (over 12 months). Serological test PrioCHECK HEV Ab porcine (MIKROGEN GmbH, Neuried, Germany) was used. The PrioCHECK HEV Ab porcine is a diagnostic test for detection of antibodies directed against hepatitis E virus in porcine serum based on ELISA technology.

Results: The serological test found positive results in both analyzed districts. The overall seroprevalence (in both farms) among piglets was 43.3% (95% CI: 8.9–95.6), in fattening group was 61.7±54.2% and among sows was 93.3% (95% CI: 86.8–99.9). The highest prevalence was found in Sofia district (80/90; 88.9%), and the lowest was detected in Yambol district (39/90; 43.3%). Doubtful results were estimated in 6.1% of all investigated serum samples.

Conclusion: The present study established that HEV is widespread in pigs from Southern Bulgaria. The seroprevalence in Southwestern Bulgaria is twice higher than in Southeastern Bulgaria. Sows are most affected by HEV, while piglets are at least. The obtained results determine preventive measures and further investigations.
Knowledge, Attitudes, Beliefs, and Practices Pertaining to Camel-to-Human Disease Risks among Persons at Camel Farms, Abattoirs, and Meat Markets in Jordan

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Purpose: Camels are the suspected source of primary human infections with MERS-CoV in the Middle East, but little is known about the pathway’s mechanisms and the human behavioral practices that promote it. Understanding knowledge, attitudes, beliefs, and practices regarding camel-to-human disease risk among camel-exposed persons will highlight potential intervention areas to interrupt camel-to-human MERS-CoV transmission.

Methods & Materials: Standardized questionnaires were administered to individuals in close proximity to camel abattoirs, camel meat markets, and camel farms across Northern and Middle Jordan from February to August 2018. Knowledge, attitudes, beliefs, and practices surrounding camel-to-human disease risks were assessed to generate prevalence ratios (PRs) and 95% confidence intervals (CIs) modeling high-risk practices using knowledge, attitudes, and beliefs about camel-to-human disease risks as independent variables. PRs were adjusted for potential confounders (age, gender, education, occupation, and site type). A subset of participants who reported working directly with camels or camel products completed an additional questionnaire to assess current biosecurity practices and attitudes on various proposed biosecurity interventions.

Results: A total of 800 individuals will complete the main questionnaire by August 2018 (409 completed to date). Hypotheses to be tested include: individuals who do not believe camels may transmit diseases to people are (1) more likely to report consumption of un-boiled camel milk; (2) more likely to report consumption of uncooked camel meat; (3) less likely to report proper cleaning of wounds resulting from an animal bite or scratch; and (4) individuals who work directly with camels or camel products who do not believe camels may transmit diseases to people are less likely to report regular use of gloves, aprons, and masks during their work.

Conclusion: Identified knowledge gaps surrounding camel-to-human disease risks will be characterized to better inform educational interventions that may be implemented at camel abattoirs, farms, and meat markets if they are strongly correlated with high-risk behaviors and practices with camels or camel products. Assessment of current usage of personal protective equipment and attitudes surrounding various biosecurity interventions among those working with camels or camel products will be used to describe the feasibility and potential sustainability of the different proposed biosecurity interventions.
Assessment Of The Impact On Paediatric Rabies At Queen Elizabeth Central Hospital, Blantyre, Malawi, Following A Mass Canine Rabies Vaccination Programme

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Purpose: Background
Rabies is a common infection in the domestic and wild animal population of Malawi. Significant numbers of children die a terrible death each year as a consequence. Many more individuals are bitten by potentially infected dogs each month and require post exposure vaccination. However, rabies is a preventable infection against which there is a good vaccine for both animals and humans. Previous work suggests that if canine vaccine coverage can reach 70%, there will be consequent fall in human incidence.

Objectives
To determine the impact of a comprehensive canine vaccine campaign on paediatric rabies cases.

Methods & Materials: Retrospective case note analysis. All historic paediatric rabies cases presenting to Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi, between May 2012 and May 2017, have been identified and analysed. Data analysis compared paediatric rabies case numbers pre and post a comprehensive canine vaccine campaign which commenced in May 2015, in the Blantyre District.

Results: In total 14 paediatric rabies cases were found during the study period. More males than females were affected (males: 10 (71%); females: 4 (29%)). The average age was seven years (range 3-11). The average length of stay in hospital was three days until death (range 1-7). Geographical locations of dog bites since data collection (May 2012) included Limbe, Lundu, Bangwe, Chileka, Mulanje, Muwalo, Nkhataumbera, Thyolo, Zomba, Mpemba and Mangochi region (four were unknown). Since May 2015 only two patients were admitted to QECH, but none of them came from Blantyre city nor district.

Conclusion: This study underlines the importance of eliminating human rabies through canine rabies vaccination. Further funding of these campaigns will prevent unnecessary child deaths.
Prevalence and Subtypes of Blastocystis in Diverse Animals from the United Arab Emirates

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Purpose: The contribution of Blastocystis from non-human hosts to zoonotic transmission is yet to be elucidated. The objective of this study was to determine the prevalence of Blastocystis and increase our knowledge of the epidemiology and host specificity of Blastocystis in different animals in the country.

Methods & Materials: A total of 114 stool samples were tested using PCR amplification of the small subunit (SSU) rRNA gene, cloning and DNA sequence analysis and subtyping.

Results: 32.5% (37/114) were identified as positive for Blastocystis, with 67.5% (77/114) being negative. All 37 positive samples generated the PCR product (~600 bp) and were consequently sequenced for confirmation. The following prevalences were observed in the different animals: cows (45.5%), sheep (72.7%), birds (16.6%), fish (11.1%), rabbit (66.7%), squirrel (50%), African spurred tortoises (36.8%), Iguana (100%), Chinchilla (100%), Shrew-faced Squirrel (100%) and Syrian Tortoise (50%). Of the 37 sequenced samples, twenty-four samples were confirmed by sequence analysis and corresponded to SSU rDNA sequences available in GenBank. Subtypes (STs) amplified belonged to ST4, ST10, and ST14. In the case of cows, two subtypes were identified ST10 (allele 43) and ST14. Similarly in sheep, two subtypes were detected ST10 (allele 43) and ST14. Two mixed infections with STs 10 and 14 were identified in sheep. Only one single ST, ST14 was found in rabbits. In the rodents order, ST4 (allele 42) was detected in squirrels, ST17 in chinchilla, and ST14 in shrew-faced squirrel. The discovery of ST4 in squirrels suggests that rodents may serve as reservoir for human infection. However, knowledge of allele is valuable since human and non-human hosts may share similar subtypes with different alleles. Interestingly, in the case of reptilian hosts, 7 tortoises and 1 iguana were positive for Blastocystis.

Conclusion: More animal groups will be screened and further studies involving the analysis of the whole SSU rDNA sequence of those identified as positive will be performed before confirming a zoonotic transmission.
A Qualitative Inquiry To Understand the Drivers Affecting Uptake Of Health Promotion Intervention To Reduce Zoonotic Infections And Non-Prescribed Veterinary Antibiotics Use In Peri-Urban Smallholder Dairy Farms In Select Sites Of India

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Purpose: India is one of the largest producers of milk and peri-urban areas are the main contributor to meet the rising demand of the cities. Lack of stringent policy and legislative provisions have contributed to adoption of questionable farming practices that not only adversely affect the outputs and profits, but also place farmers, their animals, and consumers at risk of health hazard. In light of limited evidence on the burden of the zoonotic infection and non-prudent use of veterinary antibiotics in India, a study was conducted across three sites to understand the potential risky practices and factors contributing to the burden. To address the same, an intervention package was developed and implemented for one year. The study aims to understand the drivers affecting uptake of health promotion intervention package.

Methods & Materials: All the intervention farms were considered as the sampling universe. Thematic guides were developed using the intervention package. Barriers and facilitators to the uptake of practices related to infection control as well as prudent use of antimicrobials were documented across each theme. Data was analyzed using Atlast i.v7.

Results: A total of 19 interviews were conducted across the three sites. These included 6 farmers each at Guwahati and Ludhiana site and 7 at Bangalore site. Financial stress was found to be a major barrier to the intervention related to infection control as well as prudent use of antimicrobials. Perceived risk of disease, unavailability of a trained veterinarian and lack of infrastructure in the peri-urban settings were others reasons for the non-compliance, majority in Guwahati and Ludhiana site. However, Bangalore study site emerged as positive deviance model. Subsidized feed, concentrate and easy availability of veterinarian, and incentivized system were found to facilitators of the intervention uptake.

Conclusion: Increased level of knowledge and improved attitude towards the prescribed practices could only translate behavioral change through support from health system and policy level efforts. Strengthening extension services through the better penetration in the community like cooperative movement etc. and the establishment of new veterinary colleges and other institutions to bridge the human resources gap can contribute to the relatively more prudent use of antibiotics.
New Approaches To Evaluation Of Relationship Between Epizootic And Epidemic Activity In The Foci Of Co-Circulation Of Different Orthohantaviruses

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Purpose: To determine the main characteristics, indicators and thresholds of active epizootic process in populations of different orthohantavirus (OHV) hosts and demonstrate the their correlation with long-term HFRS morbidity in Russian Far East.

Methods & Materials: During 1980-2017 about 13,000 wild rodents of 5 species (Apodemus agrarius, A.peninsulae, Myodes rufocanus, Microtus fortis and Myodes rutilus) were investigated for the presence of hantavirus infection using ELISA, RT-PCR and IF methods. Also retrospective analysis of 2638 serologically confirmed HFRS cases was performed. We determined such descriptive characteristics of epizootic process as total OHV infection (TOHVI), acute OHV infection (AOHVI) and relative acute OHV infection (RAOHVI), in relation with population of different rodents, and their quantitative ranges, that allowed us to estimate the level and stage of epizootic activity.

Results: The epizootic processes in all rodent’s populations were divided on to 3 stages: 1 stage – the increase, 2 stage – peak, 3 stage - the decrease of OHV activity. The stages 1/2 lasted no more than one year, the stage 3 was continued sometimes 3-5 even 10 years depending on rodent species. Last 15 years on all stages of cycle the threshold indexes were considerable higher for A. peninsulae, than for other rodents supporting the leading epidemiological role of this OHV host in different ecosystems mainly, forest areas. In contrast, in the early of 1980-1990 the activity of epizootic process in population of A.agrarius strongly correlated with HFRS morbidity. Despite the long-term significant fluctuations of activity of epizootic process and dynamics of related human OHV infection, the direct correlation between the HFRS vs. epizootic activity was not demonstrated, and during the study period no trends of HFRS morbidity (increase or decrease) were demonstrated.

Conclusion: Our results support more complex origin of increased HFRS morbidity, then direct relation with high levels of infection among rodents. Main socio-economics events, change of the key regional industries and high invasion of people to natural foci may be the main cause of HFRS outbreaks. Nevertheless, the analysis of threshold indicators of active circulation of OHV in rodent’s populations allows to predict the increased risk of HFRS in nature foci.
Mucormycosis In Pediatric Patients With Hematological Malignancies: Rare, But Real Threat, A Case Series

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Purpose: To highlight the importance of clinical suspicion for early diagnosis and treatment of invasive mucormycosis to optimize the patient’s outcomes.

Methods & Materials: A retrospective review of three pediatric patients with documented invasive mucormycosis between 2014 and 2017. Patient demographics, a pattern of infection, microbiological, histopathological, radiological studies, the medical and surgical treatments and prognosis were described.

Results: Three patients 5, 6, and 13 years old, all are females, with underlying acute lymphoblastic leukemia. Case 1 was on maintenance chemotherapy, while cases 2 and 3 were in the induction phase. Diagnoses for case 1, 2 and 3 were osteomyelitis of the left distal femur, invasive fungal infection of the hard palate and disseminated sinopulmonary mucormycosis, respectively. The diagnosis was established in case 2 and 3 by positive tissue culture for Rhizopus and Rhizomucor species, respectively; while tissue cultures were negative in case 1. Histopathological examination showed features of Mucor species in cases 1 and 2 and features of Aspergillus species in case 3. All 3 patients underwent serial surgical debridement of infected tissue, and received liposomal amphotericin B, in addition to posaconazole. Case 3 received caspofungin for the first 6 months because of disseminated combined mucormycosis and aspergillosis. Recovery was full for case 1 and 2 with no relapse after one year of follow-up; case 3 still on treatment with good response.

Conclusion: Although rare, pediatric mucormycosis is a clear and real threat, we emphasize the importance of early diagnosis and aggressive surgical and medical management to achieve a successful outcome.
Impact of Snakebite on Livestock and Livelihood: A Neglected Issue?

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Purpose: Snakebite is a neglected tropical disease that kills more than 100,000 humans and disables more than 400,000 each year. It primarily affects poor agricultural workers, farmers, cattle herders living in rural areas of developing countries. It is described as an occupational disease. But, the impact of snakebite on these rural communities could be even higher if we use a One Health approach and take into account the possible direct impact on domestic animals and indirect impact on livelihood (e.g. animal losses, impaired productivity). To explore this hypothesis we developed the first scoping review to identify and characterize the global literature on snakebite in domestic animals.

Methods & Materials: Three bibliographic databases (PubMed, Web of science and Agricola) were searched using terms related to snake, snakebite and domestic animals for publications up to December 31st 2016.

Results: The global literature on snakebite in domestic animals (n=143 observational studies, reviews and letters) was strongly biased with most focus on North America, Europe and Australia (69%, n=143) and less on Central and South America, Asia and Africa (31%). The attention is on pets (e.g. dog and cat) (77%, n=119) and less on livestock (e.g. horse, cattle, sheep, goat, pig) (24%). Thirty-seven snake species biting domestic animals were identified. WHO’s Medically Important Venomous Snakes were most frequently involved. The social-ecological determinants of snakebite are poorly documented and include a strong seasonality and a diversity of habitat. Snakebite in animals caused neurotoxic, cytotoxic and hemotoxic envenomation syndromes similar to humans and death. Half of publications on envenomed livestock reported a fatality rate above 47%. There was no literature on the indirect impact of snakebite in animals on livelihood.

Conclusion: This review identified major knowledge gaps with respect to impact of snakebite on livestock and livelihood and suggests a high burden in terms of mortality and potential economic impact. Filling these gaps is necessary for a full understanding of snakebite and to raise awareness on this “neglected of the neglected” issue. This data gap will be addressed by an unprecedented nationwide human-animal health survey of 25,000 households in Nepal and Cameroon funded by the Swiss National Science Foundation.
Prevalence of Antibodies to Foot and Mouth Disease Virus Infection in some Wildlife and Cattle in Bauchi State, Nigeria.

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Purpose: Foot and Mouth Disease (FMD) is an important trans-border viral disease of both domestic and wild cloven hoofed animals characterized by high morbidity with devastating consequence on the livestock economy worldwide, despite the endemic nature of FMD in Nigeria and availability of wildlife and their interactions with livestock in the same habitat during grazing activities, there is scarcity of information on the potential role of wildlife in the epidemiology of FMDV in Nigeria.

Methods & Materials: Sera were collected from chemically immobilized wildlife including 4 elephants (Loxodonta Africana), 11 waterbucks (Kobus ellipsiprymus), 1 hartebeest (Acelaphus baselaphus caama), 24 elands (Taurotragus oryx), 1 kudu (Tragelaphus strepsiceros), 12 wildebeests (Connochaetes taurinus) and 300 cattle (Bos indicus) within and around Yankari Game Reserve (YGR) and Sumu Wildlife Park (SWP) in Bauchi State, Nigeria. Sera were screened for Presence of Antibodies produced against FMDV using priCHECK® 3 ABC NSP ELISA kit and positive samples where subjected for serotyping using Solid-Phase Competitive ELISA, (IZSLER Brescia-Italy).

Results: Of the 300 and 53 sera samples collected from cattle and wildlife 197 (65.7%) and 13 (24.5%) (P<0.05) respectively tested positive for antibodies to FMDV. In cattle antibodies to FMDV were higher (P<0.05) in white Fulani 157 (72.8%) than Red Bororo 23 (39.7%) and Sokoto Gudali 17 (33.3%) breeds of cattle whereas in females FMDV antibodies were higher (P<0.05) 150 (72.8%) than in males 47 (50.0%). In the wildlife, detectable antibodies to FMDV were observed in waterbuck 2 (28.6%), elephant 1 (25.0), wildebeest 4 (33.3%) and eland 6 (25.0%) and four serotypes of FMDV–O, A, SAT-1 and SAT-2 were detected in the wildlife with serotypes A and Sat -1 being dominant.

Conclusion: This appears to be the first report of evidence of FMDV antibodies in wildlife in Nigeria and suggest that wildlife could play an important role in the epidemiology of FMD in Nigeria, and should stimulate continued surveillance at the wildlife-livestock interface level in order to acquire better understanding of their role in the maintenance and transmission of the disease in Nigeria.
Concurrent Measles Infection Of Cynomolgus Monkey During A Reston Ebola Virus Outbreak In The Philippines

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Purpose: Measles virus (MV) infections is still a major cause of vaccine-preventable childhood deaths worldwide. In the Philippines, an outbreak of measles virus occurs every 2 to 3 years. In 2015, a non-human primate facility noted unusual deaths of 6 cynomolgus monkeys (Macaca fascicularis), characterized by generalized rashes and sudden death. This is the first report of concurrent outbreaks of Measles and Reston Ebola virus among non-human primates in the Philippines.

Methods & Materials: 174 serum samples were collected from macaques in the quarantine facility and were tested for MV antibody by Enzygnost Measles Anti-IgM ELISA (Siemens, Healthcare Diagnostics, Germany). The serum samples were diluted in rheumatoid factor absorbent to remove inhibitors that might interfere with the reaction. A TaqMan RT-PCR assay was performed to amplify partial N gene of MV from serum samples. Likewise, amplification of the partial L gene of MV RNA and sequencing of the product was done.

Results: A total of 174 serum samples were tested with 8 samples with anti-MV IgM detected. Also, 1 sample was positive for MV RNA using RT-PCR and was subjected to sequencing. One macaque was found to have both anti-RESTV and anti-MV while another one had dual infection of RESTV and MV as detected by PCR. Phylogenetic analysis of the partial L gene using Mega 6 showed that the MV belonged to genotype B3, the same genotype that caused a large outbreak in the Philippines in 2014.

Conclusion: Measles virus was detected in non-human primates in the Philippines during a RESTV outbreak in a non-human primate quarantine facility. Human to non-human primate MV were reported in quarantine facilities and wildlife parks in other countries. Considering the high risk of MV infection in macaques associated with human MV outbreak in the Philippines, further studies are required to mitigate the transmission.
Purpose: In the absence of national routine surveillance for Q fever, the purpose of this study was both to evaluate the infection rate of *Coxiella burnetii* in ticks and rodents from different landscapes in four regions of Ukraine (North, South, East and West) in order to identify enzootic areas, and also to assess the risk of infection for humans in these regions.

Methods & Materials: During 2014-2016, we tested specimens of ticks and rodents collected by regionals public health facilities in four regions of Ukraine: North (Zhytomyr and Cherkasy Oblasts), South (Odessa Oblast), East (Donetsk Oblast) and West (Ivano-Frankivsk Oblast). Ticks were collected from cattle, dogs and from environment using active method (flagging). Indirect immunofluorescence assay (IFA) was used to identify *C. burnetii*

Results: During the study period, 9,792 specimens of ticks were tested. As a result, *C. burnetii* corpuscles were found in ticks pool from all four region of Ukraine: Donetsk Oblast – 21.05 ± 6.6% of pools; Odesa Oblast – 11.84 ± 2.06%; Ivano-Frankivsk Oblast – 3.86 ± 1.34%, and Zhytomyr Oblast - 14.29 ± 9.3%; samples from Cherkasy Oblast were negative.

During testing of 357 rodents from 19 rayons of Odesa Oblast, *C. burnetii* was detected in 6.3 ± 2.16% of the pools, including: Danube-Dniester area – 7.41 ± 5.04%; Transnistrian area – 5.71 ± 2.77%; the city of Odesa and neighboring areas – 6.67 ± 4.56%. Q fever pathogen was found in 3 rodent species: *Mus musculus*, *Apodemus uralensis*, and *Microtus arvalis*.

Conclusion: Our study has identified new enzootic territories for Q fever in all four regions of Ukraine and confirmed that existing natural foci of the diseases remain active. Study results have become the basis for determining the landscape and geographic areas with high and low risk of infection for human. Further studies of the pathogen circulation and risk factors are needed to prevent spread of Q fever and outbreaks in population living in enzootic areas.
20.083
Molecular Survey Of Tick-Borne Pathogens (TBPs) In Dogs In Italy

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Purpose: Dogs are common feeding hosts of *Ixodes ricinus* and infected animals may act as reservoir for human tick-transmitted infectious agents and as carriers of ticks into human settings. The aim of this work was to evaluate the presence of tick-borne zoonotic bacteria by molecular methods in dogs from North Eastern Italy.

Methods & Materials: A total of 150 blood samples were collected from dogs entering a municipal kennel. After DNA extraction, the presence of *B. burgdorferi* s.l., Spotter Fever Group (SFG) *Rickettsia* spp., *Anaplasma* spp. and *Ehrlichia* spp. was screened by real-time or conventional PCR. PCR-based assays were carried out for genes 23S rRNA (*B. burgdorferi* s.l.), *glt*A (SFG *Rickettsia* spp.) and 16S rRNA (*Anaplasma* spp. and *Ehrlichia* spp.). Species identification of *Anaplasma* spp. and *Ehrlichia* spp. positive samples was obtained by comparison of nucleotide sequences of conventional PCR products amplifying a portion of 16S rRNA and/or *groEL* genes to nr/nt database by BLAST.

Results: Thirteen dogs (8.7%) were positive to *Anaplasma* spp. (9 animals, 6.0%) or *Ehrlichia* spp. (4 animals, 2.7%); 5 showed sequence similarity ranging from 99% to 100% with *A. phagocytophilum*, 2 with *A. platys*, 2 with *Anaplasma* spp., 3 with *Ehrlichia canis* and 1 to *Ehrlichia* spp. Neither *B. burgdorferi* s.l nor SFG *Rickettsia* spp. were found.

Conclusion: The results demonstrate the presence of zoonotic TBPs in dogs from North Eastern Italy. Infected dogs, other than representing a reservoir, provide a picture of the circulation of pathogens in the territory and give an idea of the risk for humans of entering into contact with these infectious agents. The findings confirm that, in spite of the climate changes, in North Eastern Italy the prevalence of SFG *Rickettsia* spp. is still lower than in Central and Southern Italy where *Rickettsia conorii* is largely diffuse.
Molecular Survey Of Zoonotic Agents In Rodents From An Urban Environment, Hungary

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Purpose: Along with several effects, urbanization causes significant changes in the distribution of animal populations and procures a closer coexistence between animals and humans, therefore potentially induces changes in zoonotic disease transmissions as well. Rodents are reservoirs for several zoonotic pathogens. Among others, leptospirosis and hantaviral infections are the most widespread zoonotic diseases worldwide. Hepatitis E virus is a significant causative agent of acute hepatitis with various host groups, some of them may have zoonotic potential. In this study, we performed a molecular survey of pathogens among wild rodents living in urban environment, thus occur in close proximity to humans.

Methods & Materials: Small rodents were collected in the city of Pécs (Southwest Hungary) using live traps, and screened for pathogens by different types of PCR methods (TaqMan-based real-time PCR, RT-PCR, PCR), using specific primer sets, followed by DNA sequencing and molecular sequence analyses.

Results: Altogether 338 rodents, belonging to 5 species (Apodemus agrarius, A. flavicollis, A. sylvaticus, Microtus arvalis and Myodes glareolus) were tested. A total of 18% of the rodents were infected with Leptospira species (all the rodent species), 9% of them were infected with Tulahantavirus and Dobrava-Belgrade orthohantavirus (M. arvalis, M. glareolus, A. agrarius), and 3% of them (M. arvalis) were infected with Hepatitis E virus (Orthohepevirus C, presumably a novel Microtus-specific genotype). Dual infections were detected also: Leptospira with hantaviruses in 9 (2.6%) rodents, Leptospira with HEV in 4 (1%) rodents, Tula hantavirus with HEV in 2 (0.5%) animals. Multiple co-infections with all three pathogens were not found. However, dual infections suggest that the same rodent host can be infected with several pathogens at the same time.

Conclusion: In this study, rodent-borne pathogens (Leptospira spp., DOBV) with significant importance for further clinical considerations were identified. Although these pathogens were already known in Hungary, we provide important surveillance data on them from rodents within urban territories for the first time in the country, pointed out serious threat to public health. Along with these results, a new genotype of Hepatitis E virus was detected also, even though, in order to estimate the potential zoonotic capability of this virus, further investigations are needed.
Differential Distribution of *Salmonella* Serovars and *Campylobacter* spp. Isolates in Free-Living Crows and Broiler Chickens in Aomori, Japan

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**Purpose:**

*Salmonella* and *Campylobacter* cause foodborne enteritis mainly via the consumption of raw/undercooked contaminated poultry meat and products. Broiler flocks are primarily colonized with these bacteria; however, the underlying etiology remains unclear. The present study was conducted in order to obtain further information on the prevalence and genotypic distribution of *Salmonella* serovars and *Campylobacter* spp. in free-living crows and broiler flocks in a region for 2 years, thereby facilitating estimations of the potential risk of transmission of *Salmonella* serovars and *Campylobacter* spp. from crows to broiler flocks.

**Methods & Materials:** This study was conducted in Aomori prefecture, the most north-eastern part of the main island of Japan. A total of 123 crows were captured on 32 occasions between October 2012 and April 2014, using box traps placed at two sites 2.2 km from a city in the prefecture, where more than 20 broiler farms as well as a few scattered cattle and pig farms exist. The species of crows were visually confirmed by the size and shape of the beaks. They consisted of 78 jungle crows and 28 carrion crows. All 123 crows were tested for *Salmonella*, of which 89 were also tested for *Campylobacter*. The ceca were taken aseptically and divided into 2 parts of approximately 0.4 g each; one for the isolation of *Salmonella* and another for *Campylobacter*. The isolates were subtyped by PFGE and MLST analyses and antimicrobial susceptibility test.

**Results:**

*Salmonella* serovars Bredeney and Derby were isolated from 8 and 3 out of 123 captured crows, respectively, both of which are not common in broiler chickens. *Campylobacter* were isolated from all 89 crows tested and *C. jejuni* was prevalent (85 crows). PFGE showed broad diversity in the crow isolates of *C. jejuni*. However, 3 crow isolates and 2 broiler isolates showing similar banding patterns were assigned to different sequence types in MLST and different antimicrobial profiles.

**Conclusion:** These results indicate that crows do not share *Salmonella* serovars with broilers, and harbor various genotypes of *C. jejuni* that differ from those of broiler flocks. Thus, crows are not a potential vector of these bacteria to broiler flocks in this region.
Epidemiological Features of Human Cases Received Rabies Post Exposure Prophylaxis After Rabies Suspected Animal Bites in the Zenica-Doboj Canton, Bosnia and Herzegovina

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Purpose: To determine epidemiological features of human cases received rabies post exposure prophylaxis (PEP) in the Antirabies Service of the Epidemiology Department, Institute for Health and Food Safety, Zenica-Doboj Canton (ZDC) Bosnia and Herzegovina (B&H).

Methods & Materials: Data of all admitted patients (and causal animals) for the 2009-2017 period were analyzed: age, gender, municipality, anatomical bite/scratching site, type (source) of animal (stray/owns/wildlife), veterinary observation of animal, antirabies PEP received or not.

Results: Total of 1717 persons were admitted (incidence of 4.71/1000/ZDC population). Bites/scratches were the most frequently noticed during the April and May, 182 (10.6%) and 163 (9.4%), respectively. Persons admitted were mostly from Zenica municipality, 1279 (74.5%; incidence of 11.55/1000) (66.6% of urbanization). Males were frequently represented comparing to females, 1089 (63.4%); average age 37.42 years (1-86 years). Patients were mostly 50-64 and 25-49 years of age, 425 (24.7%) and 391 (22.7%), respectively. Dog bites were the most represented ones, 1634 (of 1717; 95.1%), of which 1255 (77%) were caused by stray dogs. A total of 997 (58%) patients were indicated for PEP. Only 340 (of 1717; 19.8%) animals were underwent to 10-day veterinary observation: 3% among stray and 76.1% among owned animals. Largest number of injuries were presented at lower extremities, in 716 (41.7%), of which lower leg was presented in 242 (14.1%), and upper extremities, in 221 (12.9%), of which hand was represented in 127 (7.4%) cases. In 124 (7.2%) patients the injuries were presented at multiple sites of the body.

Conclusion: The ZDC is rabies free region. But, because of large rate of stray animals no to underwent veterinary observation, no existence of unique dog register and consequently no information about stray animals number/vaccination/neuter/euthanasia, there is urgent need for improving prevention and control of the disease through One Health concept.
Purpose: A reassortant avian influenza A(H5N6) strain emerged in Europe in December 2017 which is virologically considered unrelated to the Asian lineage H5N6 associated with severe human infections in China. To date there are limited data on outcomes of human exposures to the reassortant strain. Between 8 January and 31 May 2018 there were 20 wild bird detections of avian influenza A(H5N6) in England, more than any other country in Europe. These incidents involved multiple human exposures relating to the handling of dead birds, which were managed with active health surveillance for up to 10 days after last bird contact and oseltamivir post-exposure prophylaxis (PEP) for those within 7 days of such contact. The objective of this presentation is to describe the human exposures and associated outcomes to this strain in England, to inform the emerging evidence base for the zoonotic risk of this strain.

Methods & Materials: Following animal health notification, local health protection teams managed the public health response and collected information about human exposures in the field environment, to birds with laboratory confirmed infection, which was then summarised descriptively at the national level. This included: the proportions of exposures where personal protective equipment (PPE) was worn and PEP provision; the median and range of days under health surveillance; the number of individuals who developed possible avian influenza symptoms during surveillance and influenza testing results.

Results: 67 separate human exposures were recorded. Of these, PPE was worn during 17 exposures (25.4%), not worn during 39 (58.2%) and not known for 11 (16.4%). PEP was provided for 33 exposures (49.3%), was unknown for 4 exposures (6.0%) and too late or refused for 30 exposures (44.8%). The median period of active follow-up for exposures was ten days (range: 1-42). Five individuals reported mild respiratory symptoms during their follow-up period but none tested positive for influenza virus.

Conclusion: Despite 67 identified exposures, with no PPE worn in over half of these exposures, no human infections were identified. This adds to the emerging evidence base for the low zoonotic risk of reassortant European lineage H5N6.
Occurrence Of Coxiella Burnetii Infection At Human-Animal Interface In Chhattisgarh, India

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Purpose: Coxiella burnetii, causative agent of Q fever in humans, is an emerging zoonotic pathogen. Ruminants act as primary reservoir, and serve as the most important source of infection for humans. Most of the Q fever infections in humans remain unapparent, while some manifest acute symptoms. Around 1-4% of the acute cases persist as chronic or persistent focalized infection. Chronic cases mostly persist as localized cases such as endocarditis, hepatitis, vascular infection (aneurysm) are commonly observed. The agent also causes abortion in pregnant women. The present study was conducted to determine occurrence of the agent at human-animal interface.

Methods & Materials: To perform sero-screening of C. burnetii in goats and humans using commercial i-ELISA as well as PCR employing is1111 and comT genes. Serum samples were collected randomly from goats (n=65) and humans (n=93) from Chhattisgarh state of India.

Results: Apparent sero-prevalence of C. burnetii in goats and humans was observed to be 27.6% and 46.24%, respectively, whereas, 20% of animals screened were found to be positive by PCR. None of the humans tested yielded positivity by PCR. Out of the animal samples screened, 12 were found to be positive for both PCR and ELISA, whereas, 6 samples were negative for PCR, but turned positive for ELISA. One sample which was negative for ELISA revealed positivity by PCR assay.

Conclusion: As the humans and goats samples were collected from the same geographical area, high prevalence in both the species indicated possibility of the transmission of infection from animal reservoirs to humans. Despite high serum prevalence in humans and animals, no clinical signs were observed, as most of the infection remains unapparent or with a history of mild fever in humans, remains unnoticed without overt clinical symptoms. However, risk of developing chronic infection in the apparently healthy individuals cannot be ruled out; hence, similar studies needs to be conducted across India in order to estimate actual burden of the disease.
Purpose: The continued advance of African Swine Fever (ASF) through Eastern European countries represents a threat for the European pig industry. ASF is often described as a highly contagious infectious disease of swine. In the current affected countries in the European Union (EU), the spatio-temporal dynamics of ASF spread in wild boar appear to be different; i.e. in Estonia, the whole country has been affected since its introduction in 2014, while in Poland, ASF remained within the limits of a single county for a long period. Here we quantify the velocity of spread of ASF in wild boar in the EU to elucidate whether it is constant or increasing, which can help to understand if further cases are expected in other areas as well as the rate of occurrence.

Methods & Materials: We apply a spatio-temporal kriging model to interpolate the date of a total of 7112 ASF notifications in wild boar from 2014 to 2017 taking into account the spatial variance in the study area (rasterized in 50x50 km cells). ASF was notified by the EU Member States to the ADNS (Animal Disease Notification System) quarterly, which constitute the input data (using as variable the indicatrix 0-1 by cell: ASF reported not-yes respectively). The velocity of progression of the wave-front of ASF in wild boar is expressed, by month, in km/time, through a derivative function of the surface in the wave-front (value=zero), and is compared between countries.

Results: The universal kriging surface had high velocity values on the wave-front of the first years, which is consistent with the input values. The velocity ranged between 0.6 and 54 km/month (mean=11.28km/month, SD=10.48). There are variations in velocity among countries and trimesters. The spatial continuity in the direction E-W is strong.

Conclusion: These findings have important implications for ASF control. Assuming equal efforts to combat ASF based on the EU regulation, the differences observed in the velocity of spread between countries could be explained by the different quality of available habitats for wild boar, the knowledge of which is fundamental to tailor disease management strategies in wild boar.

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Isolation Of \textit{B. ovis} From Preputial Swabs And Urine Samples In Subclinical Infected Rams

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\textbf{Purpose:} \textit{Brucella (B.) ovis} is the causative agent of contagious ovine epididymitis a chronic disease in rams. Besides reduced fertility in breeding rams, it may cause infertility or abortion in ewes and increased perinatal mortality in lambs. Transmission occurs via passive venereal infection or direct ram-to-ram contact. Routine diagnostic is based on serological testing since clinical symptoms are often missing. In addition shedding of \textit{B. ovis} is intermittent and a single negative sample does not guarantee freedom of disease. To study an association between seropositivity and shedding of \textit{B. ovis}, three seropositive rams originating from a flock with 23 rams were transferred to the NRL for Bovine Brucellosis at the IVET Moedling for further investigations.

\textbf{Methods & Materials:} The serological response and shedding of \textit{B. ovis} were followed for four weeks. Blood samples were collected in weekly interval and tested with indirect enzyme-linked immunosorbent assay (I-ELISA) and complement-fixation test (CFT). Additionally, urine samples and preputial swabs were examined with bacteriological methods and real-time PCR. After the sampling period rams were diagnostically culled for pathological investigation.

\textbf{Results:} None of the rams showed clinical signs by scrotal palpation. Two of the rams were seropositive in both serological tests, one ram only in I-ELISA. \textit{Brucella} spp. could be identified by real-time PCR in urine samples of all rams and in preputial swabs of 2 rams at each sampling point. \textit{B. ovip} was confirmed by bacteriological and genotyping methods. Gross morphological changes of the genital organs were detected in one animal only, displaying chronic periorchitis with adhesion between the parietal and visceral lamina of the tunica vaginalis, moderate scrotal serous effusion and bilateral thrombosis of testicular vessels.

\textbf{Conclusion:} An association between \textit{B. ovip} shedding and seropositivity was confirmed. Antibodies decreased weekly during the sample period, but shedding of \textit{B. ovip} still remains. \textit{In-vivo} diagnostic of preputial swabs and urine samples should be considered as an additional tool for direct diagnosis of \textit{B. ovip} in serological positive rams. To prevent transmission, positive rams have to be separated from the flock and slaughtered. Our study showed that castration does not prevent spreading of the disease since shedding via urine is possible.
Detection And Molecular Characterization Of Rotavirus A Antigen In Fecal Samples From Diarrheic And Non-Diarrheic Pigs In Maputo City And Province, Southern Of Mozambique

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Purpose: Rotavirus A (RVA) has been identified as an assured cause of severe gastroenteritis in humans as well as in pigs throughout the world. Studies on the genetic constitution of RVA strains in humans have shown a prevalence of P[6], P[8] and G8 genotypes with animal origin, in particular from pigs, goats or other ungulates. In Mozambique, to our knowledge, there are no studies on RVA in swines, which makes it difficult to trigger control measures and understand the importance of this animal species on human rotavirus diversity in the country. The aim of this study was to detect and characterize molecularly rotavirus of group A in diarrheic and non-diarrheic pigs from Maputo city and province, southern of Mozambique.

Methods & Materials: Between August and September 2016, 288 swine’s fecal samples from diarrheic and non-diarrheic pigs up to 3 months of age, from familiar and commercial herds of Maputo province and city were collected, sent to Virology Laboratory of the Animal Sciences Direction (DCA) and stored at -20ºC. The samples were tested using a commercially available ELISA kit (ProSpecT™ Rotavirus, Oxoid, United Kingdom) according to the manufacturer's recommendations and the positive samples were genotyped by nested-PCR.

Results: From 288 processed samples, 11.8% (34/288) were positive for RVA antigen, in which 85.3% (29/34) were non-diarrheal and 14.7% (5/34) diarrheal samples. From the positive samples, 35.3% (12/34) were from animals with 2 months of age, 29.4% (10/34) <1 month, 23.5% (8/34) with 3 months and 11.8% (4/34) were 1 month of age. From positive samples on ELISA, 13 were tested by nested-PCR, from which 9 were positive for P genotype (8 for P[6] and 1 for P[1]). It was not possible to characterize for G genotype.

Conclusion: The study showed that porcine RVA circulates in Mozambique in symptomatic and asymptomatic pigs, suggesting that asymptomatic animals can spread the virus to other animals and humans due the potential zoonotic of porcine genotypes described previously. The P[6] genotype was the most frequent and full genome sequencing is being performed from the positive samples for further analysis.
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Populations Of *Eimeria* *tenella* Express Resistance To Commonly Used Anticoccidial Drugs In Southern Nigeria.

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**Purpose:** Coccidiosis, caused by several species of the Apicomplexan parasite - *Eimeria*, is one of the most economically important parasitic diseases of poultry globally. This study determined the preponderance of chicken *Eimeria* in Southern Nigeria and assessed the parasite’s resistance to three commonly used anticoccidial drugs: Amprolium hydrochloride; Amprolium hydrochloride+Sulfaquinoxaline-Sodium; and Toltrazuril.

**Methods & Materials:** Multiplex PCR amplification of the SCAR region was used to confirm *Eimeria* preponderance. Resistance was assessed following the inoculation of $2.32 \times 10^5$ infective *Eimeria* spp. oocysts into 135 broiler chicks. Data on weight gain, feed intake, feed conversion and fecal oocyst shedding were recorded. At 7 days post inoculation 9 birds per treatment were sacrificed and assessed for macroscopic lesions in the four intestinal regions - Upper, Middle, Lower and Cecal Region. Percent optimum anticoccidial activity (POAA), Anticoccidial index (ACI) and Anticoccidial sensitivity test (AST) were used to access drug resistance.

**Results:** The preponderance of *Eimeria* spp. were *E. tenella* (77%), *E. necatrix* (55%), *E. acervulina* (44%) and *E. mitis* (11%), with multi-species infection occurring in 55% of samples assessed. Fecal oocyst shedding was low (P<0.05) in the medicated groups. At 7 days post inoculation 9 birds per treatment were sacrificed and assessed for macroscopic lesions in the four intestinal regions - Upper, Middle, Lower and Cecal Region. Percent optimum anticoccidial activity (POAA), Anticoccidial index (ACI) and Anticoccidial sensitivity test (AST) were used to access drug resistance.

**Conclusion:** The high preponderance of *E. tenella* in the field, and the occurrence of cecal lesions – caused mainly by *E. tenella* - despite drug administration, indicate resistance in populations of this species in our isolate. Based-on the POAA, ACI and AST values, the *Eimeria* isolate showed reduced sensitivity to toltrazuril.
Serological, bacteriological and molecular investigation of brucellosis in bovine in four Egyptian governorates

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Purpose: For the aim of validation of serological, bacteriological and molecular biology tests in surveillance of brucellosis in some governorate in Egypt

Methods & Materials: 347 known positive and negative serum samples of large ruminants with a history of Brucella melitensis infection were selected

Results: The highest relative sensitivity was achieved by the buffer acidified plate agglutination test. The assessed kappa (κ) agreement in both species indicated a substantial agreement (p< 0.05) in case of the BAPA, Rose Bengal plate, indirect ELISA and rivanol tests. According to the data obtained from the receiver operating characteristic curves (ROCs), the area under the ROCs and diagnostic odd ratio, the diagnostic performance of serological tests in cattle was arranged in descending order as follows; BAPAT, Riv.T, RBPT, iELISA, EDTA-modified micro-agglutination test (EDTA-mMAT) and MAT. The equivalent picture in buffaloes was, Riv.T, RBPT, BAPAT, iELISA, EDTA-mMAT and MAT. Eleven Brucella field isolates were recovered, whereas four isolates were recognized as Brucella abortus biovar 1 from cattle and seven as Brucella melitensis biovar 3 from cattle and buffaloes using phenotypic bacteriological typing and molecular speciation (Bruce-ladder PCR). As a result of better diagnostic performance offered by EDTA-mMAT over MAT under investigation, the authors recommended switching from MAT version locally adopted to EDTA-mMAT, and to a limited extent, Riv.T could be used to confirm reactors identified by screening tests.

Conclusion: Under the field of this investigation authors concluded and recommended the following:
It is recommended that the screening BAPA and RBPT, low cost and better performance than iELISA, shall be used in any seroprevalence programmes implemented for the control and eradication of the disease. To a limited extent Riv.T could be used in large ruminants to confirm reactors identified by screening tests if CFT is not available. As a result of better diagnostic performance offered by EDTA-mMAT in large ruminants under investigation, it is an appropriate time to shift from MAT formats locally adopted to EDTA-mMAT to avoid bias in results unfitting the native epizootological condition.
Prevalence Of *Staphylococcus pseudintermedius* In Cats Population In Poland

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**Purpose:** *Staphylococcus pseudintermedius* (*S. pseudintermedius*) is a part of the normal microbiota of dogs and cats. This opportunistic bacteria can cause a wide range of infections, ranging from pyoderma and surgical wound infections to deep infections such as osteomyelitis. The present study reports on the incidence, antibiotic resistance, and risk factors of *S. pseudintermedius* colonization in cats dependent on their state of health.

**Methods & Materials:** The study was conducted in the years 2013-2017. There were collecting samples from healthy cats (n=520) and animals with infection of the conjunctiva, upper respiratory tract or skin (n=67). Swabs from animals were taken from nares, conjunctival sacs, anus and skin. The isolates of *Staphylococcus* were classified as coagulase-positive *Staphylococcus* (CPS) using coagulase tube test. Species identification was conducted using Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The antimicrobial susceptibility was conducted using disc-diffusion method.

**Results:** There were observed a statistically significant correlation between the state of health and colonization with *S. pseudintermedius* in cats (p=0.02856, OR=4.93; CI 95%=1.84-12.56). The prevalence in healthy cats group was 3.08% (CI 95% 1.62-4.53%) and in sick animals 11.97% (CI 95% 4.48-19.4%). From cats under investigation 26 of *S. pseudintermedius* strains were isolated: 15 and 10 from healthy and sick animals respectively. Only strains isolated from sick animals shown methicillin resistance on the phenotypic level (18.18% methicillin-resistant *S. pseudintermedius* (MRSP)) whereas 26.26% and 45.45% strains from healthy and sick cats respectively harboring mecA gene were detected. Also much more often *S. pseudintermedius* isolates from infected animals were resistance to others chemotherapeutics.

**Conclusion:** Bacterial infection was a significant risk for colonization with *S. pseudintermedius* in cats. Infections caused by *S. pseudintermedius* may be a significant therapeutic problem because some strains have been characterized by resistance to the majority of chemotherapeutic drugs commonly used in domestic animals.
Patterns of Antimicrobial Resistance Bacteria from Respiratory Tract of Psittacine Pet Birds in Thailand

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Purpose: Parrots or psittacine birds are one of the most popular pets. The common problem in birds is respiratory disease, which spread mainly through contact, droplet and aerosol transmission. In particular, opportunistic bacteria normally involve in disease progression and threat of antimicrobial resistance. Moreover, close contact with a sick bird may pose health risk to owner due to spread of antibiotic-resistant bacteria. Thus, the aim of this work was to provide data on the characterization of antimicrobial resistance bacteria from upper respiratory tract of psittacine cases in Thailand.

Methods & Materials: The case records for all psittacines visiting the exotic pet clinic, Kasetsart University Veterinary Teaching Hospital Bangkhen from January to October, 2015 were used to assess type and antimicrobial susceptibility pattern of bacteria. Swab technique at choanal or nasal area of bird was used for bacteria collection and culture. Eleven antimicrobial drugs were tested using disk diffusion method, which represented 9 antimicrobial groups.

Results: A total of 80/376 psittacine cases (21%) was diagnosed to have respiratory problem. Then, eighty-eight isolates were obtained from 53 respiratory cases consisting of Streptococcus spp. (18 isolates, 20%), Staphylococcus spp. (16 isolates, 18%), Escherichia coli (15 isolates, 17%), Pseudomonas spp. (9 isolates, 10%), Klebsiella pneumoniae (7 isolates, 8%), Pasteurella spp. (6 isolates, 7%) and Enterobacter spp. (5 isolates, 6%). The staphylococci presented highly resistant to beta-lactam antibiotics (>50% of isolates) meanwhile Pasteurella spp. appeared to show susceptible almost of antimicrobial drugs except clindamycin. However, clindamycin seemed to resist with E. coli, Enterobacter spp, Klebsiella pneumoniae and Pasteurella in all isolates. Patterns of multiple drug resistance (MDR) indicated in this study, which presented in all Pseudomonas isolates, 93% of E. coli isolates, 80% of Enterobacter cloacae isolates, and 57% of Klebsiella isolates. Additionally, two isolates of Pseudomonas were resistant to 8 antimicrobial categories.

Conclusion: Bacteria isolated from upper respiratory psittacine cases presented antimicrobial resistant trend and MDR. Respiratory treating in Psittaciformes should concern with susceptibility test and prudent use of antimicrobial usage in practice for therapeutic plan. Furthermore, link between colonized bacteria in a pet bird and owner should not be overlook.
Livestock Diseases Threatening Smallholder Farmers in Lao People’s Democratic Republic

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Purpose: In Lao People’s Democratic Republic, uncontrolled animal trade, lack of animal containment and limited access to veterinary services are a growing-ground for virus spread. Foot-and-Mouth Disease (FMD) and several avian viruses are enzootic and threaten subsistence farmers. We assessed the (sero-)prevalence of several livestock viruses, and evaluated the knowledge, attitude, and practice of smallholder farmers towards FMD.

Methods & Materials: In 2018, sera were collected from 394 domestic ruminants and screened using an ELISA that differentiates between infected and vaccinated animals. Questionnaires (n=101) were statistically explored to identify knowledge gaps and risk factors related to FMD. In addition, oral and cloacal swabs, collected from 619 backyard poultry in 2011, 2014 and 2015, were tested by PCR for Newcastle disease (NDV), Influenza A, Coronavirus (CoV) and Chicken Anemia Virus (CAV). 206 poultry sera were screened by ELISA for the presence of anti-NDV and –Influenza A antibodies. Statistical and phylogenetic analyses revealed the viral infection patterns.

Results: Although most farmers had very limited knowledge about FMD, many could correctly enumerate the symptoms and observed outbreaks recently. This was confirmed by our laboratory analysis: overall 37.1% of the animals were seropositive and 72.3% of the farms had at least one seropositive animal. Approx. 90% of the farmers reported that FMD negatively affects livestock trade and health. Moreover, we found high positivity rates of CoV and CAV RNA in cloacal and oral swabs (CoV: 38.3% and 6.2%; CAV: 16.1% and 1.7%). Younger animals were more likely to shed both, CoV and CAV, and similar virus strains co-circulated in chickens and ducks. Despite serological evidence of NDV and influenza A circulation (86.9% and 1.9%), viral RNA was detected in none of the swabs.

Conclusion: A large proportion of the Lao population relies on subsistence livestock production which is, as shown here, severely compromised by the circulating viruses. To secure their livelihoods, vaccination programmes should target all susceptible hosts and achieve a high coverage throughout the country. These campaigns should be complemented by community-based sensitization to raise the awareness about prevention strategies, such as quarantine and trade restrictions.
Purpose: After introducing FETP-Frontline in August 2016, The Ministry of Health and Social Welfare (MOHSW), The Gambia strengthened Integrated Disease Surveillance and Response (IDSR). The District Surveillance Officers (DSOs) cadre was created and weekly surveillance reporting started in September 2016. Few months later, weekly reporting platform was activated on the DHIS2. Thus, when the DSOs collected surveillance data from health facilities, they reported to the health regions, where data is collated. We reviewed the reports from the regions, to describe the data collected and summarize the progress so far.

Methods & Materials: Weekly surveillance reports from the 7 health regions were collected and reviewed for 2017. We reviewed and analyzed data on 10 immediately reportable diseases and 2 unexplained clusters of events/deaths: Acute Flaccid paralysis (AFP), Diarrhea with Blood (DWB), Measles, Meningitis, Suspected Cholera, Neonatal Tetanus, Schistosomiasis, Maternal Death, Suspected VHF (Ebola), Yellow Fever, Unexplained Events and Unexplained Deaths. We assessed Timeliness of reporting, defined as punching of Surveillance data into DHIS2 by 12 noon of day 2 (Tuesday) of the next Epidemic Week.

Results: A total of 3,048 diseases and conditions were reported from week 1 to week 52, 2017. 1,725 (57%) were under 5 years and 1563 (51%) were males. 2,760 (90%) of the cases were DWB, 181 (5%) were schistosomiasis, 45 (1.4%) were meningitis, 20 (0.6%) were AFP, 7 (0.2%) were measles, 24 (0.6) were maternal death, and 11 (0.2%) were unexplained cluster of health events. Of the 7 regions, Central River Region reported majority of the cases; 1,398 (32%) and North Bank West reported the least cases; 306 (7%). Cumulative Timeliness of Reporting for all regions increased from 30% in 2016 to 90% in 2017.

Conclusion: The most frequently reported disease/conditions were diarrhea with blood and schistosomiasis. While, timeliness of reporting increased in all the regions, we recommend continuous supervision for further improvement.
Purpose: To estimate seroprevalence of small ruminant brucellosis and to assess awareness of pastoralist community about zoonotic importance of the disease.

Methods & Materials: Cross sectional study was carried out on a total of 283 (99 sheep and 184 goat) from October 2016 and April 2017. Structured questionaries was also administered to 126 respondents of 10 PA’s.

Results: The overall seroprevalence of brucellosis in small ruminant was 23(8.1%) (95%CI:5.2,11.9) by c-ELISA. The individual species seroprevalence of brucellosis was 9.2(95%CI:5.5,14.4) and 6.1(95%CI:2.3,12.7) in goat and sheep respectively. Among 126, 112(88.9) of community have no knowledge about zoonotic importance of brucellosis and ways of transmission of disease where as 14(11.1%) of them have awareness about the disease in addition physicians have no awareness about the disease. They did not consider brucellosis while treating patients admitted to health post with suggestive clinical sign brucellosis.

Conclusion: The study showed high sero prevalence in goat than sheep and high risk of human brucellosis in the area. Therefore integrated human and animal health disease control strategy should be developed and implemented to control the disease.
Measuring timeliness detection of outbreak of food and water borne diseases in Albania, 2005-2016

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Purpose: This study aimed to evaluate timeliness and data quality of national outbreak of food and water borne diseases reporting in Albania during 2005-2016.

Methods & Materials: The study used aggregated data at national level from public health surveillance reports. Data on disease outbreaks of food and water borne diseases occurring from 2005 to 2016 were obtained from the national disease surveillance database and outbreak investigation reports. The dataset consisted of basic epidemiological information, regarding time, place, and total cases of each outbreak occurrence. Time variables included date of symptom onset in the index case, date when the outbreak start, outbreak detection date, date of laboratory confirmation, date of public health response and date of report to Ministry of Health and WHO and date of first public communication. The total number of outbreaks of food and water borne diseases reported during 2005 to 2016 were described. The number of confirmed cases and suspected cases reported for each disease outbreak were also summarized by year, using median and interquartile range.

Results: Almost all date data in outbreak reports in Albania were complete but missing data were found only in the date of laboratory confirmation, date of disease treatment, date of patient hospitalization and date of disease end. During 2005-2016, 43 outbreaks were reported to Institute of Public Health, Albania. 36 (83.7%) outbreaks were confirmed by region and by pathogen. Food and water borne outbreaks are more common during the summer months and too often, outbreaks of food and water borne diseases go unrecognized or unreported or are not investigated. Salmonela is most common cause of food borne illness and most persistent.

Conclusion: Confirmation of food and waterborne outbreak is low and timeliness data related to it are often missing. There is a need to have a joint platform for surveillance data, outbreak reporting and response and communication activities to measure timeliness but also the effectiveness of the response and communication activities especially related to IHR implementation.
Purpose: The combination of albendazole and ivermectin is effective in the treatment of cutaneous larva migrans.

Methods & Materials: We present a retrospective descriptive study; reviewed the clinical histories of 04 patients, who were hospitalized in the Service of Tropical Diseases during the months of March and April 2018. They are 04 male patients aged between 27 and 44 years (Average age: 38 years). A follow-up examination was made two weeks after the treatment was completed.

Results: As main epidemiological and clinical features, all the cases had risk activity being military personnel in training course and the background of a trip to the National Reserve of Tambopata in Madre de Dios, which is a protected area in the southern Peruvian Amazon with average stay time of 14 days. The most frequent location of lesions was in lower limbs; 2 patients on one leg and 2 on both leg. Before the first consultation, the average time of lesions evolution was 18.7 days. The clinical presentation in all was an erythematous papular lesion, with serpentinaeus path and local itching. Regarding the treatment, all received 400 mg of albendazole every 12 hours for five days and ivermectin to 200 mg/kg/day for two days, with a favorable evolution and well tolerated. At the time of the follow-up examinations, the four patients presented post-inflammatory hyperpigmentation on previous treated lesions and one of them also present persistent itching without actives skin lesions. No one had recurrent infectious.

Conclusion: The diagnosis of cutaneous larva migrans is clinical, the typical presentation and the exposure to a tropical endemic area makes possible an early diagnosis. With reference to the treatment in this study we have observed that combination of albendazole and ivermectin is effective and well tolerated as drugs in the treatment of cutaneous larva migrans.
A review of Foodborne Diseases Outbreaks reported to the Outbreak Response Unit, National Institute for Communicable Diseases, South Africa, 2013 – 2017

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Purpose: Foodborne diseases (FBDs) are a major public health concern and an important cause of morbidity and mortality globally. WHO Africa region had the highest estimated burden of FBDs in 2010. The burden of FBDs in South Africa is not well established. FBDs are notifiable medical condition in South Africa. We provide a retrospective descriptive analysis of FBDs outbreaks reported to National Institute for Communicable Diseases (NICD) over five-years, January 2013 to December 2017.

Methods & Materials: A retrospective descriptive review was conducted using secondary data abstracted from FBDs outbreaks reports submitted to NICD from 2013 to 2017. Stool and environmental samples were collected during outbreak investigations and tested enteric foodborne pathogens.

Results: 327 FBDs outbreaks were reported from January 2013 to December 2017, causing illness in 11 155 individuals, with 78% (8 680/11 155) hospital visits, 4% (494/11 155) hospital admissions and 0.4% (49/11 155) deaths. Most of the outbreaks were reported in warmer months, from KwaZulu-Natal (141/327, 43%), Gauteng (63/327, 19%) and Mpumalanga (40/327, 12%) Province. Institutional outbreaks were most common (106/327, 32%), followed by households’ outbreaks (89/327, 27%), and community outbreaks (35/327, 11%). Specimens were collected in 73% (239/327) of outbreaks. Stool samples were collected in 62% (147/239), food samples in 55% (132/239) and water samples in 14% (33/239). Enteric pathogens isolated in stools included Salmonella species (29/147, 20%), Clostridium perfringens (12/147, 8%), Bacillus cereus (7/147, 5%), Shigella species (6/147, 4%) and Listeria monocytogenes (2/147, 1%). Pathogens isolated from food samples were Salmonella species (15/132, 11%), Escherichia coli species (14/132, 11%), Bacillus cereus (13/132, 10%), Clostridium perfringens (4/132, 3%), and Listeria monocytogenes (4/132, 3%). Water contamination indicators found were high Escherichia coli and total coliforms counts (3/33, 9%).

Conclusion: Although FBDs outbreaks are a notifiable medical condition in South Africa, they are likely underreported. There is great variability in outbreaks investigation and reporting throughout the country. The lack of epidemiological data limited the analysis. This review is based on outbreaks reported to NICD, is not a true representative of the burden of FBDs in the country. Strengthening and training to improve outbreak investigations, including specimen and epidemiological data collection and reporting is recommended.
Using Quantitative Microbial Risk Assessment to Explore Waterborne Pathogen Infection Risks Associated with Interventions of Agricultural Management Practices

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Purpose: Agricultural management practices in agricultural watersheds can impact how on and off-farm pathogens are mobilized and transported to downstream water bodies used for recreation and/or water consumption. This study used Bayesian quantitative microbial risk assessment (QMRA) to examine how agricultural drainage management and riparian zone conservation practices impact infection risks associated with a suite of pathogens in agricultural watersheds.

Methods & Materials: Bi-weekly surveillance of waterborne pathogens in two small (~250 to 460ha) agricultural watersheds in eastern Canada was carried out for eight growing seasons (April to November). An 11-tube most probable number (MPN) method was used to target Campylobacter spp. in water samples. Parasites were enumerated using the USEPA method 1623, sequenced, and genotyped using phylogenetic analysis. E. coli O157:H7 was determined via PCR and culture methods. QMRA was used to identify differences in mean infection risks for a hypothetical exposure scenario associated with drainage water control from fields (paired watershed design), and riparian zone protection (3m buffer) utilizing livestock exclusion fencing (upstream-downstream approaches).

Results: E. coli O157:H7 was infrequently detected but light pasturing with livestock having the capacity to interact with the water course increased relative mean infection risks. From a relative risk perspective, riparian zone protection from livestock reduced risks associated with Giardia and livestock-sourced Cryptosporidium. Adjusting for the proportion of Cryptosporidium spp. detected that are infectious to humans reduces downstream risk estimates by roughly one order of magnitude. Campylobacter spp. had high prevalence but detections decreased significantly through the protected riparian zone exhibiting a pasturing/riparian protection treatment effect. Also for Campylobacter, it was found that infection risks were higher in the watershed that was under drainage control, in relation to the one that wasn’t. Field drainage control reduced dilution and flushing which increased pathogen densities.

Conclusion: Quantifying infection risks associated with beneficial agricultural management practices is not frequently practiced. While some mean infection risk trends were expected (riparian zone study), the results of the drainage management study were unanticipated. QMRA can be useful for determining unanticipated side-effects and benefits of agricultural management practices, and therefore should be used more frequently to assess practice efficacy.

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**Purpose:** On 1st of August 2017, district Health Officer Khuzdar reported 81 suspected cases of acute gastroenteritis from village Talar Mas, Union council Drhakala, Tehsil Wadh, District Khuzdar after current flesh floods, and requested Provincial disease surveillance and response unit for investigation. On August 2nd, 2017 a team was deputed to affected area to confirm outbreak, evaluate risk factors and recommend control measures.

**Methods & Materials:** A case was defined as, sudden onset of 3 or more episodes of loose stools per day with or without vomiting in a resident of village Talar Mass UC Drakhala Tehsil Wadh District Khuzdar from August 1st to 4th 2017”. A Descriptive study was conducted for Active case finding affected village and records of Rural Health Center was checked. Two water samples were taken and sent to National Institute of Health Islamabad.

**Results:** Total 81 cases and 1 death (CFR=1.2%) were identified from district health department record and during active case finding in the affected area. Females were more affected 41% (n=33). All the cases were reported from August 1st to August 4th, 2017. The mean age of the cases was 27 years (range= 1 to 70 years). All the cases were clinically diagnosed as acute gastroenteritis. First case reported to hospital on August 1st, 2017 and last on August 4th 2017. Lab results showed that Coliform and fecal coliform organisms were present in all water samples.

**Conclusion:** The most probable cause of this outbreak was the contaminated water sources because of flesh floods in the area and habit of open defecation. Team had successfully controlled the outbreak by spreading awareness to the community about preventive measures On recommendations of this study community started boiling water before use and local authorities also distributed chlorine tablets for disinfecting water along with awareness regarding usage of latrines.
Purpose: The rates of infection by *Toxoplasma gondii* are high in Brazil but a low percentage of individuals chronically infected by this parasite develop ocular toxoplasmosis (OT). It is believed that the host immune response modifies the susceptibility for OT. This study evaluated genes involved in the innate and adaptive immune response as potential predictors with OT.

Methods & Materials: Patients with primary disease as well as with recurrence and controls both with positive serology were recruited over a period of ten years. OT was diagnosed by indirect binocular ophthalmoscope analysis and *T. gondii* serology (IgM, IgG) were assessed by ELISA. PCR-SSOP was used to identify HLA and KIR alleles and PCR-RFLP was used to identify MICA and CCR5 alleles. Cytokines expression levels were determined by qPCR and ELISA. The data were analyzed chi-square. Odds Ratio and confidence interval at 95% values were calculated (p<0.05).

Results: Respectively, the MICA*002~HLA-B*35 and the MICA*008~HLA-C*07 haplotypes lost their association with high and low risk for OT after adjusting for multiple comparisons. The KIR alleles their HLA ligands (KIR3DS1-Bw4-80Ile and KIR2DS1+/C2++ KIR3DS1+/Bw4-80Ile+) were associated with increased susceptibility for OT. The KIR-HLA inhibitory pairs -KIR2DL3/2DL3-C1/C1 and KIR2DL3/2DL3-C1- were associated with decreased susceptibility for OT while the KIR3DS1-/KIR3DL1+/Bw4-80Ile+ combination was associated as a protective factor for OT, in particular against recurrent manifestations. CCR5/CCR5 genotype and simultaneously the CCR5-59029 AA or AG genotypes have a greater risk of developing OT. mRNA levels for TNF-α and IL-12 were up-regulated in OT patients in comparison to other clinical forms of toxoplasmosis.

Conclusion: In conclusion genes involved in different steps of innate and adaptive immune response against *T. gondii* infection influence the susceptibility or resistance for the development of OT.
Epidemiological, Clinical and Laboratory Characteristics of Foodborne Botulism in Armenia

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Purpose: Botulism is a potentially lethal paralytic disease caused by botulinum neurotoxin. This toxin is mainly produced by Clostridium botulinum. One of the types of botulism is foodborne botulism, caused by consumption of improperly processed food, is a rare but potentially fatal disease if not diagnosed rapidly and treated with antitoxin. Homemade canned, preserved or fermented foodstuffs are a common source of foodborne botulism and their preparation requires extra caution. We aimed to describe the most common clinical, epidemiological and laboratory characteristics of foodborne botulism in patients hospitalized in “Nork” Republican hospital during 2000-2017 years.

Methods & Materials: This is an analytical retrospective study based on analysis of medical records of the patients registered in the Infectious Clinical Hospital “Nork” during January 2007 to December 2017.

Results: Total number of cases 51, with a mean age of 26 years old. Male 30 (58.8%), female 21 (41.2%). Case distribution by 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017 years: 5(9.8%), 7(13.7%), 7(13.7%), 3(5.9%), 9(17.6%), 3(5.9%), 9(17.6%), 1(2%), 1(2%), 3(5.9%), 3(5.9%) respectively. The main months of hospitalization were January (12 cases), February (15 cases) and March (9 cases). The mean day of illness at the moment of hospitalization was 2.5 (maximum 13). 49(96%) cases mentioned consumption of home-canned vegetables. 25(49%) cases mentioned other family member who hospitalized with the same symptoms. Main clinical symptoms: weakness 45(88.2%), dizziness 16(31.4%), fever 9(17.6%), difficulty swallowing 40(78.4%), slurred speech 19(37.3%), nausea/vomiting 19(37.3%), diarrhea/constipation 4(7.8%), abdominal pain 7(13.7%), double vision 16(31.4%), drooping eyelids 6(11.8%). Leukocytosis mean value 8.7*10³/µL (maximum 18*10³/µL), Hemoglobin mean level 130.2g/L (max 172, min 90).

Complications: 1(2%) acute gastritis, 1(2%) asthenic syndrome and 1(2%) pulmonary atelectasis.

Treatment: Antitoxin 47(92.2%), antibiotics 46(90.2%). Outcome: 51 (100%) recovered. The mean duration of hospitalization: 9.6 days.

Conclusion: According to our retrospective study, homemade canned vegetables were the main source of foodborne botulism. The most common clinical symptoms of disease were weakness and difficulty swallowing. Majority of patients was admitted in January, February and March, which is connected with common consumption of home-canned vegetables in this period.
Identification of Two Gatekeeper Proteins For *Vibrio parahaemolyticus* T3SS2

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**Purpose:** Many Gram-negative symbionts and pathogens employ a type III secretion system (T3SS) to live in contact with eukaryotic cells. Because T3SSs inject bacterial proteins (effectors) directly into host cells, the switching of secretory substrates between translocators and effectors in response to host cell attachment is a crucial step for the effective delivery of effectors. The switch of T3SS secretion between translocators and effectors is controlled by gatekeeper protein complex. *Vibrio parahaemolyticus*, one of the human-pathogenic Vibrios, causes three major types of clinical illness: gastroenteritis, wound infections and septicemia. Most clinical isolates from patients with diarrhea possess two sets of T3SS genes, designated T3SS1 and T3SS2. T3SS2 is essential for enterotoxicity in various animal models. Therefore, T3SS2 is considered as a major virulence factor of the bacteria. Several T3SS2 genes have been identified, including transcriptional regulators, effectors, and translocators. However, a number of T3SS2 proteins are still unknown such as proteins that regulate secretion of substrate proteins. Therefore, the precise mechanism of T3SS2 remains unclear. In this study, we have identified two hypothetical proteins encoded in Vp-PAI as gatekeeper proteins of T3SS2.

**Methods & Materials:** *V. parahaemolyticus* RIMD2210633 was obtained from the Pathogenic Microbes Repository Unit, International Research Center for Infectious Diseases, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

**Results:** Our functional analysis of genes encoded on Vp-PAI identified two hypothetical genes (*vgpA* and *vgpB*) that have a critical role in T3SS2-dependent biological activities including enterotoxicity, cytotoxicity to Caco-2 cells and actin stress fiber formation. Deletion of *vgpA* and *vgpB* genes also caused a failure of effector translocation. Immunoblotting of secreted proteins revealed that deletion of *vgpA* and *vgpB* genes cause an over-secretion of T3SS2 effectors but diminishes the secretion of translocators. Deletion of these genes did not affect to production of secreted proteins.

**Conclusion:** Our study demonstrated that *vgpA*- and *vgpB*-defective strains dysregulate T3SS2 secretion. The mutants promote secretion of effectors but abolish secretion of translocators without alteration of transcription and production of T3SS2-related proteins. These results indicated that VgpA and VgpB are required for translocator secretion and directly regulate secretion of translocators and effectors as gatekeeper proteins of T3SS2.
Prevalence of *Salmonella* Dublin in Veal Liver in Québec, Canada From a Public Health Perspective

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**Purpose:** *Salmonella enterica* is one of the principal causes of foodborne zoonotic enteritis. Among the different serovars, Dublin (*S*. *Dublin*) is of particular importance due to its propensity to progress to an invasive infection in humans and due to the high proportion of multi-drug resistant strains in Canada. Cattle are considered as the main reservoir of *S*. *Dublin*. This serotype has emerged since 2011 in the province of Quebec, Canada, in both cattle and human populations. First animal cases have been reported in calf production. White veal are valued for the quality of their meat, offal and liver. The liver is usually consumed mildly cooked and is considered as a probable source of foodborne exposure to *S*. *Dublin* in humans. The objective of this study was to estimate the prevalence of *S*. *Dublin* positive liver after slaughtering and the seroprevalence against *S*. *Dublin* at the calf level.

**Methods & Materials:** Samples were collected from 75 batches in two slaughterhouses from August 2016 to October 2017. For each batch, a liver sample was collected from 8 calves after evisceration and additionally, a blood sample from 15 calves. Liver samples were screened by PCR and then cultured, and serums were tested using an indirect ELISA (PrioCHECK® *Salmonella* Ab bovine Dublin).

**Results:** *Salmonella* was detected by PCR in 20 of the 547 veal livers. Typical isolates were obtained from 14 livers; all were serotyped and identified as *S*. *Dublin*, for a prevalence estimate of 3.1% (IC95%: 0.0%-7.45%). At least one seropositive calf was detected in 128 (24%) of the 75 batches. At the calf level, seroprevalence was estimated at 6.97% (IC95%: 2.83%-11.11%). *S*. *Dublin* was detected in liver of 14.0% of seropositive calves compared to 1.2% of seronegative ones (p<0.001).

**Conclusion:** Our results show that veal liver can be a source of foodborne exposure to *S*. *Dublin* in humans, especially when veal liver is consumed mildly cooked. Surveillance and prevention measures associated with calf production and veal meat, including cooking recommendations could help reduce the burden associated with *S*. *Dublin* in humans. Serology could be potentially used in surveillance effort as an indicator to assess the risk of contamination.
Study On The Effects Of Transport Stress On Fecal Shedding And Poultry Meat Contamination From *Campylobacter* spp.


**Purpose:** Animal welfare and food safety represent major issues in the production of food of animal origin. Particularly in sight of the new criterion for *Campylobacter* that since January 2018 is included in the European Union food law, it is important to find new ways to reduce poultry meat contamination from *Campylobacter* spp., also controlling the shedding of this pathogen with chicken faeces. In particular, the aim of this study was to compare and evaluate, in function of the different transport duration, its influence on *Campylobacter* fecal shedding and consequent contamination in broiler carcasses.

**Methods & Materials:** A sample of 12 flocks from an Italian poultry company, all positive for *Campylobacter* infection, was selected. For each flock it was assessed, before departure and in slaughterhouse upon arrival, the *Campylobacter* prevalence in faces using cloacal swabs. The same batches were also tested after slaughtering and cooling to evaluate prevalence and level of contamination in carcass skin. Detection and enumeration were carried out using ISO or validated methods.

**Results:** Twelve flocks from 6 different farms, were selected. Six flocks were from farms located at longer distances (over 180 km and more than 3,5 hours of transport) and the other 6 from short-distance farm (always below 30 km). In the examined batches, prevalence in cloacal swabs was highly variable, ranging from 0 to 100%. The frequency of *Campylobacter* in cloacal swabs increased after transports, in particular statistically significant differences between departure and arrival were detected only for long transports. No statistically significant differences were detected in caecal contents nor on carcass skin between batches from transports of different duration.

**Conclusion:** Although the duration of transport does not significantly affect the level of contamination of the carcasses, our results highlight that in short transports *Campylobacter* shedding is significantly less frequent: this could be an important information for poultry companies, in order to keep the problem under control.
Assessment Of Laboratory Diagnostic And Surveillance System For Enteric Infections In Azerbaijan

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Purpose: Our study sought to gain a better understanding of the etiology of infections that fall within the generalized diagnosis of enteric infections with unassigned etiological agent. We hypothesized that meticulous assessment of patient diagnostic and surveillance data from enteric infections in Azerbaijan would increase our knowledge of the enteric disease situation in the country. In consequence, our ability to assign causative agents to enteric infections would be enhanced and gaps in public health detection and reporting requirements could be identified.

Methods & Materials: Given the high number of AEI cases with unknown etiology in Azerbaijan, AEI patient data from 2014 to 2016 were analyzed using the Electronic Integrated Disease Surveillance System (EIDSS) and patient information from annual reports from the Bacteriology Laboratory at the Republican Center of Hygiene and Epidemiology.

Results: The analysis revealed that yersiniosis (0.3% of the cases), shigellosis (0.6%), and salmonellosis (5.7%) were the most common AEI cases. Opportunistic bacterial infections accounted for remaining cases. Between 2014 and 2016, notification of enteric infections improved with every year (i.e., 2014: 67.4% of timely notifications; 2015: 80.4%; 2016: 87.3%). EIDSS entry deficiencies included the lack of antibiotic treatment information in 57.4% of the cases, and an overwhelming incidence of AEI diagnosis based on clinical-epidemiological data (78.6%) and not confirmatory laboratory assays, indicating that treatment received was not etiologically substantiated. Absence of point-of-care, pathogen detection assays covering more agents, especially viral agents, might in part explain the high rate of clinical diagnosis of AEIs as “acute enteric infections of unknown etiology”. In fact, in Azerbaijan, only rotaviruses are currently tested when viral enteric infections are suspected.

Conclusion: Better communication among health system databases with established minimum data entry requirements, including treatment and disease chronology information, capability for multiple pathogen testing and improvement in the quality of EIDSS entries to reconcile electronic and paper forms of notification are needed. Use of rapid immunological or molecular diagnostic methods, or test strips using immunochromatographic technology, rather than traditional long-term culture assays, should expedite pathogen identification. Altogether, such efforts will improve diagnosis, treatment and spread control of diarrheal diseases in the country.
Spatial and Temporal Analysis of Hepatitis A Cases in Fier District, Albania, 1986-2016

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Purpose: To document long term changes in the prevalence of Hepatitis A and its use as an indicator of sanitary standards and public health capacity.

Methods & Materials: Registers of infectious disease cases maintained by Public Health authorities in Fier District, Albania for the years 1986 – 2016 inclusive were scanned and digitised as part of national project to quantify the burden of such diseases in the population. In total over 21,000 individual records covering 29 separate diseases were computerized of which Hepatitis A was the most common diagnosis with nearly 9,000 recorded cases over the 31-year period. For each case, demographic and biographic details of the patient, as well as key dates such as onset of symptoms, first visit to doctor or clinic, diagnosis, recovery and reporting to PH authorities were recorded. Spatial and temporal analysis of the data were carried out to identify long term trends and geographical distribution of cases.

Results: Overall, there was a significant decrease in the number of cases of Hepatitis A from nearly 1300 per year 1986 to less than 100 cases annually from 2003 onwards. In the 4 years 2010 to 2014, a total of 18 cases were recorded. The following year, however, the number of reported cases rose to 92, of which 50 were recorded in a single commune. This outbreak was the subject of substantial media coverage and a detailed investigation by public health authorities which identified a number of contributing factors. Throughout the time series, outbreaks of Hepatitis A occurred at intervals of roughly 4-6 years, the periodicity possibly reflecting waning levels of immunity in the paediatric population. There was also a progressive reduction in the duration of clinical cases (date of first symptoms to date of recovery) attributed to more effective therapeutic options and better overall standards of health care.

Conclusion: Using Hepatitis A as a proxy for water borne infectious diseases, the study demonstrated the effects of improvements in water and sewage infrastructure over three decades on the health of the population of Fier District. Continued sporadic cases and localised outbreaks could be used to identify communities where further investment was needed.
Methods of Decontamination of ship ballast Water with Polyguanidine Disinfectant

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Purpose: The search for chemical compounds characterized by inertness and low toxicity to people and having high activity against most microorganisms in ballast water is ongoing. The disinfectant of choice in our study were representatives of polyguanidine group. With relative safety for animals and humans, these compounds have antimicrobial, antiviral, antifungal effects. A wide range of biocide action is due to the presence in the repeated links of macromolecules of polymers of guanidine groups, which are the active beginning of some natural and synthetic drugs and antibiotics. The purpose of study is development of decontamination methods of ships ballast water with disinfectant of polyguanidine group.

Methods & Materials: Cultures of E. coli, S. aureus, toxigenic strains of V. cholera served as test for developing decontamination method. The suspension test with a neutralizer - sodium laurylsarcosinate - in accordance with the National Manual 4.2.2643-10 was used for antibacterial characteristic of the testing disinfectant.

Results: In vitro the polyguanidine disinfectant in concentrations of 0.01 - 0.1% has a pronounced antibacterial effect against test microorganisms - S. aureus, E. coli and V. cholerae, and can be considered as a promising disinfectant for the decontamination of ship ballast water. When V. cholerae O1 and O139 are detected in the ballast water of vessel, decontamination can be carried out in three methods: by spraying of empty tanks, by adding of working concentration disinfectant in the full ballast water tanks and by combined method. According to approximate calculations for a ship decontamination by spraying empty tanks working concentration of 0.01 % will require a 1.0 liter of 20 % disinfectants on 6000 m², the method of adding the working solution concentration of 1000 tons of ballast – 500 liters of 20 % disinfectants, which in monetary terms will amount to from 300,000 to 1,000,000 rubles (4750-15780 $).

Conclusion: In vitro experiment established that at concentrations of 0.01-0.1% the preparation has a pronounced antibacterial effect against the test microorganisms, and can be considered as a promising disinfectant for decontamination of ballast water, allowing us to offer methods of decontamination of ship ballast.
Purpose: We present an outbreak of Gastroenterocolitis with Salmonella group D after the consumption of mayonnaises from a public foodservice in the second half of May.

Methods & Materials: We analyzed the patient data and the medical history of each patient admitted in the “Sf. Parascheva” Infectious Diseases Hospital from Iasi, Romania and also followed the evolution of the outpatients which came in the triage room. Also we collaborated with the Public Health Department for the epidemiological survey.

Results: A total of 134 patients presented to our hospital between 14 and 18 of May 2018, and 96 of those were admitted. After the epidemiological survey conducted by Public Health Department we found out that the incubation period vary from 8 hours to 24 hours. All patients declared the consumption of fast food that contained mayonnaises on 14/06/2018 from a public foodservice. First case was admitted after 9 house after the consumption and the biggest number of presentations and admittances was after 2 days, on the 16th of May (42 presentations and 35 admittances). After this date the number begun to gradually decrease. Patients that required hospitalization presented, abdominal pain, nausea, vomiting (over 6 episodes per day), diarrhea (over 12 episodes per day) associated in some cases with fever. From the laboratory data we extracted that the patients had minor fluid and electrolyte imbalance associated in the majority of cases with minor dehydration syndrome.

For the complete diagnosis we analyzed the stool sample and the liquid from the emesis. All of the results came positive for Salmonella Group D.

The evolution of all cases was good and the average hospitalisation time was 6 days with a maximum of 10 days.

Conclusion: In the warm time, the inadequate storage of the food can start an outbreak that can affect a whole city.

A good communication between Public Health Department and hospital is very important for a complete and correct analysis of the magnitude of the outbreak.

It is crucial for the public food places to respect the standards for depositing their food especially in the summer time.
Contamination, antimicrobial resistant of *Campylobacter jejuni* and *Campylobacter coli* isolated from retail meats in Italy and comparison with human strains

**Purpose:** Campylobacter is an important zoonotic pathogen potentially causing illness in humans, following the consumption of raw/undercooked meat. The objectives of this study were to determine the prevalence of *Campylobacter* spp. in retail meat in Italy and to assess the antimicrobial resistance and genetic relatedness of *Campylobacter* strains from human cases.

**Methods & Materials:** A total of 2,596 fresh meat samples (1,393 from chicken and 1,203 from bovines) were taken at retail in randomly selected supermarket in Italy during 2016. Presence of *Campylobacter* spp. and level of contamination were determined using ISO methods and antimicrobial resistance were evaluated with the microdilution method. Isolates were typed by MLST: sequence types (STs), and clonal complexes (CCs) were assigned by submitting the DNA sequence to the MLST database website. All isolates were compared with strains from humans in the same period.

**Results:** *Campylobacter* spp. was found more frequently chicken meat 223 out of 1,393 (16.01%) than in bovine meat 7 out of 1,203 (0.58%), difference statistically significant, p<0.0001. In chicken meats were identified 132 *C. jejuni* (59.19%) and 91 *C. coli* (41.55%). In bovine meat were found 3 *C. jejuni* and 4 *C. coli*. In 41.63% of chicken samples the level of contamination was very low (1-9 cells), in 57.93% was 10-999 cfu/g and only 0.44% >1000 cfu/g. In bovine meat samples, the levels of contamination were constantly below 10 cfu/g. High resistance to ciprofloxacin, nalidixic acid, tetracycline and multi-drug pattern to CipNaTe and CipNaETe was observed in most of the strains. MLST typing identified 78 STs and 17 clonal CCs. The most prevalent CCs were 828, followed by 353, 354, 21 and 206. Many STs from poultry meat were identified in human samples.

**Conclusion:** Our study confirms that poultry meat is the main source of campylobacteriosis in humans. The red meat had a very low level of contamination suggesting a minor role in the transmission of illness. Particularly worrying is the increase of resistance against fluoroquinolones. Moreover, our results on resistance against nalidixic acid and tetracycline agreed with those in the EFSA Report and other studies. MLST molecular typing methods proved to be a helpful tool in epidemiological investigations.
In vitro Penetrability Of Anisakis simplex Larvae.

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Purpose: Anisakis larvae cause Anisakiasis and most common species are Anisakis simplex and Pseudoterranova decipiens. Penetrability of the larva play an important role in the pathogenicity because the severe symptoms result from larval invasion of the gastrointestinal mucosa of patients. Recently, using molecular biological methods, Anisakis simplex larvae can be separated three sibling species, namely, A. simplex sensu stricto, A. pegreffii and A. simplex C. The aim of this study was to investigate Anisakis larvae’s penetrability in order to clarify tendency of the ability among the each sibling species.

Methods & Materials: Anisakis larvae were isolated from Scomber japonicus fish those were caught in the sea around Goto Islands (Nagasaki Prefecture, Japan), the sea around Kanagawa Prefecture (Japan) and the sea around Jeju Island (Korea). We used Anisakis simplex larvae those were identified by morphological features in the study. For identification of the sibling species, PCR-RFLP for ribosomal DNA internal transcribed spacer (rDNA ITS) regions was performed. The penetrability of the larvae were evaluated with the agar method which was previously reported (Kojima et al. Clinical Parasitology. Vol.23, p63-65, 2012).

Results: We examined 138 Anisakis simplex larvae (third stage) for comparison of the penetrability among the species (A. pegreffii, A. simplex sensu stricto). The penetration rate of A. pegreffii and A. simplex s. s. was 79 and 68%, respectively. A. pegreffii showed rapid penetrability in comparison with A. simplex sensu stricto. Penetrability under the long term storage in saline at 4℃ was also examined. A. simplex larvae survived for over 14 weeks. The penetrability was kept for long times as long as they lives. The larvae penetrated more slowly in proportion to the storage time.

Conclusion: The results showed that our method is useful to test the penetrability of Anisakid larvae. Both A. pegreffii and A. simplex sensu stricto larvae had high ability to penetrate into agar, but the former penetrated quickly than the latter. In addition, larvae’s penetrability was kept even be an imperfect body and by the storage under the cold condition. Further studies should be done to clear the reasons why almost of anisakiasis is caused by A. simplex sensu stricto larvae.
Preliminary results on prevalence and persistence of *Listeria monocytogenes* in different dairy and meat processing plants in Central Italy.


**Purpose:** *Listeria monocytogenes* is a relevant food-borne pathogen able to cause severe and fatal infections in humans. Understanding correlations among different *Listeria monocytogenes* strains and evaluating *Listeria monocytogenes* isolates capacity to persist in processing plant environments is an essential tool for food surveillance. Most outbreaks of human listeriosis seem to be linked to strains able to persist in the environment and to replicate in foods. The aim of this study was to investigate prevalence and persistence of *Listeria monocytogenes* strains subtypes in different dairy and meat processing plants in the Abruzzo and Molise Region.

**Methods & Materials:** Totally 14 food processing plants have been selected for the study (7 for dairy products – DP - and 7 for meat products - MP). Sampling was performed in 4 session from March to October 2017, from 30 to 35 environmental sampling have been sampled in all the division of the plants, both from food contact surfaces (FCS) and non food contact surfaces (NFCS). Sampling sites were selected as niches for *Listeria monocytogenes* presence. Samples were tested with FSIS MLG8-10:2017 method. *Listeria monocytogenes* strains were serotyped using the method described in the U.S. FDA Bacteriological Analytical Manual (Bennett and Weaver, 2001). PFGE subtyping was performed according to the PulseNet protocol (Pulsenet USA, 2013) and macrorestriction patterns were analyzed using the BioNumerics 7.6 (Applied Maths, Kortrijk, Belgium).

**Results:** Results are based on 1772 environmental samples tested, 135 (7.6 %) samples were found positive for *Listeria monocytogenes* (94 NFCS and 41 FCS) in 4 DP and 6 MP. PFGE showed indistinguishable persistent strains in 2 DP and 3 MP in all sampling sessions and in one MP in 3 sampling sessions.

**Conclusion:** The persistence of *Listeria monocytogenes* underlines the ability to persist in food producing plants, in favourable niches inside the processing environment. To reduce *Listeria monocytogenes* prevalence, Good Manufacturing Practices and Good Hygiene Practices are needed. Analysis are still ongoing to model the contamination path, NGS on isolated strains will be carried out to confirm the persistence of the strains and to find the presence of gene devoted to resistance and persistence.
Purpose: A private hospital in Kohima reported 41 admitted cases of diarrhoea to State Surveillance Unit Nagaland on September 2, 2017. Our team started investigation on September 2, 2017 to describe the epidemiological characteristics of outbreak and to determine the associated risk factors.

Methods & Materials: We defined a case as “diarrhoea (>3 stools in 24 hours) or vomiting in a child studying in a private school, Kohima from August 29, 2017 to September 8, 2017”. We did a classroom to classroom survey for cases and conducted a retrospective cohort study on people who attended or ate from school farewell event on September 1, 2017. We observed exposure of different food items and risk factors involved. We collected four stool samples for testing pathogens.

Results: We identified 209 cases with an attack rate of 77%. Among them, 154 were hospitalised. There were no deaths. Attack rate was highest in age group of 16-25 years (73%). Females (59%) were more affected. Among identified cohort of 271 people, 249 attended the farewell and 187 fell ill, 22 did not attend but ate food brought home. Risk of gastroenteritis was 4.5 times (95% CI= 1.8-11.0) among those who ate chocolate cake with coffee toppings and 1.2 times (95% CI= 1.0-1.3) among females than in males. Of the four stool samples, one tested positive for Escherichia Coli, one for Bacillus Pseudomonas and two for Staphylococcus Aureus.

Conclusion: An outbreak of gastroenteritis due to mixed bacterial infection occurred following a school farewell event having strong association with food item chocolate cake with coffee toppings. We recommend Information, Education and Communication activities regarding proper cooking and food storage and timely collection of appropriate samples for testing.
Estimated Burden Of *Chlamydia trachomatis* Female Genital Infection, Italy, 2009-2016.

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**Purpose:** In Italy, the nationwide burden of genital *Chlamydia trachomatis* (Ct) is absent because of passive voluntary reporting, its frequent asymptomaticity and consequent undiagnosed cases. Therefore, no specific health intervention has been taken so far. We aimed to estimate incidence rate (IR) and prevalence of Ct female genital infection and its nationwide burden in terms of disability-adjusted life years (DALY).

**Methods & Materials:** We performed an ecological study on women aged 15-70 years affected by Ct genital infection registered from 2009 through 2016 in the two Italian sentinel surveillance systems (SSS) for sexually transmitted infections (STI): SSS-STIClin (composed by 12 public STI clinics, only symptomatic cases reported) and SSS-STILab (composed by 13 public microbiology laboratories, symptomatic and asymptomatic cases reported). Endo-cervical swab and/or first void urine were tested by nucleic acid amplification test (NAAT). We calculated IR by dividing the number of Ct-positive women reported in SSS-STIClin with the number of women resident in the cities where clinics are located. We calculated prevalence by dividing the number of Ct-positive women reported in SSS-STILab by the number of those tested. To estimate DALY (95% uncertainty intervals), we used data on symptomatic cases from both SSS by the Burden of Communicable Diseases in Europe (BCoDE) toolkit.

**Results:** In the 8-year period considered, we found 981 Ct-positive women reported in SSS-STIClin (median age 25 years, interquartile range (IQR) 22-30). Mostly (70.1%), they had Italian nationality. Their number has almost tripled from 71 in 2009 to 188 in 2016. IR increased from 2.7 in 2009 to 7.1 in 2016 per 100,000 (p<0.0001).

In SSS-STILab, 2,275 Ct-positive women were reported (median age 27 years, IQR 22-34). Mostly (81.3%), they had Italian nationality. Prevalence increased from 2009 (2.4%) to 2014 (3.2%) (p<0.05), then decreased to 2.6% in 2016.

Overall, among Ct-positive women, those aged 20-24 years had the highest DALY estimation equal to 105 DALY per 100,000 stratum-specific population.

**Conclusion:** Ct female genital infection has increased in Italy, mostly among the youngest sexually active women. Non-Italian women amounted for over one-third of cases. We recommend starting nationwide active surveillance and targeted screening programmes among women aged 20-24 years.
The relationship between hygiene-social variables and *Trichomonas vaginalis*

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**Purpose:** *Trichomonas vaginalis* (*T. vaginalis*) is the most common parasitic infection among nonviral, sexually transmitted diseases in the urogenital system. The incidence of the disease can vary according to the socio-economic status of the societies or different social groups. This research was performed on women who had vaginal complaints and who applied to Eskisehir Cancer Research Early Diagnosis and Screening Center (KETEM) and Eskisehir State Hospital Obstetrics and Gynecology Clinics.

**Methods & Materials:** *T. vaginalis* was investigated by stained and unstained direct microscopic examination, culture and Real Time PCR methods, in a total of 406 female vaginal swabs taken from women applied to these two hospitals for control examination. During the collection of samples, an information form was applied to patients, in order to investigate the relationship between the prevalence of *T. vaginalis* and some social parameters.

**Results:** *T. vaginalis* was detected by at least one of the methods in 35 (8.6%) of 406 swab samples. The distribution of 35 cases of *T. vaginalis* was; Twenty-eight (6.9%) were identified by direct microscopy and 27 (6.7%) by Giemsa staining and 31 (7.6%) by culture method and 35 (8.6%) by Real-Time PCR. In addition, direct microscopic examination, gram staining and routine culture methods were performed in all samples for other microorganisms that are likely to cause vaginal discharge. In 85 (20%) of all samples Candida spp., in 9 (2.2%) *T. vaginalis* and Candida spp. coexistence, in 25 (6.2%) Gardnerella vaginalis, in 11 (2.7%) G. vaginalis and Candida spp. coexistence was detected. There was no statistically significant relationship between the prevalence of *T. vaginalis* and social variables.

**Conclusion:** When the sensitivity and specificity of the diagnostic methods are compared with their determined performance, it was suggested that the use of PCR method in addition to direct and stained microscopic examination and culture method in trichomoniasis would increase the sensitivity and would be helpful in accurate diagnosis of the agent.
Whole-Genome SNP-based Phylogenetic Analysis of Yersinia pestis Isolates from Georgia and Neighboring Caucasian Countries

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Purpose: Yersinia pestis, the causative agent of plague, is currently established in hundreds of natural plague foci around the world. Twelve natural plague foci are registered in the Caucasus region. Previous studies have shown that two distant phylogroups of Y. pestis co-existed in the region: bv. medievalis and bv. microtus. In this study Y. pestis isolates from two natural plague foci from Georgia and neighboring Caucasian countries were characterized based on genome-wide SNP typing to infer the relationships between isolates from different Caucasus plague foci.

Methods & Materials: Whole-genome sequencing of 16 Y. pestis strains from Georgia, Armenia, Russian Federation (RF) and Azerbaijan were performed using a combination of different next generation sequencing (NGS) platforms: Illumina, PacBio and 454 technologies. Genome alignment, SNP identification, phylogenetic tree construction and evolutionary analysis were performed using phylogenetic and molecular evolutionary (PhaME) analysis software (https://github.com/LANL-Bioinformatics/PhaME). We additionally included recently released draft genome sequences of eight strains from East Caucasian mountain focus (Dagestan, RF) along with reference strains from worldwide databases.

Results: Whole-genome SNP based phylogenetic trees clearly discriminated the Y. pestis strains from different foci into different clades. Caucasian bv. microtus isolates fall in one of the most ancient branches 0.PE2 and formed two independent subclusters. The first subcluster represented by strains from Dagestan (RF) is very heterogeneous and the second by strains from Armenia and Georgia with relatively little variation indicating that population is rather monomorphic. Georgian microtus strains grouped together with PestoidesG in a different sub-cluster away from Armenian strains; the latter clustered with PestoidesF, forming their own sub group. Bv. medievalis isolates from Georgia, Azerbaijan and RF shared common features with minor differences that placed them in different sub-branches close to KIM10.

Conclusion: Data from this study suggest that Y. pestis has been independently introduced in Caucasus region two different times. Y. pestis bv. microtus strain typing data may imply that the strains from the East Caucasian mountain focus form the most ancient branch of the 0.PE2 lineage. In addition, obtaining SNP signatures with geographic correlations would enable the development of highly informative tools useful for bioforensics and epidemiological purposes.
The Potential Role of Aquatic Plants (Algae) And Insects in the Transmission of *Mycobacterium ulcerans*.

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**Purpose:** To determine the potential role of algal biofilms and insects in the transmission of *Mycobacterium ulcerans*. Buruli ulcer (BU) is a necrotizing skin disease that has been reported in at least 30 countries worldwide and associated with reverine areas. Currently research shows *M. ulcerans* as an environmental pathogen transmittable to humans from aquatic niche but the mode of transmission is unknown.

**Methods & Materials:** Biofilms from river bedrocks, floating woods, soil were collected and insects were captured with D-frame net. Swabs were used for human specimens. Three decontamination methods and two drug containing media were compared. Environmental samples were cultivated and analyzed directly with polymerase chain reaction (PCR). Isolates were confirmed by susceptibility to P-nitrobenzoic acid (PNB) and PCR analysis to detect the presence of mycobacterium specific sequence hsp65, IS2404 and were confirmed by Variable Number of Tandem Repeat (VNTR) locus ST1.

**Results:** Algal biofilms were largely diatoms and insects in the orders Hemiptera (with the most abundant insect family being Belostomatidae (20%), Coleoptera and Odonata were identified. No *M. ulcerans* was isolated from the environmental samples. A nested PCR assay to detect insertion sequence IS2404 on insects and biofilms was 44% (11/25) and 33% (5/15) respectively. VNTR assay on both insects and biofilms was 66.7% (4/6) and 33.3% (2/6) respectively. The insect families found to harbour *M. ulcerans* were Belostomatidae, Nepidae and Hydrophilidae. Ninety percent (90%) of clinical specimens contained the insertion sequence IS2404. Recovery of bacilli was best with oxalic acid and LJ-EB medium

**Conclusion:** Algae and insects were implicated as possible reservoirs of *M. ulcerans* and thus could play a role in the transmission of the pathogen to humans. This information generated may serve as a platform to devise effective control measures to prevent human contact with infected aquatic plants harbouring the causative agent.
Occurrence And Characterization Of *Salmonella* Strains Isolated From Animals Involved In Animal Assisted Interventions (AAIs)

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**Purpose:** The Animal Assisted Interventions (AAIs) are constantly increasing worldwide and require very close animal-human contacts. The surveillance of zoonotic microorganisms in these animals should be compulsory, especially when patients are immunocompromised. The aims of this study were to investigate the potential role of animals involved in several AAIs as carriers of *Salmonella* spp. and the phenotypic and genotypic characterization of isolates.

**Methods & Materials:** A total of 250 faecal samples from 13 animal species involved in AAIs (94 equids, 56 dogs, 40 birds, 37 rabbits, 20 cats and 3 other species), were collected. *Salmonella* spp. was detected by ISO 6579:2002 protocol. The isolates were serotyped by the OIE Reference Laboratory for salmonellosis (IZSVe, Italy). The phenotypic resistance profile of isolates was assessed by broth microdilution method to define the minimum inhibitory concentrations (MICs) (according to the CLSI guidelines), whereas molecular typing was determined by Multilocus Sequence Typing (MLST) and microarray analyses (Alere Technologies, GmBH).

**Results:** Of the 250 animals tested, 6 (2.4%) cats (of a single Association) were positive for *Salmonella* spp. Serotyping showed the presence of *Salmonella* Rissen and *S. Typhimurium* monophasic variant 4,[5],12:i-. All the isolates showed phenotypic resistance to β-lactams, phenicols, aminoglycosides, tetracyclines, folate pathway inhibitors and fluoroquinolones. Most of the isolates carried genetic determinants conferring resistance to β-lactams, aminoglycosides, sulphonamides and trimethoprim.

**Conclusion:** The animals involved in programs of AAIs need to be well cared of and fed with safe diet. The 6 positive cats were usually fed with dry and canned commercial food but also, occasionally, with raw meat-based products (Bone And Raw Food - BARF), that could be the source of the observed infections. The Italian national guidelines on AAIs suggest avoiding the use of raw food to feed the animals involved in AAIs. The finding of the zoonotic multidrug-resistant bacteria suggests that the animal handlers and the volunteers should be adequately trained and that systematic surveillance should be performed as part of the screening process for animals involved in AAIs.
Four-Year Secondary Data Analysis of Acute Flaccid Paralysis Epidemiology and Trend, Yobe state, Nigeria: 2013-2016

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Purpose: Nigeria is one of the 3 remaining poliomyelitis endemic countries in the world. Acute Flaccid Paralysis (AFP) Surveillance is one of the key strategies adopted by the WHO for polio eradication. The ongoing insurgency in the North East is threatening to undermine the successes in the country’s Polio eradication drive; Yobe is one of the most affected states. The aim is to describe the epidemiology and trend of Wild Polio Virus (WPV)/AFP and to assess the AFP performance indicators in Yobe state.

Methods & Materials: we carried out secondary data analysis of Yobe AFP surveillance data from 2013-2016. The State Ministry of Health and WHO AFP linelists were used for the analysis. Data were analyzed using Microsoft Word version 2016.

Results: A total of 1108 cases were AFP cases were reported between 2013 and 2016. Fifty-six percent of the cases were male and children between 0-5 years had the highest number of cases (89%). Majority of the children had received more than 3 doses of Oral Polio vaccine (93.68 %) while only 5 (0.45%) were zero dosed. Eight confirmed cases of WPV Type 1 were recorded over the period, 7 in 2013 and 1 in 2014; the last case was in Gujba LGA. One Circulating vaccine-derived poliovirus (cVDPV) was detected in 2015 in Bade LGA. Non-Polio AFP rate, Stool adequacy and other AFP performance indicators were met in most of the years of the review.

Conclusion: Transmission of WPV was interrupted since 2014 in Yobe state and the surveillance system met most of the indicators for AFP surveillance. We recommended intensification of AFP surveillance and vaccination activities, especially in security-compromised areas. Close monitoring of influx of Internally Displaced Person (IDPs) from Borno state, where last known WPV was recorded should be instituted.
Purpose: The Paediatric Active Enhanced Disease Surveillance (PAEDS) network conducts Australian acute flaccid paralysis (AFP) surveillance as part of the global effort to eradicate Poliovirus. We aimed to describe the clinical epidemiology of childhood AFP over 10 years captured by PAEDS. We hypothesised there would be no secular or seasonal trends evident.

Methods & Materials: Data were collected by PAEDS nurses at five Tertiary Paediatric hospitals from 2007 to 2017. Children aged 0-15 years with AFP-like symptoms were included. For analysis, two PAEDS datasets were unified, with AFP compatible cases stratified by demographic features and expert review panel diagnoses.

Results: Of 400 AFP compatible cases, 50.7% were male; 45% of cases were children aged 0-4 years, 7.3% aged <1 year. As expected, the most populous states Victoria (n=147) and NSW (n=128) reported the majority of cases. The most frequent diagnoses were Guillain-Barre Syndrome (GBS; 33.8%), Acute Disseminated Encephalomyelitis (ADEM; 18.3%) and Transverse Myelitis (TM; 17.3%). A variety of less common diagnoses also caused AFP; some showing age-related predominance. No secular trend was seen across all AFP cases; however there was an apparent increase in both TM and ADEM. ADEM was more frequent in winter and TM in autumn. For the three major diagnoses, 14% of cases documented a concomitant infectious organism.

Conclusion: There is a stable annual incidence of AFP in Australia children between 2007-2017 despite increasing rates of TM and ADEM. AFP diagnosis is age-related. Our ongoing work will describe the clinical features of the major diagnostic groups, in particular analysing for disease clusters that may represent previously unrecognised outbreaks of neurotropic infection (e.g. non-polio enteroviruses).
Molecular Diagnosis of Zoonotic Mycobacterium africanum Transmission between Human and Cattle: A Case Report

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Purpose: Tuberculosis is one of the most important infectious diseases of Humans and cattle in Nigeria and Africa. This study aimed at reporting a case of human to cattle transmission of zoonotic Mycobacterium africanum based on culture, SD Bioline TB Ag MPT64 and multiplex PCR known as Genotype MTBC.

Methods & Materials: A cattle herd was tested for Mycobacterium infection using single caudal fold intradermal tuberculin test and a bull reacted positively, but showed no gross TB pathological lesions after slaughter. The cattle rearer was also diagnosed with active pulmonary TB in the hospital. The cattle rearer’s sputum and the bull’s bronchial as well as retropharyngeal and mediasternal lymph nodes were obtained, processed and cultured. The isolates were analysed using SD Bioline TB Ag MPT64 as well as Genotype MTBC.

Results: The results revealed that both the rearer’s sputum as well as the bull’s lymph nodes yielded Mycobacterium africanum which is primarily a human pathogen and rarely been isolated from cattle.

Conclusion: The report highlight the importance of Mycobacterium africanum and its public health implications between humans and cattle transmission. Measures for control are also suggested.
Occurrence Of *Clostridium Difficile* In Dogs Involved In Animal Assisted Interventions (AAIs) In Italy

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**Purpose:** *Clostridium difficile* is the most important infectious agent of healthcare-associated diarrhea in patients. In the last years, there has been an increasing of community infections due to strains characterized by high virulence and/or antimicrobial resistance. Even if transmission of *C. difficile* from animals to human has not yet been demonstrated, recent studies suggest animals as possible reservoirs for human pathogenic strains.

AAIs are a multidisciplinary support, beneficial to humans and based on a closed human-animal interaction. AAIs are developing considerably in Italian healthcare facilities involving especially dogs. Given the frequent involvement of vulnerable population groups, there is a need of a deeper knowledge about the role of animals involved in AAIs as possible carriers of zoonotic or antimicrobial resistant agents. This is mandatory for an effective risk assessment to protect both the humans and the animals involved.

The aim of this study was to evaluate the presence of *C. difficile* in healthy dogs involved in AAIs.

**Methods & Materials:** 56 faecal samples from healthy dogs involved in AAIs in Italian healthcare facilities were collected. Isolation and identification of *C. difficile* were performed according to standard methods and the isolates were confirmed by PCR and characterized by agarose gel-based PCR-ribotyping method previously described.

**Results:** *C. difficile* was isolated in two out of 56 (3.6%) of the healthy dogs tested. Those dogs were involved in AAIs with children, disabled and elderly people. Two different PCR-ribotypes (RT) were identified: RT078 and RT087.

**Conclusion:** The PCR-ribotypes of *C. difficile* detected in previous studies in dogs were RT001, RT009, RT010 and RT014. RT078 and RT087 were commonly isolated from humans and sometimes from other animal species than dog; especially RT078 is considered an emerging cause of human infections in the USA and in Europe, being the 3rd most common type detected in hospitals. RT087 was identified as the predominant RT in a Hungarian hospital epidemiological study. Our results suggest that dogs may be a source of these ribotypes for humans and raise the question of the zoonotic potential of *C. difficile*. The role of dog-human interaction in the cross-transmission of this agent should therefore be further investigated.
Purpose: Prisoners are considered high-risk group for blood-borne infections and are not recommended for blood donations globally. Pakistan Prison rules allow prisoners to donate blood and get 30 days extra remission. Thus, the present study was conducted to estimate the seropositivity of human immunodeficiency virus (HIV), hepatitis B (HBV) & C (HCV) virus, and syphilis in prisoner blood donors and compare it with general population donors.

Methods & Materials: This retrospective study was conducted in October 2009 by the Inspectorate of Prisons Punjab and Phoenix Foundation for Research & Development Lahore. Donors from January, 2007 to September, 2009 from prisons and general population were included. Physically fit volunteer donors were tested for HIV, HCV, and HBV by ELISA and syphilis by VDRL. For confidentiality, personal identifiers were stripped off.

Results: A total of 14,337 blood donors were tested, of whom 3,074 (21.44%) were prisoners. Mean age of general population and prisoner donors was 21 (16-72 yrs) and 28 (17-57 yr) years respectively. Among general population, 518 (4.6%) were positive for at least one of these infections; 88% being males. Out of 11,263 general population donors 292 (2.6%) were positive for HCV, 201 (1.8%) for HBV, 22 (0.2%) for Syphilis and 14 (0.1%) for HIV. Co-infection among these infections was negligible.
Among prisoners, 434 (14%) were positive for at least one of these infections; 99% being males. Out of 3074 prisoner donors, 235 (7.6%) were positive for HCV, 114 (3.7%) syphilis, 111 (3.6%) HBV and 21 (0.7%) for HIV. Co-infection in prisoner donors as combination of any two or more of HIV, HBV, HCV and syphilis was present in 0.03%- 0.68%. Being a prisoner blood donor was significantly associated with higher seropositivity for Syphilis (OR=19.68; CI=12.2-31.99), HIV (OR=5.53; CI=2.69-11.46), HCV (OR=3.11; CI=2.6-3.73) and HBV (OR=2.00; CI=1.62-2.630) P < .05 for all.

Conclusion: Prisoner donors bear three times high risk of transmission of these infections through transfusion. Prison blood donations may be discouraged. If alternate sources are limited, stringent screening of prisoner blood donors with highly sensitive tests is recommended. I also recommend further studies and then amendments in relevant Pakistan Prison Rules accordingly.
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Host's Immunogenetic Risk Factors in Patients with Chagas Disease

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Purpose: Chagas Disease (CD) is endemic in many Brazilian regions being considered an important public health problem. About 30% of chronically infected individuals develop Chronic Chagas Cardiomyopathy (CCC) and 10% develop Digestive Tract Disease (DTD) after 20 of infection by the Protozoan Trypanosoma cruzi. Taking into account that the host immune response might be modulated by different genes, it is expected that immunogenetic markers may at least partially explain this phenomenon. This study investigated the levels of CCL3 and CCL4 chemokynes, the IL17 cytokine genotypes, CCR5Δ32 and CCR5 59029 A/G Polymorphism, the functional MICA-129 Polymorphism in patients with different clinical forms of CD.

Methods & Materials: Patients chronically diagnosed with CD and controls were recruited over a period of eight years. CC and DTD were diagnosed by clinical and laboratory routine procedures and the chronic infection (IgM, IgG anti-T. cruzi) was assessed by ELISA. PCR-RFLP was used to identify MICA, CCR5 polymorphisms and IL-17 alleles. CCL3 and CCL4 chemokines were measured using Milliplex MAP Assay. The data were analyzed chi-square. Odds Ratio and confidence interval at 95% values were calculated (p<0.05).

Results: CCL3 and CCL4 plasma levels and the CCR5 59029 A/G polymorphism were not correlated with different clinical forms of CD. On the other hand, the A allele and A/A genotype of IL17A as well as the functional MICA-129 Polymorphism were significantly increased in patients with severe Left Ventricular Systolic Dysfunction (LVSD) when compared to controls.

Conclusion: In conclusion the severity of LVSD is strongly influenced by genes involved in different steps of innate and adaptive immune response against T. cruzi.
Purpose: Molecular biology is a really good tool worldwide being used supporting the diagnosis in infectious disease, specially among that potentially congenital transmitted. Toxoplasmosis is a worldwide spread infection, causing with clinical aspects reported being different among populations. Here we describe clinical aspects presented by newborn babies with congenital toxoplasmosis from São Paulo state, Brazil.

Methods & Materials: From the 45 pregnant women with previous *Toxoplasma gondii* acute infection, were collected amniotic fluid to determine fetal infection by *T. gondii* by PCR, using B1 gene as molecular marker and blood to new serology by ELISA. At birth were noted the gestational age and the babies weight. Following WHO protocols prematurity was defined as gestational age <37 weeks and low birth weight ≤ 2499 grams. The t-test was used to compare values (p < 0.05).

Results: The fetal infection determined by positive PCR in amniotic fluid was found in 28 (62.2%) samples. The *T. gondii* antibodies were reassayed; from that still 33 IgM positive samples 19 (57.6%) were positive PCR; from the 12 IgM negative 8 (66.7%) were positive PCR. From the total 11.1% (5/45) newborns present symptoms related to toxoplasmosis at birth and the fetal infections were determined in 3 of them. Fetal deaths accounted for 11.1% (5/45) and 8.8% (4/45) of the positive PCR and 2.22% (1/45) negative PCR. The maternal age, prematurity and low birth weight were not related to fetal infection (p = 0.675; p = 0.941; p = 0.697, respectively.

Conclusion: Congenital toxoplasmosis was the cause of fetal death. Prematurity and low birth weight are not related to congenital infection by *T. gondii* in Brazil as has been reported in other countries. The results confirmed that there was vertical transmission of *T. gondii* determined by PCR.
Purpose: During the mid-transmission period of ebola virus disease (EVD) outbreak in Sierra Leone, a 19-year-old pregnant woman who was a petty trader in a health facility in Freetown, noticing no fetal movement for past 3 days, reported to a health facility. Medical history and laboratory testing showed no abnormalities except that she was sickle cell positive. She was not known to any surveillance team of having any epidemiological link to EVD case. She was then induced with oral medications as well as IV infusion. And was delivered of a macerated dead fetus in the 36 week of gestation.

Methods & Materials: The death swab from the baby was taken to the nearest laboratory and it returned positive to direct qPCR. The woman was invited for sampling and a qPCR test also conducted. Blood samples was taken from the woman and tested for both ebola virus IgG and IgM at the Chines laboratory in Jui, Freetown.

Results: EVD testing showed that the fetus was positive to EVD with a high threshold value of 21 while the woman was negative for EVD at a threshold value of 42. The woman was positive to EVD IgG but negative to EVD IgM by ELISA technique.

Conclusion: This is a rare EVD case in the period of medium transmission. We conclude that the woman would have come in contact with a low dose of the virus not enough to cause a full blown EVD and the immune system was able to stop the virus from further replication.
Hand Hygiene education, an underutilized tool in preventing emerging, re-emerging infectious diseases and seasonal epidemics.

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**Purpose:** A common thread among most emerging infectious diseases is Hand Hygiene. So why isn't more being done to introduce, educate and bring awareness of this important topic among our youngest members in the community. We created a fun scientific experiment with second and third grade students to see if education and intervention will bring about a positive behavioral change that will subsequently decrease the rate of illness during peak influenza and norovirus season.

**Methods & Materials:** Ninety students cultured their "dirty" hands on a petri dish. Proper handwashing technique was demonstrated to all students. They were then divided equally into the hand washing group and the hand sanitizer group. All students then cultured their "clean" hands on a second petri dish. The students observed, compared and documented their findings daily for a week.

We then used a simulation germ that illuminates with black light to test the efficacy of their hand washing and see areas where they could improve. We followed the illumination along the classroom and each other to see how easily the "germ" spread.

A light microscope, a microbiologist and slides of common pathogens were brought into the classroom for examination and discussion. Children were happy to share stories of infections they and their families have had.

**Results:**
1. 91% of the students observed decreased microbial growth from "dirty" to "clean" hands.
2. Education improved compliance as 89% had a favorable behavioral change to clean their hands.
3. There was a 71% decrease in incidence of illness related absences 30 days after the hand hygiene intervention.
4. Three out of the five classes reported hand sanitizer was more effective when compared to hand washing.

**Conclusion:** Hand Hygiene education is remarkable beneficial, especially in children who are at the greatest risk of illness. It is clearly effective in decreasing infectious disease risk, while teaching a valuable life-long habit. For the impact to be felt as a preventative strategy both for illness and emerging infectious diseases, its implementation into the elementary school curriculum is warranted. In the words of the Benjamin Franklin, "an ounce of prevention is worth a pound of cure".
**Purpose:** Measles is an important vaccine-preventable infectious disease causing morbidity and mortality in developing countries. Adequate vaccine coverage at least 95% required to prevent measles outbreaks. The analysis of measles laboratory surveillance data from 2010–2015 was carried out to identify the risk factors for measles and vaccination coverage rates.

**Methods & Materials:** Blood samples of suspected measles cases are sent to virology laboratory National Institute of Health Islamabad, Pakistan to detect measles specific immunoglobulin M (IgM) antibodies. Patient’s age, gender, residence and vaccination status are recorded. Measles laboratory surveillance data from 1 January 2010 to 31 December 2015 were used in this study.

**Results:** Of 28356 suspected measles cases processed in the laboratory during study period, 14938 (53%) were males, and 18563 (65.5%) were IgM positive. Median age of suspected cases was 3 years (Range; 21 days to 71 years). Children aged 1 - 5 years were the most affected (>54%). However 8% suspected cases were <9 months of age (age below 1st dose of vaccination in Pakistan). IgM positivity was 65% and 72% among <9 months and 9 - 24 months children respectively. A total of 11336 (40%) suspected cases had been vaccinated with at least one dose of MCV; 13739 (48%) had not been vaccinated and 3281 (12%) had an unknown vaccination history. The vaccination status of suspected measles cases residing in Northern areas having unrest was poor (17%) as compared to provinces of Punjab and Sindh having 56% and 38% respectively. Probability of IgM positivity for measles was 2.5 times higher among unvaccinated as compared to those were vaccinated for at least one dose of MCV ((OR 2.50, 95% CI 2.38–2.65). Cases start rising from February through June (n=19508, 69% reported during these months).

**Conclusion:** Measles continues to be an important cause of morbidity and mortality in Pakistan due to poor vaccination coverage; too below the required to prevent outbreaks. A reasonably high number of cases are <9 months of age identifying a need for policy decision; First dose be given at six months or even earlier to save the infants from this deadly disease.
Colistin resistance beyond carbapenems: molecular epidemiology of multi-drug resistant *Klebsiella pneumoniae* from an Italian hospital

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**Purpose:** Multidrug resistant (MDR) *Klebsiella pneumoniae* (*Kp*) is a worldwide concern worsened by colistin resistance emergence. In this study, molecular epidemiology of clinical MDR *Kp* strains is described, assessing clonal relationships and resistance genes profiles. Prevalence of Extended-Spectrum Beta-Lactamase (ESBL) *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes was evaluated, as well as that associated to carbapenems (*bla*KPC, *bla*GES, *bla*VIM, *bla*MIP, *bla*NOM, *bla*OXA-48) and colistin resistance (*mcr*-1,2,3,4 and *mgr*<sub>B</sub>).

**Methods & Materials:** Twenty-six *Kp* cultures were collected from “A.Cardarelli” hospital in Molise Region (Central Italy), 57.7% from Intensive Care Unit (ICU). The Minimum Inhibitory Concentration (MIC) was assessed by BD Phoenix™. Genotyping was achieved through Pulsed-Field Gel Electrophoresis (PFGE) with *Xba*I and MultiLocus Sequence Typing (MLST). PCRs for resistance genes detection and *mgr*B sequencing were performed.

**Results:** Most (n=20, 77%) strains were carbapenems resistant (imipenem, meropenem, ertapenem), and 53% (n=14) to colistin. A higher prevalence of *bla*KPC (100%) and *bla*SHV (96.2%) was found compared with *bla*TEM (88.5%), *bla*VIM (69.0%) and *bla*CTX-M (7.7%). While none colistin-resistant (col-R) strain carried *mcr*-1,2,3,4 variants, 42.8% (n=6) had an Insertion Sequence (IS) in *mgr*B, identified as IS5-like (5/14, 36%) and ISKpn14 (1/14, 7%). The prevalent Sequence Type was ST512 (50%), followed by ST101 (38.5%) and ST307 (11.5%). PFGE detected 12 clusters and 18 pulsotypes (95% similarity), and was more discriminating than MLST (Simpson’s Index 95% vs 61%). An ICU outbreak, during November 2014-January 2015, included five ST512 strains, *bla*TEM/*bla*SHV/*bla*VIM/*bla*KPC positive, and col-R due to *mgr*B IS5-like.

**Conclusion:** Molecular epidemiology study identified an outbreak caused by poor compliance with hygienic standards because of reduced personnel during Christmas holidays. Although ST258 is the most prevalent in Italy, half strains were typed as ST512 that represents its monoallelic variant. Our results confirm the endemic *bla*KPC distribution and *bla*TEM/*bla*VIM as the prevalent ESBLs in *Kp* hospital outbreaks. According to epidemiological data, plasmid *mcr* variants were not found in col-R strains. Conversely, IS elements prevalence in *mgr*B explain resistance in about half col-R strains, and was in line with Italian data. Further investigations are needed to identify *mgr*B mutations in IS negative col-R strains.
The Distribution of Intestinal Parasitic Infections in Patients of Attending to University Hospital in Eskisehir/Turkey from 1980 to the day.

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**Purpose:** Intestinal parasites are present throughout the world in different number and type of prevalence. In this retrospective study was performed to present the distribution of intestinal parasites in Eskisehir/Turkey at a university hospital of the presence of intestinal parasites change the results of the parasite species and also according to clinics to the 80s until today.

**Methods & Materials:** The stool examination results in the parasitology laboratory of the university hospital were examined retrospectively with three separate periods. These periods 1983-1993, 1995-2000 and 2000s today. The stool samples were examined by native-Lugol, flotation and sedimentation methods in the parasitology laboratory of the hospital. Anal tape method was used in suspicious cases. Trichrome and modified acid-fast staining methods were also applied to suspicious stools after the 2000s. Statistical analysis was using SPSS version 20 software. The results were presented in tables and graphs parasite species, gender and years.

**Results:** In a retrospective study between 1983-1993; a total of 32,450 stool specimens were examined, and 12.15% of them had intestinal parasites. Positive results were described as 9.35% of as protozoon cysts and 2.80% as helminth eggs. The most common parasite was *Giardia lamblia* with 9.2 %. Parasitic incident rates are most often described in the examples sent from the pediatric and internal medicine clinic. Between 1995 and 2000, a total of 16,551 stool samples were examined and 2.6% of the parasites were identified, which it has also been the most common parasites *Giardia lamblia* 1.8%. A significant increase compared to parasitological examination requests are sent to our laboratory in the past from 2000 until today, but a significant decrease in parasite incidence was detected. During this period, a total of 40,963 stool specimens were examined, respectively; 1.2% *Blastocystis hominis*, 0.1% *Giardia lamblia*, 1.4% *Enterobius vermicularis*.

**Conclusion:** Since 1983, there has been a rapid decline in parasitic rates as the infrastructure problem of the city has been eliminated and the control of city water supply and drinking water has been regulated. However, there is still a need to increase control measures.
Promising In Vitro Effects of Selected Antiseptics Against Acanthamoeba Polyphaga Strain - The Etiological Agent of Vision-Threatening Keratitis Identified in Polish Patient

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Purpose: In the last decades, Acanthamoeba keratitis, the vision-threatening corneal infection is detected with increasing frequency in Poland. As the disease is difficult to treat due to extremely high resistance of cysts to chemicals, agents of possible anti-amoebic activity are still tested. In this study, A. polyphaga isolate of T4 genotype from patient with complicated keratitis, a history of swimming in a lake, not using contact lenses and A. castellanii Neff strain were evaluated in terms of their in vitro susceptibility to selected antiseptics.

Methods & Materials: Samples of the strains cultured under bacteria-free conditions in BSC calf serum-enriched culture medium were exposed to antimicrobial agent Povidone-iodine and Aerodesin disinfectant. Effects of the compounds on Acanthamoeba strains following 48, 96 and 120 hrs exposure compared to the control cultures were monitored: the developmental form dynamics, encystation process, overall number of surviving amoebae, changes in cysts/trophozoites/proportion assessed in the exponential growth phase were determined.

Results: The diagnosis was confirmed by confocal microscopy, in vitro culture and by 18S rDNA gene sequences (T4 genotype). Both antiseptics showed some amoebostatic effects on Acanthamoeba strains. Povidone iodine after 120h from application reduced number of viable amoebae to 17% in comparison to the control samples (100%); Aerodesin indicated expected cysticidal efficacy: no amoebae after 92h exposure were detected.

Conclusion: Risk factors of Acanthamoeba keratitis diagnosed mainly in contact lens wearers include also ocular exposure to water containing cysts and trophozoites of the amphizoic amoebae. Promising in vitro effects of the chemicals against A. polyphaga strain, etiological agent of vision-threatening keratitis justifies further tests with a modified agent application pattern particularly in terms of expected cysticidal efficacy.
Use of Whole Genome Sequencing in an Outbreak Investigation of Bovine Brucellosis due to *Brucella melitensis*, Austria, 2018

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**Purpose:** Brucellosis is a disease caused by *Brucella abortus*, *B. melitensis* and rarely *B. suis*. *B. abortus* causes epidemics of miscarriages in bovines and Bang’s disease in humans. *B. melitensis* is predominantly associated with sheep and goats in Mediterranean countries. From 2013 until 2017, twenty cases of human brucellosis were reported in Austria, none of them were a documented autochthonous infection. In May 2018, a dairy farm was affected by an epidemic of miscarriage. *B. melitensis* was isolated from bovine abortus material and from a blood culture specimen taken from the farm’s veterinarian, hospitalized for febrile illness. Subtyping of the two isolates was requested by public health authorities.

**Methods & Materials:** Species was identified by BioFire assay and an in house real-time PCR test. Genomic libraries were paired-end sequenced on an Illumina MiSeq instrument. Genome assemblies were performed de novo using SPAdes, and whole genome sequence (WGS) data interpretation was carried out with SeqSphere+. A *B. melitensis* core genome (cg) MLST scheme comprising 2797 core and 310 accessory genes was created using 56 *B. melitensis* complete genomes.

**Results:** The two isolates were identified as *Brucella melitensis*. Using SeqSphere+, isolates showed 98% accordance with the cgMLST targets. Based on the cgMLST and the pan genome (cgMLST plus accessory genome), the two isolates showed zero allelic difference to one another, but differed by 1276 (cgMLST analysis) and 1386 alleles (pan genome analysis), from *Brucella melitensis* strain RV9-18 (an external ring trial isolate, EMERGE 2018), respectively.

**Conclusion:** Austria is officially recognised as being free from bovine brucellosis (*B. abortus*; since 1999) and from small ruminant brucellosis (*B. melitensis*; since 2001). The source of the present outbreak affecting cattle is still unclear. In addition to the culture confirmed case of the veterinarian, three persons living on the farm were identified as non-hospitalized brucellosis-cases by serology only. Using WGS, the epidemiologically related human and bovine isolates were indistinguishable from each other, which underlines the considerable potential of WGS to corroborate epidemiologically linked cases.
Identification of Multiple Drug Resistant Strains of Bacteria Harboring New Delhi-Metallo-\(\beta\)-Lactamase-1 (blaNDM-1) Gene from Blood Samples of Children of a Tertiary Care Hospital

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**Purpose:** Bacteria exhibit clinical threat because of their tendency to hydrolyse the carbapenems by producing carbapenemase. *New Delhi metallo-beta-lactamase* is a monomeric protein of 28kDA, it can hydrolyze all types of beta lactams. *New Delhi metallo-beta-lactamase* is encoded by *blaNDM-1* gene. Data regarding the prevalence of *blaNDM-1* gene in children in different species of bacteria from a tertiary care hospital of Lahore, Pakistan is presented in the research.

**Methods & Materials:** Blood samples of 240 children (0-15 years) suffering from different infections were screened for multiple drug resistance. Gram negative *Enterobacteriaceae* were identified by biochemical tests and API strips. Carbapenemase was detected by Modified Hodge Test and *Metallo beta lactamase* (MBL) were detected by Combined disk test and Double disk synergy test. *blaNDM-1* gene was confirmed by PCR, then cloning in a T/A vector and sequencing was done.

**Results:** Out of 240 blood samples of children 116 (48.3%) samples were multiple drug resistant (MDR). Out of 116 MDR species 16(13.8%) samples have positive *blaNDM-1*gene. MBL strains having *blaNDM-1* are 4(25%) *Enterobacter cloacae*, 3(18.75%) *Klebsiella spp.*, 2(12.5%) *Pseudomonas spp.*, 2(12.5%) *Citrobacter freundii*, 2(12.5%) *Acinetobacter Baumanii*, 2(12.5%) *E .coli* and 1(6.25%) *shigella spp.*

**Conclusion:** *blaNDM-1* gene is found to be present in *Enterobacter cloacae* and *Klebsiella spp.* predominantly. Neonatal emergency/neonatal unit has more occurrences of *New Delhi-Metallo-\(\beta\)-Lactamase-1* producers then the surgical and hematological/oncological wards. *blaNDM-1* gene is also common in neonatal sepsis. Neonatal sepsis is the major cause of mortality and morbidity in Pakistan.
Viral Suppression and Loss To Follow Up in HIV/AIDS Patients on Antiretroviral Therapy in Pakistan

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Purpose: UNAIDS has set a new ambitious target of 90-90-90 to help end AIDS epidemic. According to this target by 2020, 90% of all people receiving antiretroviral therapy will have viral suppression. We conducted this study to find percentage of virally suppressed individuals on antiretroviral therapy at our HIV treatment center.

Methods & Materials: A one-year retrospective study was conducted at HIV treatment center, Jinnah Hospital Lahore. Medical record of patients registered and put on antiretroviral therapy between 1st Jan and 31st Dec 2014 was reviewed. Patients who did not come for follow-up for ≥ 6 months were declared lost to follow-up. Patients were considered virally suppressed if viral load was undetectable at 6 months of ART. Patients with VL > 1000 copies/ml at end of 6 months of ART were declared treatment failure.

Results: A total of 162 patients were put on ART. High risk behaviors included injection drug use in 43% (n=70), heterosexual contact in 28% (n=45), female sex workers 7% (n=11), males who have sex with males 8% (n=13), contaminated needles in 4% (n=6) and blood transfusion in 3% (n=5). Treatment regimens included TDF+3TC+EFV in 84% (n=136), ZDV+3TC+EFV in 12% (n=20), ZDV+3TC+NVP in 3% (n=5) and ZDV+3TC+LPV/RTV(r) in 0.6% (n=1) patients. Viral suppression data was available for 52.5% (n=85) patients, 33% (n=54) patients were lost to follow up and 14% (n=23) patients had insufficient lab data. Out of 85 patients for which lab data was available, 78% (n=66) were virally suppressed, 12% (n=10) had treatment failure and 10% (n=9) had detectable VL but below 1000 copies/ml. Injection drug use was the strongest independent risk factor for loss to follow up and IDUs were more likely to lost-to-follow-up as compared to non-IDUs [50% vs 20% respectively; p < 0.001; OR 3.84, 95% CI 1.93 – 7.65]. On sustained ART, IDUs had similar percentage of viral suppression as compared to non-IDUs [71% vs 80%; p = 0.39; OR 0.59, 95% CI 0.21 – 1.75]. Overall, 40% patients were virally suppressed.

Conclusion: The strongest challenge in achieving treatment response is loss to follow up. Injection drug use is the strongest predictor of loss to follow up.
Markers of systemic inflammation in HIV-infected patients with different HIV RNA level

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Purpose: Immune reconstitution inflammatory syndrome is one of the main components of the pathogenesis of HIV infection. The severity of systemic inflammation and immune activation is an important prognostic indicator of disease progression.

Aim of research: to determine the diagnostic significance of systemic inflammation markers in HIV-infected patients in depend of on the level of viral replication HIV.

Methods & Materials: Materials and methods. The study involved 224 HIV patients who were divided into three main groups according to the level of HIV replication. Patients with undetectable viremia or with a viral load (VL) less than 50 copies/ml (n=82) were included in 1st group, with a defined patients with a level of HIV RNA concentration of more than 10 000 copies / ml (n=74) were in 3rd group. All patients received HAART. The content of lipopolysaccharide-binding protein (LBP), procalcitonin and cytokines (TNF-α, IL-1β, IL-6, IL-8, IL-10, INF-γ, INF-α) were detected in the blood serum using a solid-phase enzyme-linked immunosorbent assay. The concentration of HIV RNA (viral load of HIV, copies /ml) was determined in blood plasma by the RT-PCR.

Results: Significant increase in the level of LBP and cytokines IL-1β, TNF-α, IL-6, IL-8, IL-10, INF-γ, INF-α was found in groups of patients in comparison with healthy people. An average correlation was found between the level of HIV viral load and the LBP level in the blood in the groups of patients with a defined level of HIV viremia (2nd and 3rd). In patients with HIV infection with viremia, there are signs of more active systemic inflammation, accompanied by increased production of antiendotoxin proteins and cytokines (TNF-α, IL-10, INF-α and INF-γ), compared with patients with undetectable levels of viral load of HIV. Level of procalcitonin in all groups of patients had not any statistical deviation in comparison with its level in the healthy people.

Conclusion: Full suppression of HIV replication is associated with lower levels of LBP, TNF-α, IL10, INF-α and INF-γ. Antiretroviral therapy can significantly reduce the severity of systemic inflammation in patients with HIV infection, but does not ensure the normalization of the concentration of antiendotoxin proteins and cytokine status.
Late Presenter Patients with Advanced HIV Disease Associated with Tuberculosis

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Purpose: Despite the wide access to highly active antiretroviral therapy (HAART) the number of patients presenting late for care tend to increase over the past few years worldwide. However, starting therapy at an advanced stage of disease would have an impact on treatment outcomes. The goal of the study was to analyze the socio-demographic and clinical profile of Late Presenters (LPs) with advanced HIV disease.

Methods & Materials: Over the period of 2014-2016 years a retrospective cohort study was conducted among 571 patients who attended the large medical center, Department for treatment of TB/HIV patients. Demographic, clinical, and laboratory data were prospectively collected.

Results: In total the number of LPs with advanced HIV disease (CD4 < 200 cells/ml) was 43.4%(n=248): 2014 - 49.5%(n=96); 2015 - 45%(n=81); 2016 -36.1%(n=71). The median CD4 cell count was 62 cells/ml. Among the LPs the majority was male(68.5%). The median age was: men 37.4(25÷61) and women 36.7(24÷62) years. The ratio of patients in age group 50-59 years and senior was 4.8%. Sexual mode of HIV transmission prevailed in LPs group: 77.4%(MSM was 8.5%) versus 22.6% IDUs. Out of 243 patients who started HAART (5 patients discontinued therapy due to intolerance of medication) 79%(n=192) achieved a viral load <300 copies/ml. About 29.6% of LPs had a persistently low CD4 count below 200 cells/ml despite treatment. Most patients had disseminated pulmonary tuberculosis in the phase of infiltration/ decay(41.5%). Out of 248 LPs who attended the clinic 39.5% were unaware of their HIV+status; 31.1% were aware of their HIV+status but did not undergo therapy and 29.4% were aware of their HIV+status and were on HAART.

Conclusion: Thus our study revealed that a significant part of patients diagnosed at late stages of HIV infection (43.4%) started HAART with CD4+cells below 200 cells/ml. A fairly large number of patients (39.5%) were unaware of their HIV-positive status before the development of opportunistic infections. Out of 60.5% patients who knew of their HIV+ status, 31.1% did not take antiretroviral drugs. All those facts indicate the need for further strengthening of HIV/TB screening procedures and treatment, as well as a need to improve awareness about HIV-infection among all groups of population.
Female Sex Hormones Included in Contraceptives Induce Replication of HIV-1

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**Purpose:** HIV continues to profoundly affect women worldwide. According to UNAIDS report women represent about half (52%) of all people living with HIV worldwide. The WHO strongly recommends the use of hormonal contraceptives by women at high risk of HIV and women living with HIV, including women taking HAART. However, the data on affect of sex steroid hormones on virus replication and as a result on the disease progression is still controversial. The goal of this work was to study the affect of female hormones included in oral contraceptives (β-estradiol and Progesteron) on HIV-1 replication and efficacy of antiviral drugs.

**Methods & Materials:** Peripheral blood mononuclear cells (PBMC) were infected with HIV-1 subtype A6. Afterwards the cells were cultured for 6 days in the presence of β-estradiol/progesteron with or without the presence of NRTIs Lamivudin(3TC), NNRTIs Etravirin(ETR) and PIs Indinavir(IDV), which are widely used for HIV treatment. Virus production was monitored by p24 levels in culture supernatants on day 6.

**Results:** The low concentrations of β-estradiol(1.1-5.4 μg/ml) inhibited virus production (p24: 1.8 and 3.8 ng/ml correspondently; Virus alone (VA):9.0 ng/ml). The same concentrations of Progesteron did not significantly affect virus replication(p24: 6.7 and 8.0 ng/ml correspondently; VA:9.0 ng/ml). However, the 1.3-1.8-fold increase of virus replication was detected in the presence of high concentrations of both hormones (26-136 μg/ml): p24 β-estradiol - 12 and 16.5 ng/ml; p24 Progesteron - 11.7 and 16.0 ng/ml; p24 VA- 9.0 ng/ml. Incomplete suppression of viral replication was observed when infected PBMC were co-cultivated in the presence of β-estradiol or Progesterone (26, 136 μg/ml) and antiretroviral drugs(2.5 μg/ml). Suppression of viral replication: 3TC+Estradiol – 89,5%; 3TC+progesterone -73%; ETR+Estradiol -86,5%; ETR+Progesteron -76%; IDV+Estradiol -56%; IDV+Progesteron -60,5%. However, in the absence of hormones the virus production was completely (100%) suppressed by those drugs.

**Conclusion:** The high concentrations of steroid hormones induce HIV-1 replication and as a result reduce the efficacy of antiretroviral drugs in vitro. This should be considered when the hormonal contraception is assigned to women with HIV-1. And especially when it is assigned to women with high risk of HIV-infection who is unaware of their HIV positive status and not undergoing HAART.
Impact of the Real Time PCR in the Diagnosis of Acute Bacterial Meningitis in Infants in A Tertiary Health Care Center in India

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Purpose: Detection of pathogens causing acute bacterial meningitis (AMB) in infants by conventional and molecular methods (Real time PCR).

Methods & Materials: 62 Cerebro spinal fluid (CSF) were collected from March, 2015 to December, 2017. All samples were processed in the department of Microbiology, AIIMS, following standard Microbiological diagnostic procedure. Culture of the CSF was performed using Blood agar and Mac Conkey agar. Brain heart infusion broth with 0.5% yeast extract was used for culture of *L. monocytogenes*. Bacterial antigen from CSF was detected by Latex agglutination test (LAT) using Directigen meningitis Kit (BD, USA) and DNA from CSF was extracted by using QIAamp DNA mini Kit (Qiagen). Culture, latex agglutination and Real time PCR were performed to test each sample for *S. agalactiae*, *E. coli* and *L. monocytogenes*.

Results: Overall 62 CSF samples, 28 neonates and 34 Infants were included in this study. All samples were processed by conventional and molecular methods, 17 (27.41%) were positive for the different bacteria. Out of the 17 organisms, *E. coli* 5(29.41%), *S. agalactiae* 2(11.76%), *S. pneumoniae* 8 (47.05%), and *H. influenzae* 2(11.76%) were detected respectively. *S. agalactiae* were positive in neonates (only by Real time PCR). Among the *E. coli* (one was positive by latex, and one was by both LA and culture), but all were positive by Real time PCR. Similarly, three *E. coli* were observed in neonates. However, all *S. pneumoniae* and *H. influenzae* were detected only by Real time PCR in infants. Mean glucose and protein level was (45 and 225/dl) respectively. PMN cell count was high.

Conclusion: Real time PCR has emerged as a powerful molecular tool for the diagnosis of acute bacterial meningitis in infants where there is the need for urgent diagnosis and treatment. In this study, *S. pneumoniae* (47.05%) was the leading organism in infants where as in neonates, *E. coli* (17.64%), was the most frequently identified pathogen followed by *S. agalactiae* (11.76%).
Physicians’ compliance with the National Drug Policy on Malaria in a tertiary teaching hospital, India, from 2010 to 2015: a mixed method study

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Purpose: National drug policies are formulated to encourage rational use of drugs and to reduce drug resistance. This study assessed physicians’ compliance with the National Drug Policy on Malaria at a tertiary care hospital in north India

Methods & Materials: This mixed method study extracted data from adult malaria inpatient records of the hospital from 2010-2015, and assessed drug supply at pharmacies. Physicians’ practices and perspectives were explored by in-depth interviews. Compliance was assessed by severity, type of species and pregnancy status. Thematic analysis was done for the qualitative data.

Results: A total of 247 case files were reviewed. Vivax malaria (41.0%) was more common than falciparum malaria (37.2%). The majority (90.8%) of cases were severe malaria. Overall compliance for use of schizonticidal drug was 73.0% in severe malaria and was only 9.5% in uncomplicated malaria. Compliance for use of gametocidal drug (primaquine) was 15.3%. Schizonticidal drugs were available in all pharmacies except the public one. Primaquine was available in only one. The main themes emerging in the thematic network analysis were physicians’ misconceptions, physician-related factors, and hospital-related and drug access factors.

Conclusion: The degree of compliance for severe malaria treatment was reasonably good but low for radical cure. Raising knowledge and awareness among health care providers, by using written treatment protocols and continuing medical education would improve compliance.
What variables are associated with the public’s willingness to take measures to maintain a hygienic hospital environment?

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Purpose: To date, most of the studies on hospital infections have focused on the perceptions and reported behavior of the medical personnel. This research explore the practices undertaken both by Israeli patients and visitors, in order to maintain or not, a hygienic hospital environment, and to locate the variables that are associated with them.

Methods & Materials: An online survey of national representative sample of Israeli hospital's visitors and patients adult population, who were hospitalized in the five years before the interview (n=209), and who visited patients in hospitals in the three years before the interview (n=454).

Results: Only a minority of patients (24%) comment to medical personnel about maintaining hygiene, while a majority (67%-69%) took active steps to maintain a hygienic environment. The main variables that were found to be associated with patients’ making comments were level of religiousness and gender, whereas priorities, namely whether hospital infections were a high priority, and the frequency of the patient’s visits to hospital outpatient clinics, were associated with self-initiated action.

Conclusion: In order to reduce barriers to commenting to hospital personnel, we propose framing the subject of hospital hygiene as a matter of health literacy, rather than a sole medical issue, and a subject of public discourse that might even contribute to instituting new norms and generating a multi-systemic change of approach.
First report of the role of cockroaches in hospital acquired infections (HAI) in Villavicencio-Colombia

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**Purpose:** Hospital acquired infections (HAI) are of mandatory report to the public health surveillance system in Colombia (SIVIGILA). The presence of cockroaches in hospitals represents a risk since they are considered potential mechanical vectors. The constant report of HAI in health centers in Villavicencio makes necessary to establish their relationship with cockroaches. The objective was to relate the pathogenic bacteria isolated from cockroaches to HAI in a clinic of Villavicencio during the first semester of 2017.

**Methods & Materials:** Cockroaches were caught in the kitchen, maternity ward and archive room of the clinic. Primary individual isolation was done in BHI broth, and then in solid media (blood and MacConkey). Identification and antibiogram were performed by automated method. An active institutional search of the HAI cases at the clinic was made during the first half of 2017.

**Results:** A total of 95 specimens of Blattella germanica (95.79%, 95% CI 89.57-98.84%) and Periplaneta americana (4.21%, CI 95% 1.16-10.43%) were collected, from which 34 bacterium species were isolated, 20 of which were pathogenic. Enterobacter cloacae (17%, IC95% 10.78-27.10%) and Klebsiella pneumoniae (16%, 95% CI 9.94-25.90%) were the most frequent. Enterococcus faecalis presented resistance to ampicillin in 66% of isolates; Citrobacter sp. resistant to cefoxitin in 100% and to meropenem in 14%. 100% of E. cloacae isolates were resistant to cefoxitin, 12% to meropenem, 18% to imipenem and 40% to ertapenem. Klebsiella oxytoca showed 50% of its isolates resistant to ceftriaxone. K. pneumoniae presented 25% of samples resistant to cefoxitin, 7% to ceftriaxone, 31% to meropenem and 40% to ertapenem. The only isolate of Proteus vulgaris was resistant to imipenem and ertapenem, and Shigela dysenteriae to ertapenem. In the clinical records, in the same period, K. pneumoniae, P. aeruginosa and S. aureus had an isolation frequency in patients of 14.7%, 11.7%, and 8.4%, respectively. The major resistance in K. pneumoniae was to ceftazidime.

**Conclusion:** The same bacterium species were caught in cockroaches and patients at the same period time, but it is necessary to carry out molecular studies to confirm the relationship between them. This is the first report of cockroaches carrying bacteria in the eastern part of the country.
**Purpose:** In order to screen for invasive mosquito species and associated viruses, we performed a field survey for mosquitoes in the Black Sea region of Anatolia where Aedes aegypti and Aedes albopictus were previously recorded.

**Methods & Materials:** Mosquitoes were collected from 31 sites in Artvin, Trabzon and Rize provinces during 2016-2017. The specimens were identified morphologically and pooled according to collection site and species. Selected specimens were processed for DNA barcoding via cytochrome oxidase I amplification and sequencing, for confirmation of the morphological species identification. Virus screening was carried out using polymerase chain reaction (PCR) assays targeting alpha and flaviviruses, as well as recently-described novel rhabdovirus Merida-like virus Turkey (MERDLVT), followed by sequencing for characterization.

**Results:** A total of 756 mosquitoes that comprise Ae. albopictus (675, 89.2%), Ae. aegypti (61, 8.1%) and Culex pipiens sensu lato (20, 2.6%) were collected and grouped in 65 pools. No amplification was observed in any pool via generic alphavirus PCR. Generic flavivirus PCR was reactive in 7 and 8 pools collected in 2016 and 2017, respectively. Cell fusing agent virus (CFAV) sequences were characterized in 4 pools (6.1%) of Ae. albopictus (n=2) and Ae. aegypti (n=2), collected in 2016. Aedes flavivirus (AFEV) sequences were characterized in 6 pools (9.2%) of Ae. albopictus (n=5) and and Ae. aegypti (n=1), collected in 2016 (n=3) and 2017 (n=3). Sequences of West Nile virus (WNV) was detected in 5 pools (7.6%) of Ae. albopictus (n=4) and and Cx. pipiens s.l. (n=1), collected in 2017. In phylogenetic analyses, the WNV sequences clustered with local and global lineage 1 clade 1a strains. Moreover, partial L and N gene sequences of MERDLVT were identified in the Cx. pipiens s.l. pool, coinfected with WNV.

**Conclusion:** This is the initial detection of WNV and MERDLVT in field-collected mosquitoes from the Black Sea region. Although no alphavirus sequence could be demonstrated, presence of Ae. albopictus and Ae. aegypti indicates ongoing risk for potential spread.
Purpose: Campylobacter enteritis is rapidly becoming the most commonly recognized cause of bacterial gastroenteritis in man and causes 5–14% of diarrhea, worldwide. High incidence of Campylobacter diarrhoea, as well as its duration and possible sequelae, make it important from a socio-economic perspective. Diagnoses of Campylobacter infections are challenging because the organism is difficult to isolate, grow and identify. Guidelines for Clinical and laboratory diagnosis are inadequate.

Methods & Materials: A Hospital based Cross sectional descriptive study conducted in JSS tertiary care hospital, Mysuru. Study included 55 stool samples of children aged between 1 to 60 months who presented to Department of Pediatrics, JSS hospital, both as in and out patients with complaints of diarrhea and dysentery. All stool samples were inoculated on to selective and non-selective media with filtration using a 0.45 μm membrane filters and incubated in microaerophilic conditions using the candle jar at temperatures 37°C and 42°C. The culture isolates were confirmed by standard phenotypic tests. A simplex polymerase chain reaction (PCR) with universal Campylobacter primers and primers specific for C.jejuni and C.coli, was performed on the DNA extracted from the stool filtrates.

Results: Campylobacter was isolated in 5(9.1%) out of 55 stool samples using culture and the isolate were confirmed to be Campylobacter jejuni by phenotypic tests. Campylobacter Genus level PCR was positive for 10 samples (18.2%), rest of the samples were negative. The positive samples were subjected to species level PCR and all were positive for C.jejuni (100%) and negative for C.coli (0%).

Conclusion: The implication of this study is that culture is less sensitive for use in diagnosis of Campylobacter infection in our settings. Nucleic acid based diagnostics offer increased sensitivity, can determine both the presence and burden of infection, and can distinguish between Campylobacter infections at the species level. We therefore recommend PCR, if feasible, as the preferred diagnostic modality for detection of Campylobacter infection. However, the role of culture in the diagnosis of Campylobacter infections is that it allows precise identification of bacteria and testing of their susceptibilities, which are integral to management of patients.
Outbreak Investigation of Acute Watery Diarrhea (AWD) Due To Blockage Of Drain, Contamination Of Cracked Water Tank In Sector I-9/4, Islamabad Pakistan, August, 2016

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Purpose: On 26th July 2016, large number of AWD cases were reported from sector I-9/4 Islamabad. Outbreak was investigated with objectives to identify probable risk factors and formulate recommendations for control. Sector I-9/4 is an urban residential area (population 16,463) and mainly inhabited by lower middle socioeconomic class.

Methods & Materials: Case was defined as any resident of sector I-9/4 Islamabad having > 3 loose stools within 24 hours with one of the following sign/symptoms; fever, nausea, vomiting and abdominal cramps between 6th July - 5th August 2016. Conducted 1:2 matched case control study by using a structured questionnaire. Cases were identified by door to door survey. Age and gender matched controls were selected by neighborhoods. Two stool samples from symptomatic cases were collected. Drinking water samples were collected from underground water tank/kitchen of a case for analysis.

Results: Total 508 residential flats were surveyed and 46 cases were identified. Mean age was 21.43 years (range 1-65 years). Most affected age group was 45-54 years (attack rate 1.08%) with an overall attack rate of 0.28%. Cases were more likely to have consumed public tap water for drinking (Adjusted OR 6.6 CI 5.2-25.4, p < 0.002) compared to non-cases. Residents using boiled water (OR 0.32, CI 0.15-0.69, p<0.003) and water from nearby filtration plant (OR 0.086, CI 0.037-0.20, p<0.000) for drinking remained protected from the illness. Out of two stool samples tested 01 was positive for Vibrio cholera (Poly o1 Serotype Inaba biotype E/Tor). Drinking water samples were also found contaminated with fecal coliform (>240 CFU/100ml).

Conclusion: Contamination of the underground water tank due to leakages from the nearby sewerage system was the most probable cause of this outbreak. Repair of sewerage system and protective covering of the underground water tanks was recommended.
Purpose: Pregnant women represent 60% of all cases of *Listeria monocytogenes* (LM) infection in population younger than 40 years. If the infection remains unrecognized, listeriosis may result in interruption of pregnancy or premature abortions. It is considered that 22% of perinatal infections end with neonatal death or stillbirth. In neonates, mortality rate approaches 50% and it is increased if there is present early neonatal sepsis. Research indicate that fetal survival is higher if the pregnancy progress is farther away. Late onset listeriosis in pregnant women occurs particulary as newborn meningitis in 2 - 4 weeks after delivery. Purpose of this investigation was to find out number of all isolates of LM in human and non- human samples and compare it with isolates connected with clinical significance in pregnant women and newborn.

Methods & Materials: The Croatian National Institute of Public Health (CNIHP) conducted diagnostic in human and non- human samples received from several different medical institution across the Republic of Croatia by serological testing, cultivation and automated identification.

Results: In the period from 2014 to 2017. god. 103 samples were received for serological testing, mostly from pregnant women. Also, CNIPH received 5 LM isolates to confirm, previously diagnosed in samples of newborns. In this period, listeriosis was not confirmed by cultivation nor in one sample obtained from the urogenital system of pregnant women. In accordance with ISO 11290-2: 1999/A1: 2008. - out of 2817 food samples, LM was confirmed in 3 samples only.

Conclusion: During pregnancy risk of listeriosis infection is 20x higher than in the average population. Moreover, pregnant women may be asymptomatic or present nonspecific clinical symptoms (e.g. flu-like symptoms).

According to our results, there were low number of LM isolates among from all tested samples in CIPH in three years period. Because of clinical significance of LM infection, and threats for human health, especially for pregnant women and newborns, our obligation is to better monitor LM isolates in humans and all other samples including food, in order to avoid serious complications of LM infection in pregnancy.

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Purpose: Blood borne pathogens in pregnant women such as HIV, Treponema pallidum, Hepatitis viruses and Toxoplasma gondii are transmissible to the unborn babies and results in adverse pregnancy outcomes. Screening for HIV, Hepatitis and Syphilis are part of routine antenatal care in Nigeria while screening for other relevant transmissible diseases is not available to majority of pregnant women. Vertical transmission of these diseases can cause congenital infections. We carried out the study to determine the prevalence and risk factors of Toxoplasma gondii among pregnant women in Ado Local Government Area of Ekiti state.

Methods & Materials: This is a descriptive study involving 340 pregnant women attending ante-natal centre and 4 maternity homes (Traditional Birth Attendants/Faith-Based Birth Attendants) in Ado LGA, Ekiti State. Data was collected using semi-structured interviewer administered questionnaire and blood samples from enrollees were screened for T. gondii using enzyme linked immunosorbent assay (ELISA). Descriptive, bivariate and multivariate analysis of the data obtained was carried out and level of significant set at 5%.

Results: Data analysis was carried out on 337 respondents. Mean age was 29.6 years, 108 (53%) were employed, 274 (81.3%) Christian and 251 (74.5%) had lived in Ado LGA for more than 2 years. Only 23 (6.8%) have heard of toxoplasmosis. Twenty-eight (8.3%) were tested positive for T. gondii. Cat ownership (AOR 16.5, 95% CI. 3.7 - 72.4) and eating unwashed fruits (AOR 5.54., 95% CI. 1.79 - 17.21) were major predictors of T. gondii infection.

Conclusion: The prevalence T. gondii was high among pregnant women in this study population. The exposure of pregnant women to some risk factors underscores the need to intensify effort at providing health education to women of reproductive age on prevention T. gondii infections and inclusion of T. gondii screening as part of routine test for pregnant women in Ekiti State.
Purpose: Encephalitis and meningitis (EM) are severe infections of the central nervous system associated with high morbidity and mortality. In Kazakhstan, 33% to 77% of hospitalized EM cases are of unknown etiology. While cases with clinical symptoms of EM undergo routine culture for bacterial pathogens including *Neisseria meningitides*, PCR has been infrequently used to identify viruses or bacteria missed by culture.

Methods & Materials: From February 2017 to February 2018, we conducted hospital-based syndromic surveillance for EM at the Shymkent city hospital and 14 district hospitals in South Kazakhstan Region. All consenting inpatients meeting the standard case definition for EM, as defined by the Brightborn Collaboration at level 3 of diagnostic certainty, were enrolled. A verbal questionnaire was administered and samples of cerebral spinal fluid and blood were collected. Specimens were tested using routine culture as well as real-time PCR to test for both bacteria and viruses. Data were stored and analyzed using Epi Info.

Results: In PCR and culture testing of blood and CSF specimens from 549 enrolled cases, 492 (89.6%, 95% CI: 81.6% - 99.3%) were associated with a viral agent, 33 (6.0%, 95% CI: 2.8% - 14.6%) with a bacterial agent, and an additional 8 (1.4%, 95% CI: 0.2% - 6.7%) with both. Twelve cases (2.2%, 95% CI 1.2% - 3.9%) were of unknown etiology and four (0.7%, 95%CI: 0.2% - 2.0%) were suspect rabies cases based on clinical presentation and death. Enteroviruses were identified in 82.3% (n=405) of cases with potential viral etiology, and *N. meningitidis* was identified in 51.2% (n=21) of cases with potential bacterial and mixed etiologies. Incidence rate per 100,000 population for Enteroviral EM and meningococcal meningitis was 14.5 and 0.7, respectively. These rates are 2.8 and 1.7 times higher, respectively, than previously reported in Kazakhstan. Antibiotics were used in 97% (n=477) of patients who had potential viral-only etiology.

Conclusion: Incorporation of PCR into routine investigation of EM can help avoid missed outbreak detection, ensure incidence rates are not underestimated, and avoid unnecessary antibiotic treatment.

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Purpose: Sexually transmitted diseases (STDs) are epidemics and causes enormous health and economic consequences in developing countries. HIV, Syphilis and other STDs are transmissible to the unborn babies and results in adverse pregnancy outcomes. This study aimed to determine the prevalence and risk factors of HIV and Syphilis among pregnant women in Ado Local Government Area.

Methods & Materials: A cross-sectional study was conducted between February and May, 2017 among 340 pregnant women attending ANC and maternity homes. Data was collected using semi-structured interviewer administered questionnaire and blood samples from enrollees were screened for HIV and Syphilis using enzyme linked immunosorbent assay. Descriptive, bivariate and multivariate analyses were done and level of significant set at 5%.

Results: Data analysis was carried out on 337 respondents. Mean age was 29.6 years, 108 (53%) were employed, 274 (81.3%) Christian and 251 (74.5%) had lived in Ado LGA for more than 2 years. Majority, 328 (97.3%) and 204 (60%) have heard of HIV/AIDS and syphilis respectively. Eleven (3.3%), 8 (2.4%) tested positive for HIV and Syphilis respectively. Both HIV and Syphilis were found in 1(0.3%) respondent. Predictors of HIV infection includes multiple sex partners in the last one-year (AOR 13.3, 95% CI. 2.6 - 69.8), giving or receiving money or goods in exchange for sex (AOR 5.5, 95%CI. 1.0 - 31.0). Predictors of syphilis infection are forced sexual intercourse in the past 1 year (AOR 29.0, 95% CI. 3.4 - 244.1) and new sexual partner in the past 6 months (AOR 10.2, 95% CI. 1.5 - 68.1)

Conclusion: The prevalence of HIV (3.3%) and Syphilis (2.4%) was high in this study population. The exposure of pregnant women to some risk factors underscores the need to intensify effort at providing health education to women of reproductive age on prevention of HIV, Syphilis and other sexually transmitted diseases.
The first case of autochthonous Echinococcus multilocularis in human in the Republic of Kalmykia

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Purpose: Two recognized species of Echinococcus, have special medical importance - E. granulosus and E. multilocularis - causing cystic echinococcosis (CE) and alveolar echinococcosis (AE) in humans. Due to the developed pasture livestock farming, the prevalence of the rural population in the territory of the Republic of Kalmykia, cases of CE are recorded annually. However, no cases of AE have been observed in this area. Purpose to describe the first case of AE in the territory of the south of Russia

Methods & Materials: Case of AE was described in 46-year-old patient, who contracted disease in non-endemic area

Results: In September 2016, a 46-year-old patient, a resident of the rural area of Kalmykia, applied to the surgical clinic of Rostov Medical University. The patient complained of the appearance of large tumor in the right side of the abdomen. With tomography, a lumpy tumor of the right lobe of the liver was found, 18x20 cm in size, with sharp uneven contours, and an inhomogeneous density. Condition of health in patient was relatively satisfactory. Markers of pathological changes in the blood tests were absent. The level of AAt exceeded the normal values by 3 times. Patient was examined with an enzyme-linked immunosorbent assay (ELISA) and antibodies G against E. Granulosus were discovered (coefficient of positivity 1.5). A Western Blot IgG reaction detected antigen fractions 26-28 kDa specific for E. multilocularis. The tumor was removed in the surgical clinic of Rostov Medical University. The patient underwent liver resection. Alveolar echinococcosis was verified by histological examination of the tumor. The patient received chemotherapy with albendazole 800 mg per day per day in 2 doses with an interval of 12 hours continuous course during 9 months. The patient was examined in March 2018, signs of recurrence of alveococcosis were not revealed.

Conclusion: The appearance of alveolar echinococcosis in areas where it was not previously recorded in human is also noted by some European scientists. Thus, it is necessary to improve the serological methods for diagnosis AE.
Purpose: Hand-foot-and-mouth Disease (HFMD) surveillance began in the Philippines in 2012 with the National Reference Laboratory for Polio and other Enterovirus providing laboratory support for the surveillance. The Department of Health defined HFMD as any individual, who developed acute febrile illness with papulovesicular or maculopapular rash on palms and soles, with or without vesicular lesion in the mouth. Much has been known about the epidemiology of Enterovirus 71 (EV71) in HFMD, however, data in the Philippines is yet to be established. Previous enterovirus study in the country only focused among acute flaccid paralysis (AFP) and environmental samples.

Methods & Materials: From 2012 to 2017, 3,021 samples from 17 regions of the Philippines were tested using a conventional, hemi-nested, pan-enterovirus RT-PCR targeting the 5' untranslated region (5' UTR) to screen for the presence of enteroviruses and subsequently tested for EV71 targeting the VP1 region. In 2014, Coxsackievirus A6 (CA6) and Coxsackievirus A16 (CA16) were included assay targeting the VP1 region. To determine the presence of CA6 and CA16 circulating in 2012-2013, representative samples were screened retrospectively. All samples positive for EV71 were sequenced spanning the complete VP1 sequence (981 bp) and phylogenetic analysis was done to identify subgenogroups.

Results: Majority of the referred samples tested positive for enterovirus, 74% (2232/3021). Of these, 76.7% were identified as CA6 (1101/1436), 8.9% are CA16 (128/1436) and 3% are EV71 (62/2232). An increasing trend in CA6 has been observed, from 62% in 2012 to 88% in 2017 while a gradual decrease in CA16 was noted, from 15% of cases in 2012 to 0.9% in 2017.

Conclusion: CA6 is the most prevalent enterovirus causing HFMD in the Philippines from 2012 to 2017. Phylogenetic analysis showed that EV71 isolates in HFMD clustered with the C2 subgenotype and are genetically homologous to the EV71 detected in the country between 2000-2010 from AFP and environmental samples. EV71 used to be the major cause of HFMD worldwide, however, our study suggests that CA6 has been the most common circulating enterovirus among Filipino HFMD cases since 2012. This supports an etiologic shift for HFMD as seen in the Asia Pacific region.
Assessment of KAP Regarding HIV Testing Among Military Personnel in Omdurman military area
2017 - Sudan

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Purpose: According to WHO and UNAIDS estimates, the prevalence rate of HIV infection in Sudan is 25% in 2016. The AIDS epidemic in Sudan is concentrated among the most vulnerable groups (women who have sex for money and men have sex with men).

Methods & Materials: It is descriptive cross-sectional community based study was conducted among active duty service military personnel. Its objective was to establish baseline behavioral, knowledge and intervention exposure data in relation to HIV and AIDS among military personnel. A sample size of 340 military personnel in the Omdurman Military Area was determined using statistical formula. Sociodemographic data as well as information related to sexual behavior were collected.

Results: Regarding gender 100% are males, all are Muslims and have been circumcised. About 34.1% of the age group 18-24 years, 54.1% of the sample aged 25-49 years and 11.8% were more than 50 years. Regarding education 56.2% basic school, 32% high school and 11.8% illiterate. About 75% were married and 25% unmarried. The respondents were highly knowledgeable of HIV/AIDS, 100% heard about the disease, the main channel was the lectures presented by the health workers (45%). Only 35% know the symptoms and signs, and 18% know the signs of the syndrome. Modes of transmission 76.4% sexual intercourse, 36.8% blood transfusion, 37.9% skin penetration. Regarding sexual behavior, 96.5% reported their first sexual experience between 20 and 30 years. The majority 94.7% said that marriage offered protection, 72.6% said abstinence and only 7.8% said use of condom. As regards wrong believes 54.7% said the HIV virus can be transmitted by mosquito, about 51.7% by sharing food with an infected person and 49.4% said that a healthy looking person cannot be infected with HIV. All were voluntary test and the results 100% negative.

Conclusion: Findings showed that even though the respondent’s knowledge about HIV was good but also there were some wrong believes. The study showed that circumcision, religion, marital status, education level are significant predictors for HIV infections control and prevention. These findings should be considered in any intervention strategy in the country.
Inactivation of DNA- and RNA-viruses with Millisecond Technology

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Purpose: The inactivation of viruses in biological fluids especially in plasma is highly required but complicated. But the risk to be infected with dangerous viruses (hepatitis B virus, HIV, arborviruses) should not be neglected.

The objective. To study the effectiveness of millisecond technology (MST) for inactivation of various types of viruses. Earlier we showed that poliovirus and herpes simplex virus (HSV) could be inactivated with this new method. But it is not known how other viruses will be inactivated and what regimes are required.

Methods & Materials: DNA-virus- HSV, type 1, and RNA-viruses( poliovirus, hepatitis C virus(HVC), HIV) obtained from the State virus Collection were used in the study. The media (Eagle MEM, RPMI1640) or donor’s plasma were contaminated with virus and treated in the unit MST “BioPharma”. The principle of the method: microdroplets (50-150 microns) were obtained by spraying the virus-contaminated liquid into the reactor through a nozzle under nitrogen gas pressure. The microdroplets were treated with low heat/pressure (millisecond) changes of the pressure (P-12bar) and temperature (To°C in inner chamber varied from 60° to 52°). The samples of virus-contaminated media or plasma were taken before and after the treatment, and were tested for the infectious virus.

Results: The Eagle MEM media or donor’s plasma was contaminated with poliovirus (6.3 lgTCID50) and treated in the unit “2016Pharma”. No infectious virus was detected at 58°C - 56°C, at 52°C reduction rate (RR) was only 1.5 lg TCID50. RR of infectious HVC (enveloped RNA-virus) at T=58°C (100%) was 6.5 lg TCID50, 4.0 lg TCID50 at T=56°C and 1.5 lg TCID50 at T=52°C. HIV was studied at T=60°C to 52°C. RR at 60°C was 4.0 lgTCID50 (100%) but at T=58°C was 1.5 and no inhibition of HIV virus was observed at T=52°C. For HSV RR was 6.0 lg TCID50 at 58°C, 4.0 lg TCID50 (56°C) and only 1.5 lg TCID50.

Conclusion: It is confirmed that millisecond technology can be applied for inactivation of infectious viruses basically within the same range of the temperature – 60-56°C). But HIV is more resistant then others (60°C).
the Epidemiology of Norovirus and Rotavirus in the Middle East and North Africa (MENA) Region

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Purpose: Rotavirus (RV) & Norovirus (NoV) are the first and the second leading causes of severe acute gastroenteritis (AGE) worldwide, respectively. To our knowledge, there are no systematic reviews assessing the role of NoV in AGE in the Middle East and North Africa (MENA) region and the last update on the epidemiology of RV in the MENA region was performed in 2009. Consequently, we conducted an extensive systematic literature review on articles studying RV and NoV in the 24 countries of the MENA region between 2000 and 2015.

Methods & Materials: The methods and reporting were set according to the 2015 PRISMA-P and based on the elements from the international prospective register of systematic reviews (PROSPERO). To ensure the comprehensiveness and completeness of the search, six electronic bibliographic databases were used. The Global Health Library (GHL) was used to retrieve studies published on the regional databases.

Results: We retrieved 38 and 169 studies for NoV and RV, respectively, meeting our predefined inclusion criteria. Studies on NoV and RV were conducted in 15 and 19 out of the 24 countries of the region, respectively. The reported NoV infection rates ranged between 0.82% and 36.84% while RV rates ranged between 0.6% and 76%. The majority of studies were clinical observational studies assessing RV or NoV rates mainly among children. Participants were recruited from in- and outpatient clinics. These infections were reported all year round with peaks observed mainly during cold months. GII.4 was the predominant genotype detected in stool of participants studies (64%). The predominantly reported RV genotype in the region was G1[P8] followed by G2[P4] and G9[P8].

Conclusion: Overall, there is an increasing recognition of NoV as an important causative agent of AGE across all age groups in the MENA region. Further studies are needed to assess the national and the regional burden of NoV among different age groups, its molecular diversity and seasonal variability. Moreover, our review draws attention to the major gaps existing in the continuous monitoring and surveillance of RV and provides additional support to evaluate the implementation of using rotavirus vaccines in the region.
Surveillance Of Prion Diseases In The State Of São Paulo: An Evaluation Of The Information System And Epidemiology Of Creutzfeldt-Jakob Disease

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Purpose: Prion diseases are a group of neurological disorders caused by prion and can occur sporadically, genetically, iatrogenic or foodborne. The objectives of the study were to describe and evaluate the epidemiological surveillance of prion diseases in the State of São Paulo and to epidemiologically describe the cases of Creutzfeldt Jakob disease (CJD) from 2012 to 2016.

Methods & Materials: The data source used was the division of foodborne and waterborne diseases database. The methodology used was proposed by Centers for Disease Control and Prevention in 2001, Updated Guidelines for Evaluating Public Health Surveillance Systems and the qualitative, quantitative and utility attributes of the system were evaluated, for the descriptive study were measured frequency and central tendency. The data were analyzed in Epi Info.

Results: In the evaluation of the surveillance system, the same was inflexible with regard to the insertion of the notification form in the SINAN (Information System of Notifiable Diseases). The information system does not have duplications, generally with good completeness. It is noteworthy that the field of evolution, of great importance for the closure of the case, presented low completeness. The system was classified as sensitive and useful. Were classified 80 cases of CJD. The median age was 63 years and female represented 55% of the cases. All have progressive dementia, the most signs and symptoms were myoclonus (72.5%) and psychiatric disorders (57.5%). Of the nonspecific exams, 65.7% of patients with normal MRI with 37.1% of typical EEGs and 76.7% with 14.3.3 protein present. In 68% of the cases that performed the genetic analysis, the mutation was more frequent in the E200K codon. According to the final diagnosis, 74 (92.5%) cases were classified for sporadic CJD with evolution of 126 days for died and six (7.5%) as familial CJD, with a history of around 261 days.

Conclusion: The elaboration and insertion of a notification sheet of prion diseases in the SINAN, with clinical, laboratory and epidemiological data for the classification of the forms of the disease and follow-up of its evolution. Retrospective studies, with review of medical records of patients with similar clinical pictures would be important to capture more cases.
The Impact of Anemia in Prolong Culture Conversion amongs MDR-TB Patients in Persahabatan Hospital Indonesia

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**Purpose:** Anemia was known to be the complication of Tuberculosis (TB). Low hemoglobin concentration was associated with prolonged time of culture sputum conversion in TB but the association in MDR TB is still unknown. Sputum culture conversion in MDR-TB was the main predictor of successful therapy outcome. This study aims to understand whether anemia amongs MDR-TB patients could prolong the time for sputum conversion.

**Methods & Materials:** The method of this study was cohort retrospective, with total 363 patients from MDR-TB patients treated in Persahabatan Hospital from January until December 2016. The time of culture conversion was obtained from Indonesian online database e-TB Manager under supervision of Persahabatan Hospital authorized staffs. Anemia level was further analyzed into 3 categories of anemic status (anemia and non anemia), anemic type (normocytic normochrom and microcytic hypochrome), and anemic classification (mild, moderate, severe) along with their correlation towards the length of culture conversion using the survival analysis rate Kaplan-Meier curve.

**Results:** Of the total 363 medical records, only 201 datas fitted into inclusion criteria in which 83 of 201 MDR-TB patients (41.3%) have anemia with the average of age 38.3 ± 12.9 and the average of Haemoglobin level 11.4 ± 1.2. The length of culture conversion time in both anemia and non-anemia patients were mostly occurred in 2nd month of treatment with 31 (37%) and 41 (34.8%) incidence respectively. Survival analysis rate showed a significant rate difference in prolong conversion time based on the anemic status (anemia and non-anemia). However, there is no significant relation of classification and types of anemia towards the prolong conversion time. In this study we also evaluated BMI level since it could be confounding factor. The result showed a consistant relation with previous study in this setting.

**Conclusion:** Anemia increased the risk of prolonged time in culture conversion of MDR-TB patients. There should be an effort in improving the nutritional status and hematological profile in MDR-TB patients with anemia.
21.001
Chagas Disease Is Here To Stay. Seroprevalence Of Trypanosoma cruzi At A General Hospital In Madrid-Spain (Non-Endemic Area).

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**Purpose:** Chagas Disease (CD) occurs principally in the continental part of Latin America; however it has been increasingly detected in European countries. The disease is prevalent in Latin American migrants living in Europe although the classical route of transmission does not occur. The other less common transmission routes that have been demonstrated in Europe are blood transfusion, transplantation and vertically from mother-to-child. A retrospective observational study was conducted from 2011 to 2017 at Microbiology Department in our Hospital. The aim of the study is to assess the seroprevalence of CD in a non-endemic area (Latin American population attended in this area) in order to estimate risk groups and to promote an effective screening.

**Methods & Materials:** Serological screening was performed through two different methods (WHO recommendations): chemiluminiscent microparticle immunoassay (Architect-Abbott®) and then in positives, immunofluoresce assay (VIRCELL®). Samples with discrepant results were evaluated by molecular methods in newborns and pregnant women.

**Results:** A total of 3420 serum samples corresponding to 2760 patients were screened for CD. Out of these patients, 5.4% (150) were positive. Among positive cases the majority were females (76%), diagnosed during a prenatal serological screening, representing the half of the positive results. Diagnosis request was made from Primary Care Centers (including some prenatal screenings) in 39% of cases.

The distribution by age rates was the following: adults under 35 were 59 (39,3%), adults above 35 were 79 (52,6%), the 8% (12) were children: 8 out of them were diagnosed at birth, but only 2 were confirmed with PCR. Country of origin distribution was the following: Bolivia (90,6%), Ecuador (6%), Paraguay (2%) and Brazil (1,3%).

**Conclusion:** - Around us there is an awareness of a necessity of an early diagnosis of this disease especially in Latin-American pregnant women and their children in primary care as in specialist healthcare.
- Most of Chagas disease patients were from Bolivia, however the screening must be performed to all Latin Americans migrants despite of a low prevalence.
- The protocol applied in our center meets the WHO criteria.
Purpose: Lyme borreliosis is a multisystem infection caused by the spirochete Borrelia burgdorferi. A disease can affect every organ, and it is characterized by a wide range of clinical manifestations. Erythema migrans is the basic clinical marker of Lyme disease in acute or subacute form. Recurrences occur in 9-30% of inadequately treated patients. The aim of this paper was to investigate the frequency and clinical forms of skin borreliosis on our material, with the presentation of four atypical cases and to draw attention to the importance of clinical recognition of the disease.

Methods & Materials: A retrospective study was carried out on monitored and outpatient patient’s data, seen in the infectologist office during five-year period from 15.03.2013-15.03.2018.

Results: During investigated period, 958 patients were examined in infectologist office. Of this number, 7.6% were with symptoms of borreliosis. 62 patients were presented with multi-organ chronic symptomatology. 21.7% of the patients had skin changes and 39.7% of the patients with multi-organ changes had skin affected. The diagnosis of the disease was based on anamnestic-epidemiological data, clinical picture, disease flow, serological findings and on the basis of the response to ex juvantibus therapy. The average age of affected patients, only with skin borreliosis was 35.42 g. The youngest patient was 2.3 years old, while oldest was 72 years old. 12 patients had subacute course. The average time from the onset of the disease manifestation to the establishment of the diagnosis and the onset of therapy was 6.8 weeks. The chronic flow of skin borreliosis was found in 5 patients.

Conclusion: Erythema migrans was diagnosed at 23% patients, most often in atypical form. Average diagnosis time is 6.4 weeks. In the multi-organic symptomatology of chronic flow, atypical skin changes were represented in 54% of cases. Diagnosis of chronic forms of skin borreliosis is severe in the absence of positive epidemiological data. Inadequate length of therapy for first stage illness, re-bite ticks, and stress are the main causes of recurrence of borreliosis.
Intercurrent Flaviviral Viremia and *Plasmodium ovale* Infection in Ill Returned Travelers to Ontario

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**Purpose:** Similar epidemiology and clinical presentations of arboviral infections and malaria along with the typically sequential approach to diagnostic testing, with malaria excluded urgently in febrile returned travelers, may mask the true epidemiology of such dual infections. Flaviviruses have been reported to trigger relapsing forms of malaria, including *Plasmodium ovale*, long after primary malarial infection, and this may delay the diagnosis of malaria. We aim to understand the incidence of intercurrent flaviviral infection in cases of confirmed *Plasmodium ovale* infection, where the flavivirus may have triggered relapse.

**Methods & Materials:** DNA and RNA from biobanked isolates of *P. ovale* detected in whole blood at the Public Health Ontario Laboratory between 2006 and 2018 will be extracted and screened for intercurrent flaviviral infections using previously validated real-time PCR (qPCR) assays targeting multiple flaviviruses (pan-FLAV) and specifically dengue virus types 1-4 (DEN1, DEN2, DEN3, DEN4).

**Results:** One-hundred fifteen unique isolates of *P. ovale* were identified over the reporting period, of which 62 had sufficient specimen for further molecular analysis. Among analyzed cases, over half occurred in males (36/62 [58%]), over a third occurred in females (23/36 [37%]), and 5% (3/62) had unassigned sex. Median age of *P. ovale* cases was 28.8 years (range 22 mos - 72 years; IQR 18.8 – 40.1 years). Median parasitemia was < 0.01% (range < 0.01% - 0.8%). Thirty (48%) *P. ovale* cases had documented travel history exclusively to Africa, with Nigeria as the most common source country (22/54 [40.1%]). Pan-FLAV assay yielded a 1.6% (1/62) positivity rate. DEN1-4 assay results are pending.

**Conclusion:** *P. ovale* infections are most commonly imported to Ontario from West Africa, and Nigeria, specifically. Intercurrent flaviviral viremia was noted in at least 1.6%, which may suggest that primary flaviviral infection triggered a relapse of *P. ovale*. Alternatively, such co-occurrence may suggest primary infection with both organisms known to cause fever in returning travelers. Consideration of flaviviral co-infection should be given to the *P. ovale* patient with deep thrombocytopenia, lymphopenia, and high-yield arboviral symptomatology such as rash and retro-orbital headache. The influence of flaviviruses on the clinical course of *P. ovale* should be examined prospectively.
Epidemiological Update on Fever in Returning Travelers to Toronto from the ‘Rapid Assessment of Febrile Travelers’ (RAFT) Programme

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Purpose: Fever in returning travelers may indicate serious and life-threatening infections, such as malaria. However, most such cases are due to more benign, self-limited etiologies such as traveler's diarrhea or viral URIs. In the absence of a priori predictors of which febrile travelers will develop severe clinical sequelae from their imported infection, which remains undifferentiated pending confirmatory diagnostics, close follow-up and monitoring of travelers during the initial few days of illness is required. The rapid assessment of febrile travelers (RAFT) programme was implemented to standardize the evaluation and disposition of febrile returned travelers in Toronto. We herein provide an epidemiological update on travelers assessed via RAFT from 2016 to 2017, and the illnesses with which they returned from travel.

Methods & Materials: Criteria for RAFT referral include: presentation to participating EDs, reported fever, and travel outside of Canada within the past year. Exclusion criteria include Plasmodium falciparum malaria, and fulfillment of admission criteria such as unstable vital signs or significant laboratory derangements. Demographic, clinical, and travel-related data were collected, and analyzed using descriptive statistics.

Results: Between January 2016 to December 2017, 302 ill returned travelers were evaluated in the RAFT clinic, 49% of whom were men (n=147), while 51% were women (n=155). Median age was 34 years (range 16–93 years). Travelers returned from a total of 82 countries with the most represented countries being: India (n=25, 8%), Mexico (n=21, 7%), Thailand (n=18, 6%), Cuba (n=17, 6%), and Costa Rica (n=13, 4%). Common diagnoses included: viral syndrome (n=78, 26%), traveler’s diarrhea (n=34, 11%), viral respiratory tract infection (n=27, 9%), dengue (n=16, 5%), laboratory-confirmed influenza (n=16, 5%), and typhoid fever (n=10, 3%). Among lab-confirmed influenza cases, off-season transmission occurred in 1 febrile returned traveler each from Guatemala, Israel, Zimbabwe, and Saudi Arabia, accounting for a quarter of influenza cases evaluated in RAFT. Cases of Zika virus (n=6, 2%) only occurred in the RAFT population in 2016.

Conclusion: Understanding the range of illnesses imported by febrile returned travelers, and in particular, the number of off-season influenza cases, will inform pre-travel counseling and both clinical and laboratory algorithmic approaches to care of such travelers.
21.005
Low Sequence Heterogeneity of *Plasmodium falciparum* Isolates Imported to Ontario, Canada from West Africa over a 10-year Period with Increased Molecular Markers of Resistance to Proguanil

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**Purpose:** In the province of Ontario, Canada, approximately 200 cases of malaria are imported annually by returning travellers, with *Plasmodium falciparum* (Pf) being the major causative species from West Africa. We performed sequencing analyses of Pf isolates from Ghana and Cameroon over a 10-year period to understand patterns of genetic heterogeneity and molecular drug resistance markers over time.

**Methods & Materials:** We identified 52 relevant Pf isolates: 36 from Ghana (18 from 2006-2008 and 18 from 2014-2016); and 16 from Cameroon throughout 2006-2016. DNA was extracted and Sanger Sequencing was performed for regions commonly used for strain typing: merozoite surface protein (msp) 1 and 2; erythrocyte binding antigen (eba) 175; and glutamate-rich protein (glurp) regions. Sequence assembly and phylogenetic analysis were conducted. Molecular markers, cytochrome B (cytB) and dihydrofolate reductase (dhfr) conferring resistance to atovaquone-proguanil (Malarone®); atpase6 to artemisinin and derivatives; chloroquine resistance transporter (Pfcrt) to chloroquine were analyzed by Pyrosequencing.

**Results:** Pairwise and phylogenetic tree analysis revealed some sequence heterogeneity within Ghana and Cameroon isolates, however, there was no clustering of samples over-time. With regards to molecular resistance markers, all isolates were wild type on cytB codon 268. Isolates from Cameroon all had triple codon 51,59 and 108 mutant on the dhfr gene conferring resistance to proguanil whereas isolates from Ghana had an increase from 39% (7/18) in 2006-2008 to 83% (15/18) in 2014-2016 (p=0.0153). In the atpase6 gene, 8% (3/36) of isolates (2 from 2006-2008 and 1 from 2014-2016) from Ghana had mutation in codon 623 while all Cameroon isolates were wild type. No mutant was observed in atpase6 codon 769. In Pfcrt codon 76, 27% (7/26) of Ghanian isolates were mutant with no difference in the frequency over-time (p=0.1783), whereas 50% (6/12) of Cameroonian isolates were mutant in this codon.

**Conclusion:** Pf isolates from Cameroon and Ghana demonstrated dominant and increasing molecular markers of resistance to proguanil, but remain wild type to the partner drug atovaquone in Malarone®. Relatively high percentage of molecular mutants to chloroquine resistance still predominant throughout West Africa. The low sequence heterogeneity suggest there was no major evolutionary genetic changes over the years.
Malaria in Canadian VFRs and Migrants: Surveillance Report from CanTravNet, April 2013 — March 2018

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Purpose: Background: Malaria continues to be a top travel-acquired cause of morbidity among ill returning VFRs and migrants to Canada. We examined the demographic and travel correlates of Canadian VFRs and migrants with malaria over a 5-year period to illuminate the characteristics of this disease in our traveling population.

Methods & Materials: Methods: Data on returned VFR travelers and migrants presenting to a Canadian GeoSentinel Surveillance network (CanTravNet) site between April 2013 and March 2018 who were diagnosed with malaria were analyzed.

Results: Results: Of 4434 VFR travelers and migrants in the CanTravNet database over the enrolment period, 308 (6.9%) were diagnosed with malaria, representing 3.4% of migrants (84/2478) and 11.5% of VFR travelers (224/1956), and, collectively, 64% (308/484) of all malaria cases reported. Median age of VFR travelers and migrants was 32 years (range 1-83 years; IQR 19-47 years), with males accounting for 57.8% of cases (n=178), and females 42.2% (n=130). Among VFR travelers and migrants with malaria, 27% (n=84) traveled for migration while 73% (n=224) traveled to VFR. Nigeria was the most common source country, accounting for 57 cases (18.5%), followed by Cameroon (n=32, 10.4%), DRC (n=23, 7.5%), Cote d’Ivoire (n=22, 7.1%), Ghana (n=19, 6.2%), and India (n=19, 6.2%). Plasmodium falciparum was the most well represented species amongst malaria cases in migrants (n=45, 54%) and VFRs (n=169, 75%), followed by P. vivax (n=17 [20%] in migrants, and n=19 [8%] in VFRs). P. ovale accounted for 8% of total cases (n=26), with 12 cases (14%) in migrants, and 14 (6%) in VFRs. Thirty-five cases (11.4%) of malaria in the cohort of returned VFRs and migrants were severe or complicated.

Conclusion: Conclusions: VFR travelers and migrants account for the majority of malaria cases in travelers presenting to CanTravNet sites over 5 years, with severe malaria occurring in over 10% of cases. These data underscore the high potential for malaria-associated morbidity and mortality in VFR travelers and migrants, thus, clinicians should promptly exclude malaria when encountering fever in this population. West Africa continues to be the dominant source region for malaria imported to Canada.
21.007
Mapping Population Movement within Ebola Response at Points of Entry and Areas Vulnerable to Transmission as a Result of Travel and Trade, Democratic Republic of the Congo, May-June 2018

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Purpose: The purpose of this assessment was to identify priority Points of Entry (POE) and vulnerable areas affected by travel and trade, in order to inform the Ebola response in the Equateur Province of the Democratic Republic of the Congo and beyond, aimed at preventing the further spread of the disease.

Methods & Materials: This assessment was conducted through participatory mapping exercises involving key informants, in the form of group discussions. Key informants were selected based on their knowledge of population movement dynamics; they represented the formal, informal, health and non-health sectors. Topographic maps were utilized to guide the discussion.

The participants identified different categories of points of passage and congregation of travellers – POEs and internal connecting points. They located these points directly on the maps, and showed the movement flows between the points and beyond. Subsequently, they listed the most important points, based on the volume of travellers and connectivity with EVD affected areas, big cities of the country (eg. Kinshasa and Kisangani), and countries across the border.

Results: 130 key informants took part in five exercises between 20 May and 2 June 2018. Nine categories of points of passage/congregation were identified: airports, ports, markets, cross roads, schools, places of worship, cinemas, sports stadiums, and government buildings. In total, 141 points were identified and 30 were prioritized for intensified surveillance, risk communication and community engagement. The connections between these points, as well as with other parts of the country and neighbouring countries were discussed and recorded. Many of the 30 were highlighted due to their roles in short, long distance and international travel and trade.

Conclusion: The benefits of this assessment are multi-layered. The information collected facilitated a more objective allocation of resources for outbreak response in affected areas, and preparedness actions in vulnerable locations influenced by travel and trade. Furthermore, the participatory nature of the assessment empowers its participants to take an active role in guiding the response, and eventually, directly contribute to it.

Additional implementation of this assessment will enable the validation and improvement of the current methodology and tools. More data collection is needed to quantify and profile the travellers.
Confirmed Dengue Virus Imported Case To Morocco.

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**Purpose:** Dengue virus (DENV) infection is one of the important mosquito borne infections in humans. This disease is endemic in most tropical and subtropical countries. Travellers are not only at significant risk of acquiring dengue but they also contribute to its spread to non endemic countries. One of the main vectors of DENV, *Aedes albopictus*, has been identified recently in the town of Rabat, Morocco. The purpose of this work is to describe the result of infectious disease surveillance during the year of 2017 among Moroccan and tourists who presented to a reference hospital in Rabat, Morocco. We identified a male patient with signs and symptoms of dengue that has recently travelled from Côte d’Ivoire.

**Methods & Materials:** Real-time PCR examination revealed that the patient was infected by DENV-2. **Results:** Real-time PCR examination revealed that the patient was infected by DENV-2. A phylogenetic analysis based on the envelope protein indicated that the virus belongs to the Cosmopolitan genotype and highly homologous to the virus strains from the 2016 outbreak in Burkina Faso.

**Conclusion:** Our result suggests that there is an evident risk of autochtonous transmission of DENV in Morocco in the future.
AIRSAN Bibliography: Making public health action-orientated information in the aviation sector quickly accessible

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Purpose: The AIRSAN Project, amongst other objectives, aimed to facilitate the implementation of the International Health Regulations (IHR) (2005) in EU Member-States. Many organizations (e.g. WHO, IATA, ICAO) developed useful documents to support IHR implementation in the aviation sector. We aimed to list these documents and find a reasonable way to make their contents quickly accessible.

Methods & Materials: We created a searchable Excel-based list, the ‘AIRSAN Bibliography’. Only documents issued by international organizations about public health in the aviation sector were included. Structural data, e.g. issuing organizations, were compiled. Moreover, we defined 33 IHR relevant keywords, e.g. emergency planning, training, isolation. Two scientists reviewed the documents and systematically indexed all pages including information about the keywords. To allow a distinction between more or less detailed text passages, we assigned a score from 1 - 4 (indicating the level of detail) to each keyword specific page; the higher the score, the more relevant information could be found in the text passage.

Results: The AIRSAN Bibliography includes 77 documents, issued by 8 international organizations. The practical use can be illustrated by the following example: an emergency plan needs to be developed or revised. The search for the keyword “emergency planning” lists 32 documents, with references to pages that contain keyword specific information. Overall, 12 of the 32 documents enclose information of the highest level of detail which means that they are key documents in the area of emergency planning in the aviation sector.

Conclusion: The AIRSAN Bibliography makes public health action-orientated information in the aviation sector quickly accessible for practical use. We integrated it into our website (www.airsan.eu) and expect that this new tool will facilitate intra-sectoral collaboration. The AIRSAN Bibliography eases not only implementation of the IHR but also of the EU-Decision on serious cross-border threats to health.
Epidemiological and Clinical Comparison of Influenza Virus Infections Including Meteorological Parameters Affecting Influenza Activity in the Philippines, 2006-2012

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Purpose: Seasonal influenza affects a significant proportion of the global population every year. In the Philippines, influenza infection continues to be one of the top ten leading causes of morbidity. To develop an effective preventive measures, epidemiological and clinical characteristics of confirmed influenza cases collected from the national surveillance were compared in association with influenza activity and meteorological factors.

Methods & Materials: Nasopharyngeal and Oropharyngeal swab samples were collected from patients with influenza-like illness (ILI) from the national surveillance from 2006 to 2012. Samples were inoculated into MDCK cells. Isolates were further tested using immunofluorescence and PCR for IAV, IBV and ICV confirmation. Correlation of epidemiological and clinical information was analyzed using STATA software. Meteorological parameters such as rainfall, relative humidity and mean temperature were retrieved from Philippine Atmospheric Geophysical and Astronomical Services Administration (PAGASA).

Results: Influenza viruses were detected in 5,412 (10.5%) of the 54,475 cases collected from 2006 to 2012. Of these, IAV was the most frequent type identified at 6.31%, followed by IBV at 6.31%, ICV at 0.6%. Majority of ICV (85%) were prevalent in children under five years old compared with IAV (40%) and IBV (50%) wherein most of the cases were from 5 to 18 years old age group. Moreover, IAV and IBV were seen at high frequency from patients with fever during consultation compared with ICV. Correlating the monthly mean values of rainfall with influenza types, IAV counts were significantly different between seasons across study sites. Likewise, IBV showed the same result with IAV in some study sites. However, correlation of rainfall amount with ICV counts were not determined due to the small number of cases identified.

Conclusion: The data showed that ICV, IAV and IBV continued to remain significant each year and concurred with previous reports in the Philippines. Findings provide evidence that the amount of rainfall affects the seasonal distribution of IAV and IBV infection in the country. Inclusion of meteorological parameters in the surveillance system may further understand the influenza transmission patterns in the Philippines.
Epidemiology Of Respiratory Pathogen Carriage In The Homeless Population Within 2 Shelters In Marseille, France, 2015-2017: Cross Sectional One-Day Surveys

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Purpose: To assess risk factors for respiratory tract infection symptoms and signs in sheltered homeless people in Marseille during the winter season, including pathogen carriage.

Methods & Materials: Data on 479 participants within 2 shelters who completed questionnaires and a total of 950 nasal and pharyngeal samples were collected during the winters of 2015-2017. Respiratory pathogen carriage including 7 viruses and 4 bacteria was assessed by quantitative polymerase chain reaction.

Results: The homeless population was characterized by a majority of males (2260/2365, 95.6%) of North African origin (1156/2360, 49.0%) with a relatively high prevalence of chronic homelessness (175/465, 37.6%). We evidenced a high prevalence of respiratory symptoms and signs (168/476, 35.3%), a very high prevalence of bacterial carriage (313/477, 65.6%), especially Haemophilus influenzae (280/477, 58.7%) and a lower prevalence of virus carriage (51/473, 10.8%) with human rhinovirus being the most frequent (25/473, 5.3%). Differences were observed between the microbial communities of the nose and throat. Duration of homelessness (OR=1.77, p=0.017), chronic respiratory diseases (OR=5.27, p<0.0001) and visiting countries of origin for migrants (OR=1.68, p=0.035) were identified as independent risk factors for respiratory symptoms and signs. A strong association between virus (OR=2.46, p=0.012) or Streptococcus pneumoniae (OR=2.32, p=0.014) carriage and respiratory symptoms and signs was also found.

Conclusion: These findings allowed identifying the individuals at higher risk for contracting respiratory tract infections to better target preventive measures aiming at limiting the transmission of these diseases in this setting (such as against influenza and S. pneumoniae infections).
21.012
Role of Molecular Genetic Methods for Avian Influenza Diagnostics in the Epizootological Monitoring System

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Purpose: Avian influenza (AI) diagnostics is an important part of the veterinary preventive measures to control this disease. In Ukraine, AI detection performed using virological and molecular genetic methods in accordance with OIE recommendations. The purpose of the work was to assess the capacity of expanding molecular genetic methods usage in the AI surveillance system within Ukraine.

Methods & Materials: During the period from 2017 to Q1 2018, 4390 biological material samples were analyzed using real-time PCR, and 81126 blood serum samples using Hemagglutination Inhibition assay (HAI). Samples of blood serum, pathological material, cloacal and tracheal swabs, pellets from wild birds and poultry were tested.

Results: All avian influenza studies in Ukraine are performed within the State monitoring program, as a response to suspected cases, and include testing cloacal and tracheal swabs from birds collected in the areas, where positive cases were detected. In 2017, there were 8 AI suspected cases, 5 of which were confirmed by -time PCR. H5N8 HPAIV positive cases were registered in Chernivtsi (10 samples collected from wild birds and 12 from poultry), Odesa (5 samples collected from poultry), Ternopil (5 collected from poultry) and Mykolaiv oblasts (sample from zoo bird). In 2018, there were 2 AI suspected cases (Kherson and Donetsk oblasts) that were not confirmed. At the same time, all poultry samples collected in zones with AI positive cases and tested using HAI demonstrated negative results.

Conclusion: Obtained results confirm high-sensitivity of molecular-genetic methods comparing to virological ones in avian influenza diagnostics. Thus, usage of molecular genetic methods is an important element in the early stages of response to suspected cases of avian influenza. Based on results, changes are implemented in the development of the State monitoring program of avian influenza within Ukraine. A plan on regional laboratories involvement in avian influenza diagnostics using molecular genetic methods was developed and successfully introduced.
The burden of influenza and respiratory syncytial viruses in Japan, 2006-2014: A region- and age-specific excess mortality study

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Purpose: Influenza and respiratory syncytial virus (RSV) infections greatly contribute to morbidity and mortality in humans, but such epidemiological impact is yet to be assessed in the super-aged nations using up-to-date methods. Here we estimate pandemic and winter-seasonal excess mortality rates associated with influenza and RSV across prefectures in Japan from 2006 to 2014. We then assess the influence of socio-demographic, environmental and healthcare factors on our excess mortality estimates.

Methods & Materials: We used non-parametric statistical methods that capture seasonal and long-term trends to estimate excess mortality rates from time series stratified according to 6 age groups and prefectures. We then employed statistical modelling to assess the associations between excess mortality and predictor variables.

Results: Across the 8 seasons (2006-2014), seasonal influenza was on average associated with about 27,000 annual deaths in Japan, or about 20 per 100,000 population (and only about 8,100 annual deaths, about 6.0 deaths per 100,000 population in the 2009 influenza pandemic). RSV was annually associated with about 34,000 annual deaths (about 27 per 100,000 population). For both infectious diseases, excess mortality rates were highest among seniors, especially aged ≥ 80 years. Respiratory and circulatory disease mortality was found to be most associated with influenza, accounting for 89% of influenza-associated deaths and 85% of RSV-associated deaths, respectively. For comparison, the number of notified laboratory-confirmed deaths during the 2009 pandemic was about 40 times smaller than our estimate of the actual number of excess deaths associated with the 2009 A/H1N1 influenza pandemic in Japan. Variations in excess mortality estimates across prefectures for both influenza and RSV were mostly shaped by climatological factors.

Conclusion: Strikingly, we report greater estimates of influenza-associated mortality for Japan compared to estimates published over a decade ago. Moreover, our results revealed comparable mortality burdens exerted by RSV and influenza in Japan. In particular, seniors are disproportionately affected, especially those aged ≥ 80 years, indicating that a rapidly aging population could exacerbate mortality associated with these respiratory diseases.
Genetic Characterization of HPAI H5N8 Viruses Identified in Wild and Domestic Birds in Uganda, 2017

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Purpose: In early January 2017, outbreaks of H5N8 highly pathogenic avian influenza (HPAI) were reported for the first time on the shores and Islands of Lake Victoria in Central Uganda. An unusual high mortality was detected among white-winged terns in the districts of Masaka, Wakiso and Kalangala (Ssese Islands). A spill over from wild to domestic birds was identified in chickens and ducks in all the three districts. The purpose of our study was to genetically characterize the Avian Influenza viruses identified in Uganda during the H5N8 epidemic in the African continent.

Methods & Materials: By using an Illumina MiSeq platform, we generated the complete genome of three viruses isolated from a white winged black tern, a domestic duck and a chicken. We then performed a phylogenetic analysis of the eight gene segments of these viruses together with the available sequences of the H5N8 virus A/grey-headed gull/Uganda/MUWRP-538/2017, present in the public database.

Results: The phylogenetic analyses of the eight gene segments revealed that the Ugandan H5N8 viruses belonged to genetic clade 2.3.4.4, group B and clustered with the viruses collected in May 2017 in the Democratic Republic of the Congo as well as with a virus from Cameroon (A/duck/Cameroon/17RS1661-3/2017). Differently, they showed a low similarity with the H5N8 viruses detected in 2017 in South Africa, Egypt and in some of the viruses isolated in Cameroon.

Conclusion: The occurrence of H5N8 outbreaks in wild birds during the rainy season, when Uganda provides a recovery for many migratory species of birds, suggests that infected migratory wild birds might have played a crucial role in the introduction of HPAI H5N8 into this region. The low level of similarity detected among the Ugandan, South African and Egyptian strains suggests that the outbreaks in these countries were caused by separate virus introductions. However, viruses genetically related to the ones identified in South and North Africa could have been circulating in unsampled locations.

More detailed data need to be collected and combined to reconstruct the dynamics of the disease in the continent and to develop tailored control measures.
Distribution of respiratory syncytial virus subtypes A and B among children presenting with acute respiratory tract infection to Kegalla hospital.

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Purpose: Acute respiratory tract infections (ARTIs) are associated with significant morbidity and mortality of children in developing countries. Respiratory viruses are the major causative agents of ARTI and respiratory syncytial virus (RSV) is the commonest virus causing severe ARTI in children worldwide including Sri Lanka. RSV consists of two major antigenic types (A and B) and many subtypes. Furthermore, a larger study has not been undertaken to identify the types of RSV circulating in Sri Lanka and documented information is limited to few local communications in Sri Lanka.

The objective of this research is to type the RSV strains circulating in a sample of hospitalized children in Sri Lanka with RSV caused ARTI and to identify the disease severity associated with RSV type.

Methods & Materials: Demographic, clinical data and nasopharyngeal aspirate (NPA) samples were collected from 274 children less than 5 years of age and with ≤4 days history of ARTI. Study subjects were admitted to the General Hospital, Kegalle from July 2016 to November 2017. Immunofluorescence assay (IFA) was performed on the NPA using a seven-valent viral detection kit, which detects the presence of seven respiratory viruses including RSV. Out of the IFA positives, 76 patients were identified to be only RSV positives (27.7%). Those samples were directed to real-time PCR for typing using a RSV RT-PCR kit.

Results: Out of 76 RSV positive patients, 15 were infected with RSV A strain, 48 were infected with RSV B strain and 13 were co-infected with both strains. Overall a male predominance is evident in the sample as 13/15 RSV A patients, 28/48 RSV B patients and 9/13 RSV A and B co-infected patients were males. RSV type did not appear to associate with any specific age group and disease severity.

Conclusion: RSV type B is the most predominant RSV strain to circulate among the children with ARTI in the Kegalla district, Sri Lanka.
Purpose: Seasonal influenza is a serious re-emerging acute infectious diseases with its social and health impact. Understanding its temporal characteristics in different spatial perspectives is a priority in public health and disease surveillance due to its annual recurrent episodes causing significant health burden. However, national and state-level influenza temporal trends are not fully investigated yet. Therefore, we applied EARS methods to describe temporal trends of seasonal influenza in Mexico from 2007 to 2014 in different spatial level.

Methods & Materials: The number of confirmed seasonal influenza cases are reported every week at the state level from 2007 to 2014 (except in 2009) in Mexico. We use Early Aberration Reporting System (EARS) methods (C1, C2, and C3 algorithms) to detect the signals from influenza outbreak as temporal trends investigation in two different spatial level: national level and state level.

Results: At national level analysis, C1 method could catch up 22 signals, C2 method could catch up 73 signals and C3 method could catch up 116 signals during 2007-2014 seasonal influenza surveillance. At state-level analysis, C1 statistics could catch up total 570 signals in 32 states. The average number of signals from C1 statistics is 17.8 signals/state ranged from 11 to 28. C2 statistics could catch up total 1612 signals in 32 states. The average number of signals from C2 statistics is 50.4 signals/state ranged from 31 to 67 signals. C3 statistics could catch up total 3163 signals in 32 states. The average number of signals from C3 statistics is 98.8 signals/state ranged from 72 to 120 signals.

<table>
<thead>
<tr>
<th></th>
<th>Number of C1 Signals</th>
<th>Number of C2 Signals</th>
<th>Number of C3 Signals</th>
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<tbody>
<tr>
<td>Minimum (per year)</td>
<td>11</td>
<td>31</td>
<td>73</td>
</tr>
<tr>
<td>Maximum (per year)</td>
<td>28</td>
<td>67</td>
<td>119</td>
</tr>
<tr>
<td>Average(SD)</td>
<td>17.8(4.09)</td>
<td>50.4(10.6)</td>
<td>88.5(14.6)</td>
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Conclusion: National level surveillance system and its signals may not meet the state level outbreak signals. Public health professionals should understand the difference of seasonal influenza temporal trends. Public health professionals and governments should apply state-level surveillance system to provide better guidelines for seasonal influenza prevention and control.
Farmers’ Preparedness for Avian Influenza: Assessing Changes in Biosecurity Level in a ten-year period


Purpose: Densely populated poultry areas (DPPA) and flaws in biosecurity measures at farm level has been identified as the main risk factors for the introduction and spread of avian influenza (AI) viruses among domestic poultry holdings. This study aimed at comparing changes in biosecurity measures at farm level over time and thus to identify those factors that are amenable to improvement.

Methods & Materials: Eighty-three turkey farms in northeastern Italy were assessed via a standardized questionnaire in 2004 and, after 10 years, in 2014. The information collected concerned seven macro areas: personnel, animal species reared, cleaning and disinfection materials and procedures, removal of manure and dead birds, shed characteristics and outer environment. Each macro area relates to a different aspect of farm biosecurity, which served to score and rank the farms using the Simple Additive Weighting (SAW) and Analytical Hierarchy Process (AHP) methods. The higher the score the lower was the level of biosecurity. To compare the biosecurity level over time, scores were standardized and then grouped into four classes defined by the quartiles. Farms that showed a change in the ranking were grouped in the 1st (lowest score) and 4th (highest score) quartiles, respectively. Whilst the farms which keep the same position or showed minimal variation in the ranking, were placed in the 2nd and 3rd quartiles.

Results: The average score changed significantly, with a mean value of 1.28 (sd 0.44) and 1.13 (sd 0.33) in 2004 and 2014, respectively. In both the 1st and 4th class, a lower score associated with hygienic procedures was observed. Farms in the 2nd and 3rd class, showed a significant difference associated to shed characteristics and outer environment, with a higher average score in the 2nd class.

Conclusion: This study provides a baseline assessment of biosecurity practices on fattening turkey farms in Italy before the last intercontinental wave of highly pathogenic AI of the subtype H5N8 hit Europe in 2016/17. It is advised to upscale the current assessment system to an objective risk-based scoring tool for quantitatively evaluate biosecurity at farm level. This would help developing a better evidence base for biosecurity strategy.
Contribution Of Influenza Virus And Respiratory Syncytial Virus To Community Cases of Influenza-like Illness In Northern Italy During Four Consecutive Influenza Seasons (2014-2018)

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Purpose: Influenza viruses (IVs) and respiratory syncytial virus (RSV) have a relevant epidemiological impact in influenza-like illness (ILI). The contribution of IV to community ILIs was assessed in Lombardy (Northern Italy) during four consecutive seasons in the framework of the Italian Influenza Surveillance Network (InfluNet); the impact of RSV was evaluated in IV-negative ILI cases.

Methods & Materials: From week 46 to week 17 of the following year, nasal/throat swabs from ILI outpatients were collected in Lombardy within InfluNet during four consecutive seasons (from 2014/2015 to 2017/2018). IV was typed (A/B) and subtyped (H1N1pdm09/H3N2) by real-time RT-PCR. RSV-A/B-RNA was detected in IV-negative specimens. Nearly 20% of IV- and RSV-positive samples was sequenced and phylogenetically analysed.

Results: Overall 53% of samples were IV-positive: the rate of IV-positive samples ranged from 45% (2015/2016) to 64% (2017/2018). All IV types/subtypes were detected in each season: A/H1N1pdm09 predominated in 2014/2015 (43% of IV-positive samples), A/H3N2 in 2016/2017 (94%), B in 2015/2016 (71%) and 2017/2018 (60%). 13% of IV-negative specimens was RSV-positive: ranged from 10% (2015/2016) to 15% (2016/2017 and 2017/2018). RSV-A was the main type identified in 2016/2017 (63%), RSV-B predominated in 2014/2015 (76%) and 2017/2018 (82%), while the two types co-circulated in 2015/2016 (43% and 50%). In all seasons, the highest risk of infection from IVs and RSV was observed in children aged 5-14 years and 0-4 years, respectively. Phylogenetic analysis is ongoing.

Conclusion: Although IV remains the main contributor to ILI particularly in schoolkids, also RSV contributes especially among the youngest children. Both IV and RSV circulation was heterogeneous. Phylogenetic analysis will be useful to better define clades/subclades of circulated strains, to study selective pressure on viral protein and to improve vaccine and therapy strategies.
Purpose: From 2005 through 2012, the Center for the History of Medicine at the University of Michigan Center was contracted by the Defense Threat Reduction Agency and the Centers for Disease Control and Prevention to conduct several studies on the 1918 influenza pandemic. As a result of our work, we amassed a trove of important primary source material, which we decided to make publicly available via an online encyclopedia and archive.

Methods & Materials: We visited dozens of archives and special collections across the nation and gathered photocopies of documents related to the influenza pandemic in the 50 cities in our study for the period between September 1918 and March 1920. Documents included annual (and sometimes monthly) municipal health reports, state health reports, federal reports and bulletins, records from military bases and camps adjacent to cities in our study, weekly pneumonia and influenza mortality reports for each city, correspondence between health officers and other officials, and other miscellaneous documents pertaining to the pandemic. We also gathered copies of each and every article on the pandemic from the two highest circulating newspapers in each city in our study, going through the reels of microfilm day by day.

Results: We created high resolution digital scans (with OCR) of each document. Next, we abstracted and created a set of metadata for each document using Dublin Core tagging scheme. The data was then imported into the University of Michigan Library's digital platform, DLXS, to serve as the "back end" for the digital archive. Simultaneously, we authored essays for each of the cities in our study, detailing their individual pandemic experiences and providing historical context. Then, we created a web "front end" to serve as an entry point into the archive. Documents are searchable by a variety of means, including title, subject, and keyword.

Conclusion: Our digital archive contains approximately 20,000 pages of documents pertaining to the deadly 1918 influenza pandemic, each freely accessible at http://www.influenzaarchive.org. Since its launch we have added several thousand pages of documents to the collection and will continue to add more in the future as we gather them from additional archives.
Purpose: In Japan, the current influenza vaccination programme is targeting older individuals. On the other hand, epidemics of influenza are likely to be mainly driven by children. In this study, we consider the most cost-effective target age group for a seasonal influenza vaccination programme in Japan.

Methods & Materials: We constructed a deterministic compartmental SEIR model with data from the 2012/13 to 2014/15 influenza seasons in Japan. Bayesian inference with Markov Chain Monte Carlo method was used for parameter estimation. Cost-effectiveness analyses were conducted from the payer perspective.

Results: A scenario targeting children under 15 was expected to reduce 9,948,008 symptomatic cases on average over one season. A scenario targeting adults (15-59 years of age) was expected to reduce 10,969,091 cases and a scenario targeting elderly population (age over 59 years) was expected to reduce 4,198,470 cases, respectively. Each scenario demonstrated negative incremental costs (-1,121.0 million USD for the scenario targeting children, -660.5 million USD for the scenario targeting adults, -129.2 million USD for the scenario targeting elderly).

Conclusion: A vaccination programme which targets children 0-14 years of age is predicted to have much larger epidemiological impact than those targeting elderly only.
A Multiple Regression Analysis of Number of Influenza Patients during 2009/2010 and 2017/2018 in Takamatsu City, Japan.

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Purpose: The H1N1 2009 influenza were found in the United States and Mexico in April 2009. It spread almost all countries of the world. The World Health Organization announced that the status of the influenza was a pandemic in June 2009. H1N1 influenza spread rapidly in Takamatsu city Japan also. The H1N1 influenza has been epidemic form yet. We analyzed number of influenza patients during 2009/2010 and 2017/2018 in Takamatsu city and on a university campus in Japan.

Methods & Materials: We analyzed number of patients infected with influenza in Takamatsu city and on a university campus in Japan from August 2009 to February 2010 and from September 2017 to June 2018. We used SPSS 24.0 for Windows (IBM Inc., Chicago, IL, USA) for multiple regression analysis.

Results: According to multiple regression analysis results, temperature and humidity were significantly correlated with number of daily patients in Takamatsu city 2017/2018 ($p < 0.001, R = 0.763, AIC = 162.374$). It was suggested that temperature, humidity, a holiday the next day after the lecture and a holiday 2 days after the lecture were significantly correlated with number of daily patients on a university campus 2009/2010 ($p < 0.001, R = 0.348, AIC = 441.986$).

Conclusion: We made influenza models in Takamatsu city and on a university campus by multiple regression analysis. The following models were obtained. In Takamatsu city 2017/2018; $y = -2.2x_1 + 2.1x_2 + 18.1$; ($x_1$: temperature, $p < 0.001$, OR $-3.3$ $-1.2$; $x_2$: humidity, $p = 0.017$, OR $0.4$ $-4.0$). On a university campus 2009/2010; $y = -0.07x_1 + 0.06x_2 + 2.2x_3 + 2.3x_4 +0.02$; ($x_1$: temperature, $p = 0.029$, OR $-0.14$ $-0.007$; $x_2$: humidity, $p = 0.004$, OR $0.02$ $-0.11$; $x_3$: a holiday the next day after the lecture, $p = 0.011$, OR $0.5$ $-3.8$; $x_4$: a holiday 2 days after the lecture, $p = 0.011$, OR $0.5$ $-4.0$).
The Bat Influenza H17N10 Is Neutralized By Broadly-neutralizing Monoclonal Antibodies And Its Neuraminidase Facilitates Viral Egress

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Purpose: The diversity of subtypes within Influenza A has recently expanded with the identification of H17N10 and H18N11 from bats. In order to further study the tropism and zoonotic potential of these viruses, we have produced lentiviral pseudotypes bearing H17 and N10 glycoproteins. Influenza pseudotypes are powerful tools that permit safe, sensitive serology and other cell-based assays to be performed, including at high-throughput. Different permutations of these pseudotypes permit specific measurement of anti-HA head, anti-HA stalk and anti-NA directed antibodies enabling new antivirals and mAbs to be screened.

Methods & Materials: H17N10 lentiviral pseudotypes were produced by co-transfection of 293T/17 cells with HIV Gag-Pol, H17, N10, protease (to cleave HA), and luciferase-carrying vector plasmids. HA neutralization assays were carried out with mAbs on 96 well plates using a Glomax luminometer. IC50 were calculated using GraphPad Prism. A novel NA pseudotype-based ELLA assay was used for NA inhibition determination.

Results: These H17N10 pseudotypes were shown to be efficiently neutralized by the broadly neutralizing HA stalk monoclonal antibodies CR9114 and FI6. We confirm that H17 can infect MDCKII cells and that it does not use sialic acid as its cellular receptor, as pseudotypes bearing H17 HA glycoprotein are released into the cell supernatant in the absence of neuraminidase. H17 pseudotypes are also unable to transduce cells that are permissive to non-chiropteran influenza A and B pseudotypes. We demonstrate that N10 can facilitate H5 and H7 influenza pseudotype release in the absence of another source of neuraminidase. Despite this, the N10 protein shows no activity in an enzyme-linked lectin assay.

Conclusion: This lentiviral pseudotype system will permit extensive new research on bat influenza tropism, therapeutics, restriction and seroepidemiology, without the constraints or safety issues with producing replication competent virus, to which the human population is naïve.
African Green Monkey Model of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Infection

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**Purpose:** Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a highly pathogenic zoonosis that emerged in 2012, causing lethal respiratory disease in approximately 35% of human cases. MERS-CoV continues to emerge on the Arabian Peninsula with the possibility of travel-exported cases to other regions of the world including prior confirmed cases in the Republic of Korea, the United States, England, France, and China. Currently, there are no specific countermeasures for MERS-CoV that have proven efficacious at ameliorating disease. Animal models that recapitulate severe MERS disease signs are needed to support development of therapeutics or vaccines to protect vulnerable populations.

**Methods & Materials:** For initial development of a MERS-CoV primate model, twelve African green monkeys (AGMs) were exposed to $10^3$, $10^4$, or $10^5$ PFU target doses of aerosolized MERS-CoV. Disease progression was followed with daily health observations, weights, body temperatures, blood and throat swab collection.

**Results:** In this study, infection of the $10^3$ PFU dose group was associated with minimal disease signs in AGMs, including lack of fever, lower viral titers and minimal clinical scores over 28 days of observation post-exposure to MERS-CoV. In contrast, the $10^4$ PFU dose and especially the $10^5$ PFU dose were associated with significantly more observable disease signs of MERS-CoV infection, although all AGMs survived for the 28 day duration of the study.

**Conclusion:** Clinical symptoms of MERS in humans range from asymptomatic to severe respiratory syndrome and death. Severe cases of MERS present initially as fever, cough and shortness of breath, but progress to more severe respiratory symptoms including end-stage lung disease. Although biological factors including advanced age (>65 years) and comorbidities are associated with severe MERS disease in humans, few animal models exist that demonstrate biomarkers of severity in MERS-CoV infection as end-points for therapeutic testing. This study is the first to describe dose-dependent effects of highly pathogenic coronavirus infection of primates and uses a route of infection (aerosol) more relevant to MERS-CoV transmission in humans. Aerosol exposure of AGMs at higher doses may provide a lethal model of MERS in African green monkeys with potential utility in therapeutic development and viral pathogenesis studies.
Purpose: Avian influenza A(H7N9) is considered an important zoonotic pathogen, human cases of which have been increasing in multiple epidemics waves since it was identified in 2013. Characterising the temporal pattern of mortality or clinical severity of human infection can identify changes in viral pathogenicity and help inform emergence and pandemic risk assessments. Here, we consider how the severity of A(H7N9) in humans has changed by epidemic wave, after adjusting for demographic and spatial factors, and including cases up to June 2018.

Methods & Materials: Using data published by the Hong Kong Centre for Health Protection, the association of epidemic wave with death and being clinically severe (defined as a case being characterised as fatal or critical) was estimated using generalised additive models, adjusting for age, sex and province of cases.

Results: We found significant changes in case fatality between waves, but no evidence that the largest epidemic wave (2016-17) was associated with increased mortality compared to previous waves. Mortality was significantly associated with age, with older ages tending to have higher mortality, and with province. The risk of being clinically severe in more recent waves (2015-16 and 2016-17) was significantly lower than during the previous two waves; there was a significant association with age, and significant differences between provinces.

Conclusion: We found no evidence of an increase in mortality of human cases of A(H7N9) in the most recent waves, despite a marked increase in the number of cases between 2016 and 2017 and the emergence of a highly pathogenic viral variant, suggesting that the average pathogenicity of the virus has not changed. However, the risk of being a clinically severe case was lower in more recent waves, possibly due to improved clinical care or more rapid diagnosis and treatment.
Knowledge about Influenza and Compliance with the Recommendations for Influenza Vaccination of Pregnant Women in Greece

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Purpose: Pregnant women and young infants are at increased risk for influenza-associated severe disease, complications and hospitalizations. In Greece influenza vaccination of pregnant women is recommended since the 2009 H1N1 influenza pandemic however vaccine uptake rates by them remain extremely low (<5%).

Methods & Materials: We prospectively studied the compliance with the recommendation for influenza vaccination of 304 pregnant women (mean age: 31.5 years, mean gestational age: 27.8 weeks) in a large maternity hospital before the 2017-2018 influenza season, following an educational intervention. Educational intervention consisted of a five-minute discussion with their obstetrician and the distribution of an informative leaflet. A standardized questionnaire was used. Their knowledge was evaluated with a total of 11 questions about influenza in pregnant women and young infants and maternal influenza vaccination during pregnancy. A total knowledge score (%) was calculated for each woman.

Results: Sixty pregnant women (19.5%) pregnant women were vaccinated against influenza. Their mean knowledge score was 87%. Multiple regression analysis revealed that influenza vaccination in the past and past information about the need to get vaccinated were statistically significantly associated with an increased probability for influenza vaccination during pregnancy. Maternal and gestational age, nationality, education level, residence area, number of household members, number of children, underlying disease, pregnancy problems, programmed caesarean section, history of smoking, intention to breastfeed the baby, and level of knowledge about influenza were not statistically associated with an increased possibility for influenza vaccination. “Fear of adverse events” (for them or the fetus) was the prevalent reason for refusing influenza vaccination, followed by the statements “influenza vaccination is not necessary” and “not at risk to get influenza.”

Conclusion: In our setting, an educational intervention was associated with an overall influenza vaccination rate of 19.5% among pregnant women compared to <5% the past years. In order to improve vaccine uptake by pregnant women and protect them as well as their babies, more intensified educational interventions should be explored.
Purpose: There are scarce data in determining the epidemiology and etiology of, and risk factors for severe acute lower respiratory infection (ALRI) in middle and low-income countries. Brazil developed a surveillance system for severe cases of ALRI after influenza pandemic in 2009. Therefore, we evaluated and analyzed data from a pediatric sentinel site for influenza and other respiratory viruses in Brazil.

Methods & Materials: We performed a descriptive study in a pediatric intensive care unit, which is a sentinel unit for influenza and other respiratory viruses surveillance, located in Brasilia, Brazil. The study included all cases of ALRI in children ≤24 months old, with a positive result of a viral pathogen obtained from a nasopharyngeal sample, from January 2013 to June 2017. Samples were processed by polymerase chain reaction (PCR).

Results: There were 314 cases of ALRI in the study period, 148 had samples collected and 69 (47%) were positive and, thus, included in the study. Fifty-eight (84%) were RSV, 8 (11%) were influenza and 5 (5%) were other viruses. Forty-three children (62%) were male, the median age were 4 months (1-20) and 42 (61%) were < 6 months-old. Fifty-six children (81%) presented at least one comorbidity: including preterm birth or low birth weight. RSV cases predominated from March to July. The median time from the beginning of symptoms to hospital admission were 3 days (1 – 12) and to ICU admission were 5 (1 – 30). The median time to obtain the laboratory results was 7 days (2 – 23).

Sixty-two children (90%) needed mechanical ventilation, 40 (58%) received oseltamivir and 63 (90%) received antibiotics. There were 3 deaths (4%).

Conclusion: The burden of viral severe ALRI in young children is high in middle-income countries, posing a challenge to health services, especially during seasonal epidemics. RSV is the main pathogen of ALRI in children ≤24 months old; the high usage of oseltamivir and antibiotics is mostly useless for children in this age, new preventive methods as vaccine or antivirals are urgently needed. Surveillance systems for respiratory viruses should improve their timeliness to health services access, including ICU and their laboratory capacity.
Evaluating a New Approach to Managing Seasonal Influenza Outbreaks in Care Homes in the East of England

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**Purpose:** To evaluate a novel approach to managing outbreaks of influenza-like-illness (ILI) in care home settings.

Over the winter months outbreaks of ILI in residential care settings puts a strain on public hospital services, through increased emergency admissions, extended closure times for care homes and delays to patient transfer. Public Health England (PHE) Health Protection Teams (HPTs) are responsible for advising on appropriate management to protect residents and support reopening of care homes to avoid delays in patient transfer. During the 2017-18 flu season the East of England HPT adopted a new approach to managing the increased work load from influenza outbreaks, by using the emergency response framework to declare a local incident. This subsequently led to the establishment of an incident control centre (ICC), incident management team, and provision of a dedicated outbreak response team including support consultant, health protection practitioners and business support staff.

**Methods & Materials:** A mixed methods approach was used to gather data and feedback, using the Donebedian Framework of "Structure-Process-Outcome" to assess different aspects of the response. Outbreaks of ILI were notified based on case definition and confirmed via nasopharyngeal swabs sent to local hospital laboratories for PCR testing. Adherence to guidance was assessed through an audit of ILI outbreaks recorded using specialised web-based software, HPZone; used by HPTs across England. Small group discussion and surveys were used to capture internal stakeholder feedback. External stakeholder feedback was collected via online survey.

**Results:** The incident was declared on 3rd January 2018 stood down on 30th April 2018. A total of 258 respiratory situations were recorded (131 confirmed influenza virus); there were 256 recorded hospitalisations and 56 deaths, the average length of outbreak was 13 days, average home closure was 10.96 days. Data is currently still being collected for the evaluation, and the completed evaluation report is due by the end of July.

**Conclusion:** The evaluation assesses the merits of applying this novel approach by measuring the impact on length of outbreak and closure time, alongside stakeholder perceptions and acceptability. Information gathered will be used to improve future seasonal response and if effective, recommend the approach for wider adoption across England.
21.028

Characteristics of SARI Patients in Jordan (2013 - 2018)

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Purpose: Globally, routine monitoring of severe influenza utilizing sentinel hospital-based surveillance of severe acute respiratory infections (SARI) has been established. Little, however, is known about the epidemiology of SARI patients in developing countries, including Jordan. This study describes the main characteristics of surveyed SARI patients in Jordan between 2013 and 2018.

Methods & Materials: Syndromic case definition of patients with SARI admitted to four hospitals in Jordan between January 2013 and May 2018 was used to identify study population. Standardized SARI surveillance data (demographics, clinical presentation, and diagnoses) combined with laboratory results of the nasopharyngeal and oropharyngeal swabs tested for influenza using real-time reverse transcription polymerase chain reaction were utilized to establish the epidemiological profile of SARI patients in Jordan.

Results: A total of 3,816 Patients were enrolled in SARI surveillance system between January 2013 and May 2018 (females represented 41.9%). Of these, 11.1% required treatment in intensive care unit (ICU) and 161 Patients died (SARI specific case fatality rate was 4.2%). The distribution by age groups showed an increased frequency in children under five (59.0%) and Patients older than 60 years (11.0%). Comorbidity and pregnancy, respectively, were reported in 28.6% of Patients and among 9 Patients. Seasonal influenza vaccination rate during the last 6 months was 1.1%. All SARI cases were tested for influenza, among other viruses. Positivity for influenza was 15.3% (n=586) and influenza case specific fatality rate was 8.1%. Among positive SARI cases, the most frequently identified viruses were influenza A viruses (77.3%, n=453), and Flu B (23.7%, n=140). The most frequent identified virus was Pandemic (2009) H1N1 virus (60.5%, n=355).

Conclusion: The epidemiologic profile of SARI patients in Jordan seems to mirror that in the Northern Hemisphere. Continued and extended monitoring of SARI is necessary in order to better assess the risk of Influenza, identify risk groups and establish better control measures.
Epidemiological and Clinical Survey of Influenza in Albanian Adults

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Purpose: Highlight the epidemiological, clinical and therapeutic aspects of influenza viruses infection

Methods & Materials: This study included 162 patients hospitalized at the Infectious Diseases Service of the University Hospital Center Mother Teresa in Tirana, during a 5 month period (November 2017 - March 2018). The age group varied from 14 to 79 years. RT-PCR was used to detect the viruses from the nasopharyngeal swabs from each patient. Demographic data (age and gender), clinical data (clinical signs and symptoms) and presence of accompanying diseases or risk factors for serious illnesses are described and analyzed.

Results: The study included 162 patients, 82 (50.5%) were males and 80 (49.5%) females. The average age of patients in the study is 40 years. We encountered the highest percentage of patients hospitalized - 37.7% and the peak of morbidity in February, followed by November 6.8%, December 12.3%, January 22.2%, March 21%. All patients had fever (either febrile or subfebrile), 85% had frissons, 67.9% of patients had asthenia, 58.6% myalgia, 49.4% arthralgia, 33.3% headache, 17.9% vertigo, 2.2% nausea, 14.8% vomiting and 9.9% diarrhea. The symptoms of local flu syndrome encountered in our study include: cough in 73.5% of cases, sore throat 51.2%, rhinorhea 21%, and chest pain in 32.1% of patients. The chest x-rays evidenced 56 patients (34.5%) with pneumonia and 39 patients with bronchopneumonia (24%). The average time before the onset of antiviral therapy for the total of patients is 3.7 ± 1.8 days. The average length of hospitalization was 6.6 ± 4 days. Antiviral therapy was applied to 11.1% of total patients. Only one case had a fatal outcome.

Conclusion: The influenza virus infection is associated with a high prevalence and morbidity. The wide clinical and complications, such as the infection of the respiratory tract must be detected as soon as possible in order to avoid fatal outcomes in our patients. The antiviral therapy and the use of antibiotics in complication such as respiratory tract infections resulted to be very efficient.
The Mortality Predictive Model for Tuberculosis Patients In Malaysia: An Application of Surveillance Data 2013-2014

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Purpose: Mortality due to tuberculosis (TB) remained a challenge in Malaysia. Its mortality burden has been persistent and the highest among all notifiable infectious diseases for the last thirty years. We made use of the nationwide TB surveillance data to develop a mortality predictive model for TB patients managed in Malaysia.

Methods & Materials: A content analytic exercise was conducted by using a two-year notification data in 2013 and 2014. The dataset was processed and the characteristics of mortality were explored.

Results: Out of 48,780 notified TB cases, there was 6.01% mortality cases whereby 32.82% (n=931) had died directly due to TB. The mean age was 50.84 (SD=18.67) years old, male (69.1%), Malaysian (88.5%), Malay (46.5%), with low education (95.1%) and without permanent income (65%), non-diabetic (81.5%), non-smoker (62.9%) and without HIV co-infection (88.1%). The majority of cases were new cases (90.9%), pulmonary smear positive (70.7%) and with BCG scars (71.4%) who died during the first two months of intensive treatment phase (87.8%). Further analysis revealed that age, late presentation, pulmonary severity, presence of secondary infection and meningitis were significant predictors of mortality for TB patients in Malaysia [p<0.05]. The risk of mortality significantly increased with late presentation, worsening of pulmonary severity, presence of meningitis and secondary infection respectively [Adjusted OR 95%CI: 16.078% (11.796%-21.913%); 2.022% (1.682%-2.431%); 1.634% (1.101%-2.426%); 1.46% (1.182%-1.792%) respectively]. Age was also found to be a significant predictor, however, at a small and low risk of 2.4% with increase in age [Adjusted OR 95%CI: 0.986% (0.981%-0.991%)].

Conclusion: This is a significant TB mortality model that represented TB patients managed in Malaysia with strong predictive probability [95%CI: ROC AUC 73.6% (71.5%; 75.7%); Hosmer and Lemeshow chi-squared = 7.313 (p>0.503)]. Further work on risk scoring and characterization of TB mortality is warranted to develop a predictive mortality checklist tool for early and targeted interventions. Further analyses on mortality according to high risk subpopulations such as TB-diabetes, TB-elderly and TB-healthcare workers are also highly recommended for in-depth understanding to formulate and implement subpopulation-targeted interventions.
Outbreak Investigation of Influenza A Virus H9N2 among Commercial Poultry flocks in Islamabad, Pakistan during 2017

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Purpose: On October 09th 2017 few suspected cases of AIV H9N2 were reported to provincial reference laboratory for poultry diseases, Poultry Research Institute (PRI), Rawalpindi from Badhana Kalan, Islamabad. The cases were presented with complaint of respiratory distress, loss of production and unusually high mortality. The objectives of this investigation were to know the magnitude, reasons of the outbreak and to control it through targeted interventions.

Methods & Materials: The team from PRI visited the affected village and adjoining areas on October 13th, 2017. Active cases finding was done in the area using a standard case definition. Meeting sessions were conducted with poultry farmers, live bird market professionals and local administration. Socio-demographic information, clinical features, risk factor information, immunization status and biosecurity practices were recorded on a pre tested questionnaire. Rates and frequencies were calculated using Epi Info™ 7 Software.

Results: During active case finding 28 farms qualified as suspected for Avian Influenza H9N2 infection as per case definition, having a total poultry population of 273,100 and mortality of 16000 birds (5.86%). The mean age was 25 weeks ±10.25 SD with a range of 47. Only 7% (2/28) of the flocks had complete vaccination. Only 39% (11/28) had the farm boundary wall and 14% (4/28) had mortality disposal pits. Similarly 32% (9/28) of the farms had restricted visitor’s movements. Approximately 4% (1/28) of the farms had wheel dip at the farm entrance while 50% (14/28) had foot dip.

Conclusion: AI H9N2 outbreak occurred in the area with very low vaccine coverage, regular vaccination of the flocks was recommended. Standard biosecurity measures were recommended. Farmers of the surrounding area of 3 km radius were provided with one time free vaccine to avoid spread of the disease. Immediate safe disposal of mortality was ensured. Active surveillance for detection of sero-conversion in unvaccinated flocks and flock monitoring in vaccinated flocks was started in the affected area. A seasonal alert letter on Avian Influenza was issued by PRI to sensitize the relevant stakeholders.
Influenza Vaccination in Albania

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Purpose: Influenza vaccination program on health care workers was established during 2014 - 2015. Influenza seasons and influenza vaccine has been recommended for several risk groups. Our purpose is to present an overview of the current influenza vaccination program and evaluate vaccination administration and coverage among health care workers and other risk groups and influencing factors.

Methods & Materials: Questionnaire were prepared based on WHO recommendations such as TIP Flu and distributed to health care workers before the influenza vaccination season. The influenza vaccine supplied by national immunization program was monitored and reported to national level based on a framework and several prepared forms. Data were reported weekly on paper-based forms and processed and stored in an excel database.

Results: Administration of influenza vaccine within the cardiovascular target group was ~ 36% in 2016-2017, while in 2017-2018 season was increased to 42%, and in pregnant women from 0,5% in 2016-2017 to 1% in 2017-2018 season. Elderly above 60 years old are more interested in getting vaccinated against influenza and are the group with the higher administration percentage, in 2016-2017 season was 68,4% while in 2017-2018 season reached 69%. A positive trend was noticed on the vaccination coverage for health care workers from 60% in 2016-2017 to 72% in 2017-2018 season.

Conclusion: During the last three years there is an increase commitment on influenza vaccination from government and other partners. Nevertheless, there is still need to keep influenza vaccination, among the priorities within the health agenda. The administration of influenza vaccine among target risk groups has been increased but remains very low among them due to lack of access to the vaccine. Meanwhile the well-functioning government influenza vaccination program of health care workers is showing good results.
21.033
Umifenovir in treatment of influenza and acute respiratory viral infections in outpatient care

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Purpose: Umifenovir - antiviral drug of a wide range of activity, acting in the early stages of virus reproduction. It disrupts the process of fusion of the viral lipid membrane with intracellular membranes. The virus-specific target of the drug in the cycle of reproduction of the influenza virus is hemagglutinin

The aim of study was to evaluate the efficacy of antiviral therapy with umifenovir in outpatient patients with influenza and acute respiratory viral infections (ARVI) without risk factors for severe course of disease.

Methods & Materials: 215 patients aged 18-74 years with influenza and ARVI within 5-36 hours of symptoms onset were randomized into 2 groups. 1st group was treated with umifenovir 200 mg qid 5 days (n=109), 2nd group (=106) received placebo according to the same scheme. Duration of disease, duration and severity of the main clinical symptoms, frequency of complications and speed of the virus elimination from the nasopharynx were estimated.

Results: The number of cases with complete recovery within 96 hours was 58.7±4.7% in 1st group and 44.3±4.7% in 2nd group (p=0.04), after 108 hours - 67.9%±4.5 and 50.4±4.9% (p=0.04). The duration of fever was 67.0±2.7 hours in group 1 and 76.9±2.5 hours in group 2 (p=0.02), muscle pain-51.1±2.1 and 59.7±2.5 hours (p=0.023), headache-51.3±2.5 and 64.4±2.3 hours (p=0.01), weakness – 75.3±2.2 and 89.8±2.3 hours (p=0.001), respectively. On the 4th day of treatment the virus was isolated in 34.9±4.6% of cases in the 1st group and 57.8±4.8% of patients in 2nd group; p=0.03. Bacterial complications were observed in 3.7±3.3% and 13.2±3.3% accordingly, p=0.04

Conclusion: The study is demonstrated the effectiveness of umifenovir in the treatment of influenza and ARVI in adult outpatient patients. The antiviral effect of the drug, which was administered within 36 hours of the symptoms onset is manifested by a more rapid reduction of clinical symptoms and their severity, frequency of bacterial complications and reduction in the time of virus elimination.
21.034
Strengthening Influenza Surveillance In South East European Countries
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Purpose: South East European (SEE) countries presented different influenza surveillance systems based on universal surveillance for influenza, different influenza diagnostic capacities and some of them less data compared to other European countries. The aim of the study was to identify influenza surveillance systems in SEE countries and challenges related to establish sentinel surveillance systems.

Methods & Materials: The surveillance of influenza and specifically influenza-like illness (ILI), acute respiratory illness (ARI), severe acute respiratory infections (SARI) and virological surveillance were assessed by using Sentinel surveillance system review tools and Sentinel SARI surveillance: Scaling implementation of WHO Regional Office for Europe guidance for sentinel influenza surveillance in humans and CDC International Influenza Surveillance Assessment Tool. The tools and questionnaires were translated and adapted into countries and discussed into focus groups of professionals and stakeholders. A desk review was performed by a national and international team. Data collection were analysed based on WHO Euro Guidelines for sentinel influenza surveillance.

Results: All countries had virological surveillance and had capacities to use molecular diagnostic methods for influenza diagnosis. All countries had universal ARI surveillance and influenza activity was measured by laboratory-confirmed cases. Only 50% of the countries had ILI sentinel surveillance. SARI sentinel surveillance was established only in 44% of the countries. Virus isolation and identification was missing in 44% of the countries. Baseline and epidemic thresholds to indicate a level of disease activity were developed only in some countries. Only three countries had web based ARI surveillance systems but the challenges of influenza surveillance data base management were enormous and shared by all countries. Only three countries had systems to monitor and strengthen influenza surveillance but even such systems were not well developed. Avian influenza surveillance activities were lacking in cross border areas since 2010.

Conclusion: None of the countries had performed assessment of their influenza surveillance system as a crucial activity for monitoring the timing and severity of influenza and documenting the change in the epidemiology of influenza infection. The countries need to increase their capacities on ILI sentinel surveillance especially to establish and improve SARI surveillance and their database management systems.
Epidemiological Characteristics Of Severe Acute Respiratory Illness Cases At National Influenza Central Laboratory Pakistan (NICLP) Centre Of Sentinel Surveillance. September 17 To February 2018

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Purpose: To describe the epidemiology of SARI, identify influenza positivity and circulating influenza subtypes among SARI patients.

Background: Severe acute respiratory illness (SARI) is recognized as a leading cause of morbidity and mortality globally. SARI is a rapidly progressive illness caused by pathogens like influenza having pandemic potential. In 2008 a sentinel lab-based influenza surveillance network was established in Pakistan in collaboration with CDC having objectives to assess trends of Influenza-like-Illness (ILI) and Severe Acute Respiratory Illness (SARI).

Methods & Materials: A descriptive epidemiological study was conducted based on influenza surveillance data obtained from NICLP from September 2017 to February 2018. Study was done from data records and samples of suspected SARI patients received at NICLP. Patients who met standard WHO-SARI case definition (fever=38+cough with or without any other cause in the last 10 days in a hospitalized case) Samples were tested at NICLP for confirmation by RT-PCR. Frequencies were calculated and data analyzed as per time, place and person.

Results: A total of 1500 suspected samples were received out of which 471 met SARI case definition. Most affected age group was 30-39 years with n=94 (20.0%). Median hospital stay was 5 days. During hospitalization 124 (26.3%) were ICU admissions, out of them 2 (0.42%) were on ventilator. 83 (17.6%) were mechanically ventilated. Of all SARI admissions 50.1% (236) were positive for influenza viruses, among positive 95.0% were Influenza A. 61% were A/H1N1, 33.9% were A/H3N1, 5.0% were influenza B. Travel history was positive in 8.9%. Contact history with flu patient was 11.5% (54). Males were 50.1% (263). Among females 1.25% females were pregnant. Among positive cases 176 (74.6%) were reported from Rawalpindi. Majority of cases were reported in January (277) followed by February (112).

Conclusion: Influenza viruses are frequent cause of SARI admissions, so developing strategies to control influenza is a key stone to reduce SARI burden. Based on the results, policy for inclusion of flu vaccination on annual basis for improved vaccination coverage is recommended for general community especially high risk groups.
21.036
Diagnostic Devices to Reduce the Threat Posed by Antimicrobial Resistant Pathogens

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Purpose: The U.S. Department of Defense (DoD) is developing novel field-forward medical diagnostic systems to combat the threat posed by antibiotic resistant biological threat agents. The Defense Threat Reduction Agency (DTRA) is addressing this by (1) discovering host-response biomarkers that can discriminate between bacterial and viral infections, and (2) developing a system that can perform antimicrobial susceptibility testing in less than 6 hours.

Methods & Materials: DTRA has partnered with industry to develop two separate medical diagnostic systems with different objectives and goals. The first device will discriminate between bacterial and viral infections by measuring the levels of three different host-based protein biomarkers in a drop of blood within 15 minutes. The second device combines single-cell rapid microfluidic channels with electrochemical detection for identification of resistant bacteria and determination of minimal inhibitory concentration (MIC).

Results: Resulting data shows that the first device can measure protein biomarkers in whole blood at concentration levels ranging from µg/ml to pg/ml. Furthermore, the set of biomarkers demonstrated sensitivity and specificity of 94% and 93%, respectively. The set of biomarkers have been validated in over 10,000 patients in multiple studies between 2009 and 2018. The second device showed real-time single-cell antimicrobial susceptibility testing by measuring growth activity in the presence of varying concentrations of different antibiotics. Preliminary data demonstrated that \textit{E.coli} was inhibited in the presence of clinically relevant levels of ampicillin versus non-inhibited \textit{E.coli} in control broth. In addition, MIC was obtained in less than 6 hours versus that of more traditional methods (24-48 hours).

Conclusion: While both of these systems are in various stages of research and development, DTRA’s goal is to seek U.S. Food and Drug Administration clearance within the next 3 years. Both devices support antibiotic stewardship and inform better medical treatment using different tactics. The success of these two complimentary approaches will significantly impact the way the DoD and public health sector combats threats posed by antibiotic and multi-drug resistant bacteria.
Purpose: Portal hypertension (PH) is in charge of a large portion of the aggravated conditions, namely variceal hemorrhage, ascites and hepatic encephalopathy that denote the transition from compensated to decompensated cirrhosis. Viral hepatitis B is one of the major causes that leads to hepatic cirrhosis. In patients with cirrhosis, PH is associated with increased intrahepatic vascular resistance and increased portal flow. Hepatobiliary fibrosis results in vascular compression due to collagen deposition around the sinusoids and the formation of regenerative nodules, which play a major role in increasing intrahepatic vascular resistance. Due to the invasiveness, requirement for advanced technical expertise and high costs associated with HVPG measurements, the introduction of simple, noninvasive screening and diagnostic methods would represent a great clinical advancement. The purpose of the study is to evaluate the diagnostic efficacy of noninvasive liver fibrosis indexes in the diagnosis of PH.

Methods & Materials: This is a prospective study conducted in the period 2012-2016 including 87 cirrhotic patients. These patients are partly admitted at the Department of Gastrohepatology at University Hospital "Mother Teresa" in Tirana and partly also followed at the polyclinic of specialties in Tirana. A multivariate logistic model of serum markers showed that AST-to-platelet (PLT) ratio index (APRI), AAR (AST/ALT ratio), Fib-4, fibrosis index (FI) were associated with PH. Also portal blood flow was measured and upper endoscopy was performed. Actually they couldn't undergo hepatic venous pressure gradient (HVPG) evaluation.

Results: For the diagnosis of cirrhosis AUCs were 0.879 and 0.851 for APRI and Fib-4 respectively and predicted the presence of clinically significant portal hypertension (CSPH), with the highest PPV (94%) and (93.3%). From the comparison of the ROC curves between the fluximetry (echo Doppler) and the seric markers APRI and Fib-4, no significant difference was found between them (pairwise comparison of ROC APRI-Echo_Doppler curves p=0.8, Echo_Doppler ~ Fib_4 p = 0.5) suggesting that these serial markers can be successfully used in clinical practice.

Conclusion: Noninvasive liver fibrosis indexes could be used not only as a first-line screening method for CSPH but also for predicting esophageal varices (EV) in cirrhotic patients as well as proxy for fluxmetric measurement.
Pre-analytic Assessment of Lassa Fever Blood Samples at Irrua Specialist Teaching Hospital Laboratory, Irrua, Edo State, Nigeria, 2017

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Purpose: Pre-analytic errors account for about 68% of laboratory errors and may affect test result, increase turnaround time, increase treatment cost and may lead to death. Early diagnosis and treatment of Lassa fever improves clinical outcomes. Irrua Specialist Teaching Hospital (ISTH) is a Referral Center for the diagnosis and treatment of Lassa fever. We assessed the pre-analytic quality of blood samples, their effects on test results and determined the difference between samples from ISTH and samples sent from other facilities.

Methods & Materials: We conducted a cross-sectional study from January to April 2017. All samples were assessed with a checklist until sample size of 400 was complete. Independent variables included documentation, volume, packaging, time in transit, temperature and sample container while the dependent variable was test result (negative or positive, assuming test result to be >95% sensitive). Sample quality (good, fair, poor) was determined by maximum performance score of 5, 4 and ≤3 respectively. Logistic regression analysis was done to determine effects of sample quality on test result at P value ≤ 0.05 and 95% confidence interval.

Results: Of the 400 blood samples, 228 (57%) were from ISTH. Poor packaging was the most frequent (77.5%) observed error while wrong container was the least frequent (0.25%). Sample rejection rate due to spilled sample, wrong container and peeled labeling was 1.3% and were referral samples. Mean score of sample quality was 3.74 ±0.75. Based on performance score for each sample, 52 (13%) of the samples were of good quality, 209 (52.2%) were of fair quality, while 139 (34.8%) were of poor quality. Eighty-three (20.8%) were positive for Lassa virus. Only documentation was not significantly associated with the test result after bivariate analysis. After multivariate analysis, volume (O.R=0.46, p=0.05) and packaging had significant effects on test result. (O.R=2.62, P<0.01). There was no difference between quality of sample from ISTH and other health facilities.

Conclusion: Pre-analytic error ranged from 34.8% to 52.3%. This fell within WHO range of pre-analytic errors. Samples with sufficient volume and triple packaged are more likely to have positive results. Training of health workers on sample management by ISTH was recommended and implemented.
Evaluation of Diagnostic Potential of Recombinant D-erythulose 1-phosphate Dehydrogenase Using Indirect Enzyme-linked Immunosorbent Assay for Diagnosis of Bovine Brucellosis

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Purpose: Serological tests used for diagnosis of bovine brucellosis are usually depending on smooth lipopolysaccharides (S-LPS) as a diagnostic antigen which usually gives false positive reactions. So, our study aims to produce and evaluate a diagnostic kit for accurate diagnosis of bovine brucellosis differentiating between vaccinated and infected cattle and exclusion of false positive cases. Idea of this kit depends upon the fact that The eryC gene is absent in Brucella abortus S19 only but it is present and functional in all other Brucella strains and isolates so according to these facts, the use of ELISA kit coated with single subunit (recombinant) EryC protein may be useful, rather than S-LPS, as an alternative diagnostic antigen in diagnosis of bovine brucellosis and differentiation between S19 vaccinated and Brucella infected cattle. The present study evaluated antibody responses of brucellosis infected and S19 vaccinated cattle to purified recombinant EryC protein in an indirect enzyme-linked immunosorbent assay (I-ELISA). Cattle sera were screened using Rose Bengal Plate test (RBPT).

Methods & Materials: 114 samples of naturally infected cattle (Rose Bengal test positive), 78 sera from S19 vaccinated cattle and 25 sera samples from Brucella free cattle were used in this study. I-ELISA using S-LPS and periplasmic proteins as a coating antigen were used as a gold standard test.

Results: The results revealed that in case of sera of naturally infected cattle, sero-positivity was 94.7%, 100%, 100% and 100% with EryC-ELISA, LPS-ELISA, periplasmic-ELISA and Rose Bengal test respectively. Where in case of sera of S19 vaccinated cattle, all samples were negative when tested with EryC-ELISA while in case of LPS-ELISA, periplasmic-ELISA and rose Bengal test, sero-positivity was 92.3%, 84.6% and 100% respectively.

Conclusion: It could concluded that the EryC protein could be used in serological tests for diagnosis of bovine brucellosis and differentiation between infected and Brucella abortus S19-vaccinated cattle but more studies are needed to be done on large cattle populations accompanied with bacteriological isolation to detect the sensitivity and specificity of this protein as a diagnostic antigen and also for validate this test.
Generation, Lyophilisation and Epitope Modification of High Titre Filovirus Pseudotyped Lentiviruses for use in Antibody Neutralisation Assays

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Purpose: Filoviruses, such as Ebolavirus, are zoonotic pathogens causing disease outbreaks with high mortality rates, requiring scarce high containment facilities for research. Nevertheless, pseudotyped viruses (PV), consisting of a lentiviral core (plus luciferase reporter) and the envelope glycoprotein (GP), allow basic and translational virology to be conducted under low containment. Consequently, filovirus PVs were generated and viability assessed after lyophilisation and long-term storage. Next, antibody neutralisation tests were performed using native and hybrid GPs to assess differentiation between genera and species.

Methods & Materials: PVs were produced using a 3-plasmid transfection system (representing core, reporter and envelope) in HEK293T/17 cells, and supernatant titrated. Supernatants were then lyophilised in sucrose cryoprotectant solution, stored under various conditions, reconstituted and titrated. For antibody neutralisation tests, serially diluted, polyclonal convalescent sera (NIBSC, UK) or anti-GP monoclonal antibodies (Xiangguo Qiu, PHA, Canada; Erica Saphire, Scripps, USA) were incubated with PV for 1h at 37°C, prior to titration. To create artificial GP antigens, EBOV neutralising epitopes were inserted into the GP of another genus (Cuevavirus; LLOV) by mutagenesis, PVs generated and infectivity and neutralisation assessed.

Results: High titre PVs were produced with titres between ~1x10⁸ RLU/mL (Ebolavirus/Cuevavirus) and ~1x10¹⁰ RLU/mL (Marburgvirus). Lyophilised PV titres remained constant stored at -20°C and 4°C for 12 months, while PVs kept at room temperature (22.5°C) demonstrated titre decreases of up to 3 orders of magnitude after 6 months. At 37°C, five log (Marburgvirus) or three log (Ebolavirus and Cuevavirus) decreases occurred after one month.

Zaire Ebolavirus (ZEBOV) antibodies showed no cross reactivity with native LLOV PVs. Furthermore, ZEBOV epitopes inserted into the LLOV GP and expressed on PVs had no significant impact on PV infectivity, and ZEBOV neutralising epitopes were successfully reconstituted in these chimeric antigens.

Conclusion: In this study, high titre PVs were generated. These were found to be amenable to lyophilisation and long-term storage. Reconstituted PVs retained their function in neutralisation assays suggesting their structure is not compromised during freeze-drying. Insertion of epitopes in heterologous GPs did not impact infectivity or functionality. This data suggests a PV-based serological kit could be utilised in resource-limited countries for serological studies, after simple refrigeration storage.
Purpose: Background: Subdural spinal abscess is a rare pathology, the cause of which is generally found to be Staphylococcus Aureus, mostly affecting the thoraco-lumbar part of the vertebral column. RM is a means of diagnosing the pathology. We will be presenting the medical case of a diabetic patient with a paraspinal abscess in the lumbar region of the vertebral column.

Methods & Materials: Case overview: 46 y.o. and has been diagnosed with Mellitus Diabetes. He came to our emergency room disoriented, with a high fever, and nuchal rigidity.

Results: Five days history of fever and lower back ache. After the initial examination, a CT scan of his head was conducted; seeing that it did not show an issue, we went through with the lumbar drilling, the result of which showed an abundance of polymorphonuclear and bacteria, which turned out to be Staphylococcus Aureus. Through an RM we were able to detect intraspinal abscesses on the levels of L3 to L5 with inflammatory differences on the parasternal muscles. Inflammation of the Posterior Dura with the aspect of Epiduritis. Arachnoidal cyst spreading from T8-T11, accompanied by a compression of the Spinal Medulla. The patient was treated with Vancomycin and Insulin-therapy. The patient recovered 37 days later.

Review of the information: including our patient, there are 66 documented and published cases of the paraspinal abscess.

After reviewing these cases we conclude that: The main cause in 34 cases is Staphylococcus Aureus. The most frequent affected area is the lumbar region. The patients are mostly 60 to 70 years old, but the vast majority of them have one or more of these predisposing factors: mellitus diabetes, chronic etilism, tumour, HIV, or abnormalities of the vertebral column.

An outline of the clinical events:
1. Fever and pain
2. fever, lower back pain, and neurological deficits
3. total paralysis below the abscess
CT and RM scans are used in the placing of the diagnosis.
The treatment depending on the different stage of the illness includes therapy with antibiotics and/or surgical drainage.

Conclusion: Even though the patient was immunocompromised, the early diagnosis and the immediate therapy brought about the healing of the patient.
An Investigation Of The Use Of Gas Chromatography Mass Spectrophotometry (GCMS) For Diagnosing Cryptosporidium Infection In Children In Malawi

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Purpose: Cryptosporidium spp., which infect a broad range of vertebrates, are major causes of diarrhoeal disease in humans worldwide. 1. To define and analyse VOCs emitted from the faeces of patients with gastrointestinal disease. 2. To compare the VOCs present in the Cryptosporidium positive faecal samples with the VOCs present in the control samples. 3. To identify any potential biomarkers of Cryptosporidium spp. infection.

Methods & Materials: Faecal samples were collected from children <5 years of age in Malawi. Stool samples from patients with confirmed isolated Cryptosporidium infection and negative controls, stool samples were also collected from patients that had diarrhoea but no Cryptosporidium infection. We tested the hypothesis that the pattern of excreted Volatile organic compounds (VOCs) could be specific to certain types of diarrheal infection. VOCs are a group of very small carbon-based molecules which have a high vapour pressure at room temperature due to their low boiling points. A novel method, Gas Chromatography/Mass Spectroscopy (GCMS) was used to examine the VOCs from human faeces from Malawi that contained Cryptosporidium. The technique for extraction of VOCs has been developed especially for use in faecal samples. The VOCs have been catalogued from faeces in negative control (non-infected controls). Volatiles were collected by solid-phase microextraction (SPME) and then they were analyzed by GCMS. AMDIS and MetaboAnalyst 2.0 programme was used for data analysis. Statistical analysis has been used to compare the VOCs of infectious diarrhoea by Cryptosporidium spp. from humans.

Results: When compared to non-infected controls, statistical analysis found several VOCs the abundance of which were significantly different in the two groups, cyclopentane and 3-hydroxy-2-butanone were associated with Cryptosporidium positive samples, 1-propanol with negative controls. The presence of cyclopentane and 3-hydroxy-2-butanone and the absence of 1-propanol in Cryptosporidium positive faecal samples show the potential to be biomarkers of the infection and could form the basis of a diagnostic test.

Conclusion: The discovery of two biomarkers for Cryptosporidium spp. was a significant finding of this work, demonstrating the possibility for detecting disease states utilising the “smell” technology”. This would lead to an easy and quick diagnostic tool being available for the investigation of the disease.
Assessment Of The Hepatitis E VirClia® System For The Detection Of IgM And IgG Antibodies Against HEV

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**Purpose:** HEV is transmitted to humans through the faecal–oral route, mainly by the consumption of contaminated water or undercooked meat from infected animals. Diagnosis of acute hepatitis E infection is based on the detection of specific IgM antibodies to the virus in serum. Additional tests include RT-PCR to detect the HEV RNA. The IgG detection is used to define prior contact and sometimes to confirm seroconversion, for follow-up of acute cases. The aim of this study is the assessment of a new serological method that focuses in sensitivity and it is compared to other methods previously assessed.

**Methods & Materials:** Samples have been processed in VIRCLIA equipment of Chemiluminescent CLIA (HEV based on the capture of IgM present in the sample by antiimmunoglobuline antibodies and IgG, by antigens attached to the surface of polystyrene. Two different panels have been configured:

- **IgM Panel:** 52 samples including 13 serum samples from patients with acute hepatitis and genotype 3 virus infection (confirmed by Immunoblot and molecular methods) and their serial dilutions 1:10, 1:100 and 1:1000.
- **IgG Panel:** 40 samples including 10 serum samples from seroconversion-phase patients after acute infection and their serial dilutions 1:10, 1:100 and 1:1000.

These panels have been used to evaluate other screening methods (Wantai Biological Pharmacy, Euro-Immun Medizinische, MP biomedicals, Dia.Pro diagnostics bioprobes, DSI S.R.L., Saronno, Italy. Mikrogen GmbH Neuried and HEVTOP All Diag), which results have already been published.

**Results:** Anti HEV-IgM: All tested specimens had positive results in undiluted specimens, at 1:10, and at 1:100. Of all samples 77% were positive (40/52).

Anti HEV-IgG: All tested specimens had positive results in undiluted samples and dilutions 1:10 and 7 of them also reached the 1:100 dilution. Of all samples 77.5% were positive (31/40).

**Conclusion:** Compared to other methods The HEV-IgM VirClia® System has greater sensitivity and it is only exceeded by the DSI system. The HEV-IgG VirClia® System has greater sensitivity with the exception of the Dia.pro system, which has the same sensitivity.

The Chemiluminescence VIRCLIA® method is presented as a reliable serological method for the screening of acute infection by HEV in clinical specimens.
Purpose: The spread of infectious disease continues to present a challenge for modern global public health initiatives, as was evidenced by the recent Ebola outbreak in West Africa. In disease outbreaks, diagnostics are the first line of defense in identifying the causative agent, treatment decisions, and control and prevention of future outbreaks.

Methods & Materials: We designed a multiplexed immunoassay centered on detection of viral antigens in serum/plasma samples for EBOV, LASV, MARV, CCHFV, RVFV, alphaviruses, and flaviviruses. This flexible, immunoassay system, based on the MAGPIX® platform has faster sample-to-answer time over traditional methods and higher throughput, as assays can be multiplexed. Assays were developed and validated against live and inactivated viral supernatants and other antigen types. Inclusivity and exclusivity panels were run to ensure no crossreactivity between assays.

Results: Each assay was able to detect the appropriate target, whether in inactivated, recombinant, and live agent form. Limits of detection were generated in plaque forming units (pfu) per ml of live agent. Limits of detection were also established for appropriate recombinant reagents when available, as these reagents serve as the positive controls for the multiplexed assays when deployed to overseas partners.

Conclusion: Development of robust and sustainable immunoassays is important as they have great potential for use during outbreak events to provide a more complete picture of the presence of viral agents. Immunoassays are capable of providing further insight to shape outbreak response. The recent Ebola outbreak demonstrated the great sensitivity of RT-PCR, but often patients remained positive by PCR well after other symptoms subsided. An integrated diagnostic system that uses sensitive and specific RT-PCR in combination with higher throughput, multiplexed immunodiagnostic methods can provide the highest confidence in a diagnostic result.
Purpose: Pre-analytic errors account for about 68% of laboratory errors and may lead to testing and retesting, increased cost and patient death. We assessed the knowledge of health workers in the endemic states of Ondo and Edo on Lassa fever pre-analytic sample management and also determined the effect of training on their practice of sample management.

Methods & Materials: We conducted a hospital-based cross-sectional study in March and April 2017. Ninety-five health workers from four selected health facilities were self-administered questionnaire out of sample size of 157 due to low number of health workers handling Lassa fever samples. Maximum performance score was put at 14 for knowledge of Lassa fever and 6 for knowledge and practice of sample management respectively. Univariate, bivariate and multivariate analysis was carried out to determine effect of training and socio-demographics on knowledge and practice at P value ≤ 0.05 and 95% confidence interval.

Results: Of the 95 respondents, 44 were male. (M: F ratio= 1:1.3). Majority (43%) were doctors. Mean age was 34.0 ±8.8. Ninety-six percent had good knowledge of sample management and 88.4% had good practice. Seventeen percent of doctors had poor practice while no laboratory worker had poor practice. Mean score of knowledge of sample management and practice of sample management was 4.7±1.1 and 4.0± 1.3 respectively. All 49 (53.9%) trained health workers had good knowledge. Of these, 47 (95%) had practice above 50%. Fourteen (93.3%) of Scientific officers were trained while 14 (34.2%) of doctors were trained. Health facility, education and training had significant effects on knowledge and practice of sample management (p<0.01). Correlation coefficient between knowledge of sample management and practice of sample management was 0.84 (t = 10.57, p<0.01).

Conclusion: Health workers generally had good knowledge and practice. Training is associated with good practice of sample management. Continuous training of health workers on sample management is therefore recommended.
Mass Campaigns For HIV, HBV (HBsAg) and HCV Screening by Multiplex Rapid Diagnostic Test in Sub-Saharan Africa Using Mobile Units: The game Changer

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Purpose: Chronically infected individuals by HIV, HBV or HCV are at-risk of spreading their infections and developing AIDS-related illnesses and viral hepatitis-associated liver cirrhosis and cancer. HIV, HBV and HCV testing is fundamental for both prevention and treatment services, in order to reach an effective response to these three chronic viral infections. We herein report our experience using mobile units in combination with multiplex rapid diagnostic test (RDT) for simultaneous detection of HIV, and HCV- specific antibodies (Ab) (IgG and IgM), and HBV surface antigen (HBsAg) during several free screening campaigns in health districts of urban and rural cities of Cameroon.

Methods & Materials: From February to April 2018, 1206 volunteers (665 men, 541 females; mean age 31.9 years and range, 18-91) were enrolled. Blood samples were collected after informed consent and submitted to parallel screening by multiplex HIV/HCV/HBsAg (Triplex, Biosynex, France) and CE-labeled ELISAs for HIV, HBV (HBsAg) and HCV (Human Diagnostics, Germany), as reference assays. Volunteers were screened and their results given back to them.

Results: Out of these 1206 volunteers screened, 104 (8.6%) were positive for HBsAg, 25 (2.1 %) for HIV and 27 (2.2%) for HCV. All samples positive and negative by multiplex RDT were further confirmed by reference HIV, HBV and HCV ELISAs, demonstrating 100% sensitivity and specificity, as well as excellent concordance between multiplex RDT and ELISAs (Cohen’s k =1). Only one blood sample was positive for both HIV and HBsAg. All volunteers who were seropositive by multiplex RDT for HIV, HBV or HCV were referred to counselling and treatment centers. There was in our series no lost to follow-up in the transition to reference health care centers, demonstrating that a potential benefit of multiplex RDT results is the ability to immediately establish continuum of care.

Conclusion: In populations at high-risk of chronic viral infections such as people living in sub-Saharan Africa, the simultaneous use of low cost multiplex HIV, HBV and HCV RDT in combination with mobile units may clearly improve the “cascade of screening”, the prevention strategies and the linkage-to-care with reduced cost and also contribute to achieve the United Nations 90-90-90 targets.
Purpose: To develop antigen detection test for active tuberculosis.

Methods & Materials: The selected novel proteins named as Rv2145c (SS1), Rv1437 (SS3), Rv1827 (SS4), and Rv2970c (SS5) were expressed in E.coli BL21DE3 cells and purified by affinity column chromatography. Furthermore, Mice were injected with recombinant proteins and Freund's adjuvants. The monoclonal antibodies (mAbs) were developed using these antigens via hybridoma technology. Hybridoma clones were screened and positive clones was selected for further experiment. mAbs was purified from cell culture supernatant using protein A affinity chromatography. The diagnostic potential of these mAbs were investigated using a well characterized cohort of tuberculosis patients (Pulmonary-TB, Extrapulmonary and healthy subjects sera) using sandwich ELISA.

Results: Our experimental results show admirable sensitivity of 94.59% (SS1), 86.49% (SS4) and 93.24% (SS5) while specificity was 98.29% (SS1), 96.57% (SS4) and 97.71% (SS5) in antigen detection test.

Conclusion: The developed mAbs could be used as novel biomarker for detection of active TB directly from patient sera.
Purpose: Biological confirmation of viral diseases requires the detection of the presence of viruses in cell culture. Given the delicacy of the technique, very few institutions are specialized in the production of cell lines. By using the bone marrow of pigs, we managed to set up a cell line from macrophages.

Methods & Materials: A 3-month-old Large White swine from a non-African swine fever infected farm was used in this study. After anesthesia, it was euthanised with phenobarbital, the humeral bone of the swine was removed and 10 ml of jugular vein blood was collected in an anticoagulant (sodium citrate) for the extraction of red blood cells. After decantation, the red blood cell pellet was stored in a refrigerator at 4 °C. To be later used in the preparation of the growth medium 2. This growth medium is changed and used after 24 hours of incubation of the cells before the infection of these.

Results: After a series of operations, cells were collected from the humeral bone in a microplate and placed in a CO₂ incubator at a temperature of 37°C. Twenty-four hours later, all the cells had settled at the bottom of the microplate thus forming a cell mat visible with the aid of an inverted microscope. These cells were then infected with African swine fever virus and incubated under the same atmospheric conditions. Seventy-two hours after incubation, the reaction of the red blood cells was clearly visible around the macrophages indicating a viral infection.

Conclusion: These results show that cells (macrophages) obtained from porcine bone marrow can be used to isolate African swine fever virus. Subsequent studies are required to test the susceptibility of this line to other viruses, including those of emerging diseases such as Ebola, Marburg, RVF (Valley Fever. of Rift) etc.
Purpose: The endorsed conventional methods for the diagnosis of tuberculosis are cumbersome and time consuming whereas molecular methods are expensive. The serological methods are easy and cost effective, but after finding currently available serological methods very inferior, these tests were banned in 2011 by Government of India and WHO. In this study, we identified the protein biomarkers by comparative proteomic analysis of sensitive and MDR clinical isolates and cloned four specific genes, which were overexpressed during drug resistance development.

Methods & Materials: The selected four genes were cloned in expression vector and expressed proteins were purified and named these as SS1, SS3, SS4 and SS5. The diagnostic potential of these antigens was investigated using a well characterized cohort of pulmonary and extrapulmonary tuberculosis patients using dot-blot, ELISA and flow through immunoassay methods. The sera from confirmed pulmonary-TB (n=110), extrapulmonary-TB (n=50), MDR-TB (n=50) diseased controls (n=60) and healthy subjects (n=50) were included.

Results: Our experimental results of dot-blot and flow through immunoassay shows sensitivity of 99.1% (SS1, SS5), 98.2% (SS4) and 93.7% (SS3); specificity were 100% (SS1), 98.2% (SS5), 93.6% (SS4) and 87.5% (SS3). While in ELISA for MDR-TB prediction shows the sensitivity of 100% (SS1, SS4, SS5) and 79.2% (SS3). The specificity was 98.2% (SS5), 97.3% (SS1), 94.5% (SS3) and 86.7% (SS3).

Conclusion: Our results indicate that these proteins could be used as novel biomarker for development of point-of-care device for detection of both form (PTB and EPTB) tuberculosis directly from patient sera.
A new approach to outbreak management for Bovine Cystic Echinococcosis cases in hypo-endemic areas

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Purpose: Cystic Echinococcosis (CE) surveillance in Italy is based on detection of the larval phase (hydatid cysts) at the slaughterhouse. In northern Italy, a hypo-endemic area, local health authorities investigate each individual farm with positive animals to treat their dogs with cestocidal drugs, but this system is time-consuming and poorly effective for bovine farms. This study applied a new approach based on targeted epidemiological surveys in areas with aggregation of bovine CE cases, and compared the outcome with that of two control areas with farms individually investigated.

Methods & Materials: The presence of territorial cluster of bovine farms with CE cases was investigated for three consecutive years (2013-2014-2015) in a high-risk area of Veneto Region (north-eastern Italy), using a spatial scan statistic. Epidemiological investigations, consisting of a questionnaire survey and canine faecal samples collection, were conducted in cluster and control areas. All faecal samples were analyzed for taeniids eggs and positive samples were sequenced and identified at the species level.

Results: In total, 99 farms were surveyed and 208 faecal samples were retrieved from dogs. Sixty-two farms (42 bovine and 20 sheep) were investigated in cluster areas and 37 farms (33 bovine and 4 sheep) in control areas. Based on the results of the cestode egg isolation procedure, 14 animals (6.7%) were positive to taeniid eggs. For molecular analysis, two dogs resulted positive to Echinococcus granulosus, and seven to Taenia hydatigena. Twelve positive dogs were found in targeted survey areas and ten of these dogs were shepherd dogs, belonging to transhumant sheep flocks known to pass in cluster areas.

Conclusion: The new approach demonstrated to successfully identify the probable source of infection of CE positive bovines. Most of positive dogs belonged to transhumant flocks, underlining the importance to include shepherd dogs in the surveillance system for CE.
Purpose: Detecting disease caused by novel pathogens is a challenge in surveillance. Syndromic surveillance is recognised as a key tool, agnostic to cause that may augment outbreak detection of emerging pathogens. Potential target syndromes include: rapidly progressive pneumonia, fever and rash, and acute neurologic syndromes. The Australian Childhood Encephalitis (ACE) study involves nationwide, active, syndromic surveillance that aims to define the epidemiology of this severe disease. Here we present its demonstrated contribution to outbreak detection and response.

Methods & Materials: We prospectively identified children aged 0 to ≤ 14 years with suspected encephalitis at five tertiary paediatric hospitals from May 2013 using the Paediatric Active Enhanced Disease Surveillance (PAEDS) network. Monthly reports of case numbers are generated, and compared with expected site case numbers. Confirmed encephalitis cases were categorised into the etiologic sub-groups: infectious, immune-mediated or unknown.

Results: Five distinct epidemics of encephalitic infectious disease have been identified by the ACE study since 2013. In mid-2013, during its pilot phase, the ACE study identified cases of encephalitis associated with an enterovirus 71 (EVA71) outbreak in New South Wales. Subsequently in late 2013, the ACE study contributed to the identification of the first outbreak of human parechovirus (HPeV Type 3) in Eastern Australia. It has subsequently captured the first signals of two further biennial, national outbreaks of HPeV (2015/16; 2017/18). In mid-2015, the ACE study identified a cluster of M.pneumoniae associated encephalitis that revealed otherwise unrecognised epidemic M.pneumoniae disease Sydney, NSW. Additionally, across 5 influenza seasons, the ACE study has identified cases of influenza associated encephalitis/encephalopathy (IAE) and comprehensively described a previously inadequately recognised burden of severe disease in children. In each context, we liaised with public health authorities, contributed to development of public health responses, and facilitated information sharing across jurisdictional boundaries.

Conclusion: Our surveillance has shown childhood encephalitis to be associated with epidemics of important emerging pathogens amongst children in Australia. The ACE study has contributed real-time, comprehensive data to enhanced outbreak surveillance and so supported public health responses. We provided detailed clinical review of outbreak associated encephalitis cases and thereby defined the most severe end of the disease spectrum.
Purpose: The detection, confirmation and monitoring of cholera outbreaks in many developing countries, including Mozambique, is a major challenge due to the lack of resources needed and the time required for confirmation. Culture method, which is the gold standard, requires 48 to 72 hours, qualified laboratory personnel and expensive reagents.

During October 2016, a mass vaccination campaign against Cholera (Shanchol ™) took place in the city of Nampula. The campaign aimed to reach 193,403 individuals from the six most vulnerable neighborhoods for Cholera in the city, after which surveillance for the evaluation of the effectiveness of the OCV was implemented to detect and document the occurrence of possible cases.

Methods & Materials: The surveillance took place between March and December 2017 at the local central hospital Pediatrics and the city’s Diarrheal Disease Treatment Centre. These cases were confirmed laboratorially by culture and rapid test, and for the latter, collected stools were incubated in APW for 6 hours at room temperature prior to testing following the manufacturer’s instructions and then cultured to isolate *V. cholerae* in the standard procedure.

Results: Stools were tested from 171 patients with suspected cholera in the pediatrics (n = 86) and in CTDD (n = 85). Forty-four (44) samples were positive and one hundred twenty-seven (127) samples were negative. Of the 171 samples, 75 were tested for both methods and produced the same results.

Conclusion: Our findings have shown that the rapid Cholera test modified after APW enrichment is an accessible and rapid alternative tool for monitoring cholera outbreaks in resource-limited settings. However, the culture method should remain to confirm the cholera epidemic, to monitor antibiotic susceptibility and to produce pure isolates for molecular characterization.
The impact assessment of Schedule H1 on the sales of over-the-counter antibiotics in India: Evidence from quasi-experimental research design

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Purpose: The Schedule H1 of Drugs and Cosmetics Act of Government of India sought to regulate over the counter antibiotic sales in the private market. The objective of this research is to conduct impact evaluation of this policy intervention.

Methods & Materials: The intervention is a policy for reducing over-the-counter sales of antibiotics named Schedule H1 of Drugs and Cosmetics Act of Government of India implemented through/on retail pharmacies on selected antibiotic notified under the new Schedule H1. Monthly data on the antibiotics sold in the private retail market were studied. An ITS model controlling for seasonality and autocorrelation was used to examine the dataset for significant level and trend shifts.

Results: Our preliminary analysis suggest a post intervention decrease in the average monthly market share of antibiotics under Schedule H1 by 1.56 percent which was significant (p<0.05) and a decrease in the trend by 0.041 percent which was not significant (p>0.05). The ITS model adjusted for seasonality and autocorrelation suggests slight reduction effect size i.e. -1.53% from -1.56% in level change but no change in the trend estimate.

Conclusion: The segmented regression analysis of pharmaceutical sales is a robust method for measuring the impact of interventions directed towards medicines utilization. The Schedule H1 intervention was associated with significant decrease in over-the-counter sales of the notified antibiotics initially but the reduction in sales or the impact of the intervention could not be sustained over long term.
Purpose: To make a priority of approach for Diphtheria outbreak in Samarinda City using GIS for all cases and analyze with SatScan

Methods & Materials: 106 cases were GIS tagged. 3 variables were analyzed ie. nutritional status, ages, gender. All data were analyzed using SatScan (licensed). 60 children under 7 years old with undernutrition were enrolled and GIS Tagged, an were put into two groups based on their gender. Data is analyzed to apply statistical methods using Survival Analysis in STATA 9.2 (licensed).

Results: SatScan analysis revealed that 3 clusters of diphtheria with undernutrition status. The outbreak priority management and cases approach were based on this analysis.

Conclusion: Diphtheria cases with undernutrition status were clustered and revealed based on GIS analysis. The management of outbreak based on GIS analysis were efficient enough and useful to shortened the outbreak period.
Infection Prevention Decision Support For Emerging Infectious Diseases: A Qualitative Assessment From The 18th International Congress On Infectious Diseases

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Purpose: The International Society for Infectious Diseases (ISID) is a non-profit organization committed to the prevention of infectious diseases around the world. Since 1998, the ISID has produced and distributed “A Guide to Infection Control in the Hospital” - an open access resource for Infection Prevention and Control (IPC). In the wake of the Ebola and Zika epidemics, and in addition to prior feedback received from more than 1,100 Guide users, focus group interviews were conducted. In this study, we provide a qualitative assessment of focus group responses, with an emphasis on the relevance of the guide to infection prevention and surveillance decision support for emerging infectious diseases.

Methods & Materials: Focus group interviews were conducted at the International Congress on Infectious Diseases in Buenos Aires, Argentina in March 2018. All Congress attendees were invited to participate. Moderators in small group settings used standardized questions, and insights from those discussions were transcribed. Qualitative analysis was performed to evaluate areas of need in regards to IPC resources in general, and the Guide in particular. Results pertaining to emerging diseases and IPC are highlighted here.

Results: Forty health care workers from 12 countries participated in the focus group discussions. 57% of participants identified country of residence as Argentina. Participants were divided into five groups in order to facilitate discussion. A moderator affiliated with the Guide led each group. Four distinct themes emerged in relation to IPC needs for emerging infectious diseases; (1) Update recommendations frequently to address new emerging diseases and reflect the evolving understanding of established outbreaks (2) Target recommendations towards resource-limited settings (3) Provide strategies for IPC practice implementation and surveillance practices across various resource levels (4) Ensure accessibility through various channels including mobile and desktop.

Conclusion: Our focus group responses demonstrate the need for practical IPC at the point of care, while addressing best practices and disease surveillance across a wide range of resource settings. Infection prevention and effective surveillance supported by current, evidence-based, and easily accessible decision support resources are critical in the setting of outbreaks of emerging infectious diseases.
Purpose: In Albania, regular epidemiological telephone conferences (EpiTel) between surveillance staff from national (Institute of Public Health, IPH) and subnational levels (12 prefectures and 24 districts) are a new tool for communicating, discussing, linking information related to infectious diseases events. Following the experience of Germany, Albania developed and established EpiTel in May 2017 in the scope of the German Global Health Protection Programme. After seven months of implementation, IPH conducted an evaluation in order to measure usefulness and identify strengths and weaknesses of EpiTel.

Methods & Materials: In a first step, IPH analyzed the level of participation, reporting, and the most frequently discussed events from the weekly EpiTel minutes. In parallel, standardized email questionnaires targeting all EpiTel participants at national, prefecture and district level were used to assess the process further. They included open and closed questions rating logistics, content, minutes and usefulness of information exchanged.

Results: In total, 28 weekly minutes were analyzed. Mean participation rate per prefecture was 86%. At IPH next to surveillance staff, the laboratory participated in 82% of conferences, the vaccination program and other sectors in 54% and 7.2%, respectively. On average, each prefecture presented an event in 59% of conferences. The most frequent international topics in EpiTel were Measles, Influenza, West Nile virus and Yellow Fever, while from the national and subnational level events reported via syndromic surveillance, influenza, a nationwide measles outbreak and challenges related to contact tracing and vaccinations were most frequently discussed. Overall, 10/15 staff members at national level, all prefectures and 50% of districts completed the questionnaires. The organization of the conference was found to be very well managed and Skype was agreed to be the best medium for communication. The information exchanged and shared was evaluated as accurate, informative, timely and facilitating the harmonization of control measures. All participants rated EpiTel as very useful for their daily work.

Conclusion: EpiTel is an efficient way to communicate and discuss international and national infectious disease events routinely and in a condensed way between all levels of the public health system in Albania. The participatory process fosters horizontal exchange within an otherwise centralized surveillance system.
Purpose: Model-based geostatistics (MBG) is a branch of spatial statistics that is increasingly used to support disease control programmes in low-resource settings. MBG methods allow modelling of the spatial variation in disease prevalence and can thus be used to inform intervention policy. In this study, our focus is to identify the best practice for MBG when the goal is to estimate the likelihood of exceeding relevant thresholds for intervention policy implementation. Continuous data on disease indicators, collected from surveillance programmes, are typically dichotomised to positive or negative for the disease of interest before MBG analysis is conducted. Our conjecture is that this dichotomisation reduces the accuracy of spatial mapping of disease prevalence due to loss of data.

Methods & Materials: Consequently, we carry out a geostatistical analysis of data on both simulated data, and on malnutrition and anaemia data from a cross-sectional survey conducted in Uganda and Ethiopia. We then compare two possible approaches for disease mapping: the first defines a model for a continuous measurement and uses this to predict the probability of exceeding a predefined threshold. The second approach first dichotomises the continuous measurement into a binary outcome indicating the exceedance or not of the threshold, and then develops a geostatistical logistic regression based on this binary outcome.

Results: Our results indicate that these two approaches lead to a substantially different quantification of uncertainty associated with the estimates of disease prevalence.

Conclusion: Dichotomisation of the data leads to higher uncertainty in the spatial predictions of prevalence. Modelling with continuous data should therefore be applied whenever this is feasible.
Pertussis notifications in the Netherlands: workload versus benefits


Purpose: Pertussis is highly endemic and forms the bulk of mandatory notifications to public health authorities in the Netherlands. This results in a significant workload for Public Health Services (PHSs), while the benefits for prevention and control are deemed modest. However, data are very important for surveillance. We made an inventory of PHS’s actions, associated costs and control measures taken after pertussis notifications.

Methods & Materials: First, a questionnaire was sent to all 25 PHSs in order to identify different approaches to handling pertussis notifications. Subsequently, six PHS regions were selected by contrasting incidence, vaccine coverage and way of handling notifications, i.e. elaborate and less elaborate approaches. In each region, 200 pertussis notifications were retrospectively studied and all (administrative) actions and control measures taken were scored by two trained research nurses.

Results: Results of the questionnaire showed a striking heterogeneity in handling notifications between PHSs, precluding the identification of a few common approaches. In the retrospective study, 1205 cases were included. PHSs with an elaborate approach more often gave case-specific control advice (17%) than PHSs with a less elaborate approach (4%). Median time spent per notification was 42 and 15 minutes for elaborate and less elaborate approaches, resulting in staff costs of €23.80 and €6.32 per notification, respectively. Apart from case-specific control advice, control measures as prescription of chemoprophylaxis or vaccination were performed by any PHS. Not unexpectedly, elaborate approaches resulted in more complete surveillance data.

Conclusion: Striking variation exists between PHSs in the Netherlands regarding approaches of handling pertussis notifications. A considerable workload and costs are associated with these notifications. More nationwide consensus on the best approach to pertussis notifications with maintenance of high quality surveillance data is warranted. A proposal was developed and discussed with PHSs, which led to a consensus approach, in which workload is balanced against prevention and control of pertussis for under ones, while maintaining surveillance quality.
The Power of Evaluations: One Year Review of FAO’s Surveillance Evaluation Tool (SET) and Tracking Future Impacts

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Purpose: With funding from the United States Agency for International Development, the Food and Agriculture Organization of the United Nations developed a standardized Surveillance Evaluation Tool (SET) to assess national animal disease surveillance systems and provide targeted recommendations in the form of a feasible and time-bound action plan. After completing the first year of missions in African countries, work is well underway to implement these plans. FAO is piloting a tracking system to measure the impact of SET recommendations at country-level and define success.

Methods & Materials: SET was adapted from the Oasis toolkit by French agency ANSES, and includes components of Joint External Evaluations related to animal health surveillance. Official documentation is reviewed and interviews are conducted with actors at all levels. Outputs highlighting the strengths and weaknesses are generated and a participatory approach used to develop locally-relevant recommendations. Some SET recommendations have already been incorporated into FAO country work plans to ensure continuous support and capacity building. A tracking system is now being piloted to monitor countries’ progress towards completing SET-derived action plans.

Results: A total of 13 evaluations were conducted and individual mission reports provide national veterinary services with an in-depth assessment of their disease surveillance system along with a detailed action plan for improvement. Assessments also provide a baseline to which future assessments can be compared. In addition, evaluation results can contribute to the development of national and regional strategies for animal disease surveillance and help technical and financial partners identify key priorities for capacity building. Thus far, several countries have already begun implementing specific SET recommendations, such as the development of an epidemiology unit and standardized disease reporting materials in Liberia. In future, common strengths and weaknesses across regions will be monitored, indicators created to assess impacts on local health systems, and success stories on recommendation implementation shared between countries.

Conclusion: While there are known benefits from conducting evaluations, there is a growing push from the international community to develop methods that result in policy support and allow for sustainable capacity building. With a tracking strategy for implementation and impact of animal health surveillance capacity building, tangible change can be achieved.
Establishment Of An Electronic Integrated Disease Surveillance And Response System In Sierra Leone

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Purpose: The 2014–16 Ebola epidemic exposed weaknesses within Sierra Leone’s disease surveillance system and served as a catalyst to improve detection systems. In 2015, the national Integrated Disease Surveillance and Response (IDSR) system consisted of health facilities (HF) calling/texting or hand-delivering reports to their District Health Management Team (DHMT), DHMT staff entering IDSR data from all HF into a spreadsheet, and each DHMT emailing the file weekly to the national level. When districts lacked adequate bandwidth to upload attachments, DHMT staff emailed a curtailed IDSR data summary to the national level, which jeopardized data timeliness and accuracy. In 2016, Sierra Leone introduced an electronic IDSR (eIDSR) system to gauge feasibility and efficacy in a resource-constrained setting.

Methods & Materials: The U.S. Centers for Disease Control and Prevention and eHealth Africa supported the Ministry of Health and Sanitation to establish eIDSR, which transmits data into the web-based District Health Information System (DHIS2). In 2016, district-level eIDSR was piloted then rolled out to all fourteen districts. District-level eIDSR entails HF staff calling the DHMT, then DHMT staff entering data directly into the national DHIS2 database. From 2016–17, facility-level eIDSR was piloted in one district to enable data entry using tablets at all 112 HF in the district, and in 2018, a national scale-up of facility-level eIDSR is underway to reach all 1285 HF in Sierra Leone.

Results: After implementing district-level eIDSR, IDSR reporting increased from <40% of HF in 2016 to >97% in 2017, an increase maintained in 2018. The district-level pilot revealed that data-entry time decreased by 63% and errors decreased by 45%. Analyses from the facility-level eIDSR pilot found that data accuracy was 12% higher than data reported through district-level eIDSR. The Joint External Evaluation (JEE) score for real-time surveillance went from “limited” to “developed” capacity between 2016 and 2017.

Conclusion: District-level eIDSR improved the timeliness and accuracy of surveillance data. Additional benefits are anticipated with completion of the national scale-up of facility-level eIDSR, and JEE scores are projected to reach the next level of “demonstrated” capacity. Lessons learned in Sierra Leone can inform other low-resource settings considering implementation of electronic disease surveillance systems.
Purpose: Implementation of IHR is a commitment and developing minimum core capacities like detection, identification and response to the public health events are essential for all members states. The study objectives are assess IHR implementation arrangements in Pakistan.

Methods & Materials: The qualitative study conducted during December 2017. Literature, documents and available record were reviewed. The stakeholders were engaged. Focus group discussions were carried out with representatives of IHR federal & provincial focal points and Points of Entries.

Results: Based on “One Health” approach, national and provincial Focal Points has been notified. To build national surveillance and response capacity, all stakeholders are accordingly kept engaged to undertake the desired capacity building efforts. Strategic framework for public health laboratories network, Integrated Disease Surveillance & Response and legal framework for surveillance have been developed. Emergency Operation Centers and Coordination units for disease surveillance & response have been established at federal and provincial levels aiming to provide meaningful information for actions. One Health Hub has been officially notified to overcome Zoonosis and environmental threats. Joint External Evaluation (JEE) was conducted in 2016 and gross infrastructural and human resource deficiencies in all 19 areas identified. Based on the JEE recommendations, five year costed National Action Plan aiming to develop a strong public health system with the standards and competencies required for implementing IHR. Anti Microbial Resistance (AMR) surveillance and monitoring activities have been planned. Sentinel Surveillance sites for AMR have been identified and networking been started. Federal Reference Lab upgraded as well as work on establishing four provincial reference labs have been started out of which one provincial lab has been made operational.

Conclusion: Despite varied progress in certain areas, an adequate legal framework, proper institutional mechanism to detect, identify, report and respond to the public health events is lacking. An adequate legal framework, strengthening proper institutional mechanism for surveillance and response, infrastructural and human resource capacities at POEs, improved laboratory detection, surveillance and response capacities at all levels, coordinated mechanism based on all hazard approach, control of Zoonosis and mechanisms for detecting & responding to chemical emergencies are recommended.
The Feasibility Of Evaluating Varicella Vaccine Effects In Oman Using An Electronic-Based Patient Record System: A 5-Year Retrospective Analysis.

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Purpose: In Oman, varicella vaccination was introduced in 2010 for one-year old age group. The aim of this study is to assess the feasibility of evaluating the effectiveness of this vaccination using an electronic-based patient record system (Al-Shifa).

Methods & Materials: Five years retrospective data from Al-Shifa system was extracted for Varicella, from January 2012 to May 2018. Variables considered were: patient identification number, date of consultation and age. Omani population data were acquired from mid-year census for the respective years. Those who were born after 2010 were labelled as ‘vaccinated’ in the data.

Number of cases by age group and vaccination status was tabulated and time trend for seasonality was graphed. A generalised additive model (GAM) with negative binomial distribution and control for autocorrelation was used to model age and population adjusted monthly varicella counts. Covariates considered were: month, age group and vaccination status. Statistical analyses were conducted using R software.

Results: A total of 117,974 cases of varicella were reported in Oman through Al-Shifa system, from January 2012 to May 2018. 52% were above 10-year old and in the ‘not vaccinated’ group, thus, they were not modelled. The 5-9 years age group, had comparable proportions of both vaccination groups, 22.4% in the ‘vaccinated’ and 32.7 % in the ‘not vaccinated’ groups.

Clear seasonality of varicella incidence rates was detected peaking around April and declining around October. In addition, incidence rates of varicella are markedly higher in the ‘not vaccinated’ group compared to the ‘vaccinated’.

Compared to the ‘vaccinated’ group, and controlling for seasonality and age groups, GAM showed a 60% reduction in the incidence rates of varicella after the initiation of vaccination program, (risk ratio: 0.4; 95% CI: 0.3- 0.5). In addition, compared to the >1-year-old group, and controlling for seasonality and vaccination status, the age groups of 1-9 years have 40 % greater incidence rates of varicella (RR: 1.4; 95% CI: 1.04- 1.75).

Conclusion: Our study showed the possibility of using data from Al-Shifa system to assess vaccination effects. There is marked reduction in varicella cases in Oman after the introduction of varicella vaccination program.
21.063
Google Earth™: A Low-Cost Geospatial Tool For The Outbreak Management Of Lassa Fever In Poor-Resource Settings.

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Purpose: To demonstrate that where there are no funds and no expertise to employ a Geographical Information System (GIS) as a decision-support system for the control of Lassa fever outbreak in a poor-resource setting, Google Earth™ could be used as an alternative tool to locate and track the spread of the disease.

Methods & Materials: Satellite imagery and feature-making tools in Google Earth™ were utilized for the location and visualization of hotspot locations of 2008-2013 Lassa fever outbreaks in Edo Central District area of Nigeria. Georeferencing was done with a Garmin eTrek-Global Position System (GPS) handheld device. A published cluster and hotspot data of geographical coordinates of the 2008-2013 Lassa fever hotspots in Edo Central Senatorial District of Nigeria were used as baseline data to locate the house where the outbreaks occurred. Six Medical students with no previous knowledge of GPS and Google Earth™ but with minimal supervision and hands-on training on location tools for disease surveillance, handled the location tools.

Results: With the use of Google Earth tools, and with little expertise, 31 out of the 33 previously located hotspot zone of Lassa fever in Edo Central Senatorial District of Nigeria, (X coordinates = 6.14057; Y-coordinate 6.74169 (decimal degrees)) were successfully traced using a GPS device. A satellite imagery of the 2008-2013 Lassa fever hotspots was subsequently generated. The Google Earth™ map was comparable to the ArcGIS hotspot map of the study area.

Conclusion: Where it is not economically feasible to use a Geographical Information System (GIS) software to generate and represent locations of hotspots of Lassa fever and other diseases with the same spatial characteristics, Google Earth may be an alternative and cost-effective technology to utilize for the tracking of areas of increased clustering of the disease. More so, The spatial information generated from Google Earth™ may be more understandable than a GIS map to those without mapping or GIS geospatial skills.
A Rare Malaria Case Presents With Features of Haemorrhagic Fever (Ebola) In Uganda Causing a Nationwide Threat; Watch Out For Complicated Malaria In Your country.

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Purpose: Malaria is the leading cause of morbidity and mortality in Uganda and the country has the world’s highest anopheles mosquito entomological inoculation rates at 1586 bites/year (Moses Kamya, Mallo Rao 2011). In 2016 alone the country reported 7.77million malaria cases and 12060 deaths (WHO World malaria report 2017). An undetected malaria-HIV co-infection generated a nationwide threat when it presented with features of haemorrhagic fevers (Ebola) in May this year.

Methods & Materials: We did a retrospective review of patient data for a twenty three year old female who was initially managed at an upcountry district hospital (Nakaseke district) for nonspecific symptoms of fever, headache, malaise and anorexia for 3 days before she develop sudden haemoptysis, per vaginal bleeding, bleeding from rectum, mouth and nose that prompted referral to Kampala. Patient had no prior bleeding disorders. Malaria microscopy and RDT at the district hospital were negative, and HIV serology was not done.

Results: At the referral hospital the patient was isolated as a suspected haemorrhagic fever (HF) case and several tests done. Patient tested HIV positive and had a positive mRDT for both P. falciparum and P. Vivax. Malaria microscopy was negative. Haematology results (CBC) showed a bicytopenia with haemoglobin (Hb) of 4.3g/dl, platelets (PLT) 66 x10*3/ul, total white cell count (WBC) 4.9 x10*3/ul, total red cell count (RBC) 1.43 x10*3/ul, haematocrit (HCT) of 13.6 x10*3/ul, neutrophils 2.43 x10*3/ul. Patient was started on Intravenous artesunate 2.4mg/kg BW given at 0, 12 and 24 hours and blood transfusion with one unit of fresh whole blood. Patient registered very remarkable improvement within 24hours and started oral ACT thereafter. A repeat CBC after 24hours showed Hb 6.9g/dl, PLT 156 x10*3/ul, WBC 6.27 x10*3/ul, RBC 2.3 x10*3/ul, HCT 21.0 x10*3/ul, NEUT3.47 x10*3/ul. Serum urea, creatinine and liver transaminases were raised while HF tests came out negative after more than 24 hours. Malaria RDT and microscopy were negative after 72 hours.

Conclusion: Complicated malaria can occur in special risk groups (HIV-malaria co-infection) even with very low or no parasitemia, thus treatment should not delay in absence of parasitological diagnosis.
Purpose: The West African Ebola outbreak and Zika virus outbreak in the Americas were both initially reported as undiagnosed disease events, underscoring the importance of monitoring and detecting early reports of undiagnosed diseases. The purpose of this study was to examine undiagnosed disease events captured by Public Health England’s epidemic intelligence system (EIS) between 2013 and 2017 to inform future epidemic intelligence practices.

Methods & Materials: Descriptive analyses were conducted on undiagnosed disease data captured by PHE’s manual open source EIS between 2013 and 2017. Undiagnosed disease events were defined as cases or outbreaks of disease where an infectious agent was suspected but the aetiology unknown at the time of initial assessment.

Results: 377 unique undiagnosed disease events were recorded between 2013 and 2017. The majority (47%) of reports were from Africa, followed by Asia (21%). For first reports, 258 (68%) were in the English language and 219 (58%) were initially identified through aggregate open source surveillance websites (eg ProMED and FluTrackers), followed by media sources (12%). 215 (57%) reports were subsequently validated by a definitive diagnosis; the vast majority (74%) were from informal reports quoting a national authority. The most commonly reported symptom categories were Ebola-like/haemorrhagic (45%), gastrointestinal (12%), respiratory (10%) and febrile (9%). Of the symptom categories, Ebola-like/haemorrhagic (54%), respiratory (10%) and gastrointestinal (9%) received the most definitive diagnoses. 26 (12%) of the undiagnosed disease events with a definitive diagnosis are on the WHO list of priority diseases. On average, diagnoses were reported 5.77 days (SD= 11.56) after the initial report, but ranged from 0 to 84 days.

Conclusion: PHE’s robust EIS has been identifying and gathering information about incidents of new and emerging infectious diseases since 2003. The system was able to quickly and accurately validate over half of the undiagnosed disease events detected, greatly contributing to national preparedness for events of potential public health significance. The high proportion of first reports identified through open-source surveillance websites and media reinforce the importance of monitoring grey literature when conducting surveillance for new and emerging infectious diseases.
Purpose: The dangerous diseases: dengue fever, yellow fever, Zika virus and Chikungunya, which affect hundreds of millions of people each year, are transmitted by female Aedes mosquitoes. To prevent the spreading of the Aedes mosquitoes, the Aedes larvae should be under close surveillance, so that the responsible agencies can make a warning or prepare for the epidemic. In order for anyone without the expert knowledge to detect and report the existence of the Aedes larvae, which are very responsive to the change of light intensity and move abruptly, we propose the Aedes Larvae Surveillance System using Smartphones.

Methods & Materials: The system comprises an application on smartphones that record a video at the time of flash-on and then the video is processed for counting the number of the Aedes larvae at the server. The video processing algorithms are based on the detection and tracking of the moving objects, which can be simply differentiated from the unmoved background. The proposed algorithm contain the following steps: frame extraction, frame stabilization to get rid of the jerky image motion caused by platform movement, background subtraction to capture the moving Aedes larvae when they expose to the flashlight, images segmentation using local threshold, modified Hungarian algorithm for the track assignment, larvae path classification based on pattern of the movement, and finally counting the valid paths. In order to investigate the performance of the algorithm, the 120 recorded videos of Aedes larvae in the light-color container taken by the developed smartphone application are processed in MATLAB. The detected Aedes Larvae obtained from the algorithm are compared with the ground truth obtained from visual inspection.

Results: The result of the processing algorithm by means of Recall (Hit Rate) = TP/(TP+FN) is 88.02%, where TP is the true positives and FN is the false negatives. The False Alarm Rate (FAR) = FP/(FP+TP) is 43.29%, where FP is the false positives.

Conclusion: The proposed system has a promising performance in detecting the Aedes larvae in the test environment. However the FAR is still quite high, thus we will try reduced it in the future.
Purpose: Global surveillance systems are crucial for early detection, assessment and response to public health threats. The National Health and Medical Research Council Centre for Research Excellence, Integrated Systems for Epidemic Response is developing a new epidemic observatory, Epi-watch. Epi-watch monitors and provides critical analysis of global outbreaks and epidemics of public health significance for use by policy makers, government and other stakeholders. To inform further development of Epi-watch, we implemented a stakeholder survey to understand the global outbreak surveillance needs of stakeholders involved in epidemic response and surveillance in the Asia-Pacific region.

Methods & Materials: We designed an online semi-structured stakeholder questionnaire. Purposive and snowball sampling methods were used to identify 128 participants who use epidemic intelligence and outbreak alert services in their work in government and non-government organisations in selected countries in the Asia-Pacific region.

Results: All respondents (N=91) agreed that it was important to remain up to date with global outbreaks. The main reason for following outbreak news was as an early warning for serious epidemics (83/91; 91%). Mainstream media and specialist internet sources such as WHO (n=54/91; 59%), ProMED-mail (n=45/91; 49%) and CDC (n=31/91; 34%) were the most common sources for global outbreak news, while use of rapid intelligence services such as HealthMap were less common (n=9/91; 10%). Only 51% (46/91) of respondents thought their sources of outbreak news were timely and sufficient for their needs. Not enough critical appraisal (38/91; 42%) and lack of time (36/91; 40%) were reported as limitations of outbreak sources.

Conclusion: Epidemic intelligence is important and widely utilised. The choice of sources for global outbreak news varies. There is less use and awareness of rapid sources such as HealthMap and more reliance on less timely, traditional sources. Users identified a need for more timely and reliable epidemic intelligence, but were unaware of existing rapid sources. Better methods to deliver user-friendly epidemic intelligence to end-users should be explored. Results from this study will be used to further develop Epi-watch to meet end-user needs.
New Approaches To Facilitate Reporting Of Dengue Cases In Taiwan

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Purpose: Early detection and timely reporting of dengue cases are important strategies for the dengue prevention and control. To shorten the time between case reports submitted from medical institutions and received at public health authorities (PHA), Taiwan has established an electronic notification disease surveillance system (NDSS) since 2002. However, paper-based reporting is still preferred by most clinics. This may cause delays in implementing dengue control measures and increase the data entry workload for PHA. Frontline interviews indicated that complicated NDSS account application procedure and the lengthy reporting form are major reasons hindering clinics to use NDSS. To encourage clinics using NDSS, we proposed two approaches by providing convenient NDSS login and a data exchange API.

Methods & Materials: We developed two reporting applications based on the existing NDSS system architecture, the widely-available clinic information systems (CIS), and the virtual private network (VPN) of the National Health Insurance (NHI) program. The first application allows doctors to enter NDSS accountlessly by having their RSA cards verified inside the NHI VPN. The second application allows clinics to use their CIS to report dengue cases to NDSS through a secure data exchange API and a shortened report form. To evaluate the effectiveness of the two applications, we analyzed the NDSS system logs and performed questionnaire surveys among clinics.

Results: We found that doctors are more willing to use NDSS by accountless login, which saves login time and reduces login failures. Clinics without their own internet connections are more willing to report cases by NDSS via NHI VPN. Meanwhile, the direct API connection of the CIS with NDSS could reduce at least 70% of data entry workload.

Conclusion: The study showed that these two new approaches effectively solved the NDSS login issue and significantly reduced time spent on data entry in clinics. The clinics’ willingness to report dengue via NDSS was improved. With decreasing paper-based reports, PHA staffs also benefited from less time spent and fewer entry errors while entering data into the NDSS. More time could be saved for dengue prevention and control measures.
Purpose: 21st-century intelligence gathering and assessment measures are required to stay abreast of the rapidly changing risk environment. “Infodemics”, the “rapid spread of information of all kinds, including rumours, gossip and unreliable information” contribute to confusion as well as inappropriate actions and reactions. An increasing amount of information is available and there is a "growing tension between information and noise". International SOS has been operating a specialized global medical unit to monitor and report on emerging and active health security risks since the Severe Acute Respiratory Syndrome (SARS) outbreak in 2003.

Methods & Materials: The unit continuously screens hundreds of sources including news reports, official government information and social media, and leverages the organisation’s network of global health professionals (both in-house experts and specialist consultants). Signals are filtered and validated. Algorithms are applied to analyze the events, which include a medical “risk rating” framework. When triggers are reached, the event is communicated to stakeholders, through a variety of channels.

Results: Of the thousands of incidents which are monitored, on average, 300 incidents are communicated to stakeholders. Data on these communications and feedback will be presented.

Conclusion: Timely, accurate and credible assessment and communications are increasingly valuable for organisations to make informed decisions and take appropriate actions to protect their staff and operations.
Mortality, Malignancy, and Comorbidities Associated With Chronic Pancreatitis: A Nationwide Database Study in Taiwan

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Purpose: This study aimed to investigate the risk of death, malignancy, and comorbidities in patients with chronic pancreatitis (CP).

Methods & Materials: We conducted a nationwide retrospective matched-cohort study using data from Taiwan’s National Health Insurance Research Database (NHIRD) collected between 2000 and 2011. The CP was defined if patients had at least one admission diagnosis or more than three outpatient clinic visits within a year with ICD-9-CM code 577.1. Each CP case was compared with 10 age-, sex- and index year-matched controls without CP, who were randomly selected from the whole claims data of the NHIRD set. Next, the incidence rate ratio (IRR) based on Poisson regression analysis of mortality, malignancy, and comorbidities were compared between the CP cohort and the non-CP cohort.

Results: During the study period, 12,605 persons (76.8% men) with CP were identified and matched with 126,050 controls with a mean age ± standard deviation of 48.4 ± 14.5 years. The incidence rate of peptic ulcer disease, liver disease, diabetes, and hyperlipidemia were statistically higher in the CP cohort than that in the non-CP cohort. Twenty percent of the CP group died during the follow-up period, compared with 7.7% of the non-CP group, corresponding to the incidence of death in the CP cohort was 41 per 1,000 person-years, and compared with the non-CP cohort, the IRR was 2.84 [95% confidence interval: 2.724 – 2.961] (P < 0.001). Malignancy was a frequent cause of death in the CP group (IRR per 1,000 person-years: 4.1) and non-CP group (IRR per 1,000 person-years: 2.4). The incidence rate of malignancy in the CP group and the control group was 13.8 and 9.9 per 1,000 person-years, respectively (P < 0.001).

Conclusion: The nationwide matched-cohort database study in Taiwan revealed that patients with CP have a higher risk of death from malignancy and a higher incidence of comorbidities than the general population without CP. This study was supported by grants from the Ministry of Science and Technology, Taiwan (MOST 107-2634-F-002-015 and 106-2628-E-002-004-MY3).
21.071
Epidemic Intelligence Activities During Major International Incidents Involving Emerging Infections

**Purpose:** To review and describe sources of information used by Public Health England’s epidemic intelligence (EI) system during the Ebola virus disease (EVD) outbreak in West Africa, and Zika virus outbreak in the Americas. Outputs from this analysis will be used to refine EI practices to inform risk assessments and policy during future major incidents.

**Methods & Materials:** Since 2003, Public Health England has maintained a manual event-based surveillance system of mostly open source data for detecting and monitoring incidents involving emerging infectious diseases. Where possible, initial alerting sources for data (eg media) are validated by obtaining a definitive information source (eg national authorities). Data collated during the West African EVD and the Americas Zika virus outbreaks were analysed to assess the relative importance of different sources for information gathering.

**Results:** EI activities during these two incidents resulted in the collection of 5,072 and 2,631 entries for EVD and Zika virus respectively, comprising 41% and 18% of total EI entries recorded during their respective outbreak periods.

For EVD and Zika virus, 51% and 33% of entries respectively had different initial alerting sources compared to definitive information sources. Of these, aggregate open-source surveillance websites (eg ProMED) were the principal sources of initial alerts (40% for EVD and 46% for Zika virus).

For EVD, 41% of definitive information came from media outlets, 19% from the World Health Organization (WHO), 12% each from scientific journals and national authorities. For Zika virus, 33% of definitive information came from scientific journals, 29% from media outlets, 21% from national authorities, and 13% from WHO.

**Conclusion:** Although resource intensive, EI activities are an important feature of national preparedness to inform risk assessments, policy and guidance during major infectious disease incidents.

While national and international authorities are recognised as principal sources of EI, aggregate surveillance systems, traditional media outlets, and scientific journals remain essential sources of information during international incidents.

This analysis highlights the importance of comprehensive but flexible EI systems, particularly for incidents caused by emerging infectious agents. In particular, it emphasises the utility of manual EI systems readily adaptable to the specific requirements of individual incidents.
Viral Etiology of Community-Acquired Infection in Vietnam: Unraveling the Unknown by Next-generation Sequencing Analysis

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Purpose: Community-acquired (CA) infection is a major public-health problem worldwide. Yet despite extensive laboratory diagnosis, the etiology remains unknown in >50% of the patients. Improving our knowledge of the causative agents is essential for improving disease burden.

Methods & Materials: A deep-sequencing based viral metagenomics approach was employed to analyze 493 clinical samples (384 plasma, 92 pooled nasal- and throat swabs, 10 stools and 7 CSF) from 386 CA infected patients with unknown origin (children and adults) recruited from 6 hospitals cross Vietnam in 2014-2015. Sensitive specific PCRs were used to confirm deep sequencing results.

Results: 22 viruses were detected in 54/493 samples (including 11 viruses in 32 plasma samples), corresponding to a detection rate of 14% (54/386). The detected viruses included enteroviruses (n=14), hepatitis B virus (10), rhinovirus (5), rotavirus A (3), measles virus, respiratory syncytial virus, parainfluenza virus, adenovirus, hepatitis C virus, dengue virus, influenza A/B virus, parechovirus 1/6 (2 each), metapneumovirus, human immunodeficiency virus, coronavirus, WU-polymavirus, saffold virus, salivirus (1 each) and recently described viruses including human pegivirus 2 and gemycircularvirus (1 each).

Conclusion: Multiple viral pathogens were detected by deep sequencing in 54/386 (14%) CA infected patients with unknown origin. Metagenomics can be a sensitive pan-pathogen assay for unbiased/sequence-independent detection of known/unknown pathogens in clinical samples. The results warrant further active surveillance for novel pathogens in Asia where there is a high risk of emerging infections.
Purpose: Canine leishmaniosis is caused by *Leishmania infantum*, or also called in the New World *L. chagasi*. It is considered to be a major potentially fatal zoonotic infection where the domestic dog is the main reservoir. This infection is worldwide and reported with a higher incidence in tropical and subtropical areas such as South America where the number of infected dogs is estimated in the millions. No molecular data has been available on this disease in French Guiana. The aim of this study was molecular investigation of occurrence of leishmania in the blood of dogs in this region.

Methods & Materials: Since 2016, blood samples were collected on a total of 98 dogs from French Guiana and 26 other dogs coming from continental France, were sampled before and after a 4-month mission in the same region. The samples were tested by a qPCR to quantify *L. infantum* kinetoplast DNA. Some positive samples were confirmed and species were identified, after sequencing, by using two standard PCR systems: i) a pan-*Leishmania* system was designed, targeting 28S rRNA gene and ii) PCR generic primers to amplify a segment of the rRNA internal transcribed spacer 2 (ITS2) from multiple *Leishmania* species.

Results: The results show at least 4.08% (3/98) were positive to this pathogen and two (2/26) dogs returned positive although they were negative to begin with; one of them had an ulcer on the pastern. This last had $9 \times 10^7$ Leish/mL of blood, $1.3 \times 10^{10}$ Leish/mL from ulcer swab and around $4.3 \times 10^9$ Leish/g of bone marrow. In general, the parasite load was from $2.5$ Leish/mL to $8 \times 10^{13}$ Leish/mL of dog’s blood. Sequencing analyses identified *L. infantum* specie.

Conclusion: The detection of *L. infantum* in local dogs in French Guiana and in dogs from metropolitan France after coming back from French Guiana, provide evidence that this region is endemic for canine leishmaniosis. It highlights the need for active surveillance in canine population and implementation of control measures. Competent vectors in this region are yet to be identified.
Serological Evidence of Rift Valley Fever Virus Circulation in Some Wildlife and Domestic Animals in Bauchi State, Nigeria

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Purpose: Rift Valley fever is an arthropod borne zoonotic disease responsible for severe outbreaks in livestock and humans. Information on Rift valley fever virus in wild and domestic animals is limited in Bauchi State, Nigeria despite the availability of Yankari Game Reserve and Sumu Wildlife Park with wildlife, livestock and people in communities surrounding them competing for resources. The study therefore investigated the seroprevalence of RVFV among wild and domestic animals

Methods & Materials: Sera samples were collected from 4 elephants (Loxodonta Africana), 11 waterbucks (Kobus ellipsiprymus), 1 hartbeest (Alcelaphus baselaphus caama), 24 elands (Taurotragus oryx), 53 zebras (Equus quagga crawshayi), 1 kudu (Tragelaphus strepsiceros), 12 wildebeests (Connochaetes taurinus), 300 cattle (Bos indicus), and 200 horses (Equus caballus) within and around YGR and SWP in Bauchi State, Nigeria, and were immobilized using Etorphine Hydrochloride (M99® Krüger-Med South Africa) at 0.5-2 mg/kg and Azaperone (Stresnil®, Janssen Pharmaceuticals (Pty.) Ltd., South Africa) at 0.1 mg/kg using a Dan-Inject® rifle (Dan-Inject APS, Sellerup Skovvej, Denmark) and Naltrexone (Trexonil® Kruger-Med South Africa) at 1.5 mg IM was used for reversal, domestic animals were restrained by the owners for samples collections. Sera were evaluated for the presence of anti-RVFV IgM/IgG antibodies using ID screen® RVF competition multi-species ELISA kits (ID-Vet Innovative Diagnostics, Montpellier, France).

Results: The results showed higher RVFV seroprevalence in cattle (11.3%) than wildlife (8.5%) and none of the horses sampled were positive. In wildlife, higher seroprevalence was observed in waterbuck (36.0%) followed by elephant (25.0%), eland (12.5%) and wildebeest (8.3%) and none of the 53 samples from zebra and 1 sample each from kudu and hartebeest showed detectable antibodies to RVFV. Classification of cattle into sex and breed showed higher RVFV antibodies in females (13.6%) than males (6.4%) whereas white Fulani breed had higher RVFV antibodies (12.6%) than red bororo and sokoto gudali breeds (12.1% and 7.8%) respectively

Conclusion: The results indicate exposure of wild and domestic animals to RVFV, raising concern about the possibility that these species may pose a significant health risk for wildlife, livestock and or humans
Purpose: West Nile virus (WNV) IgM antibodies have been shown to persist for up to 500 days or even longer in some patients. Therefore, additional tests are needed to confirm primary infection. The aim of this study was to analyze the value of IgG avidity in diagnosis of symptomatic and asymptomatic WNV infection.

Methods & Materials: A total 54 WNV IgM and/or IgG positive serum samples collected from 39 patients with neuroinvasive disease (meningitis/encephalitis/myelitis) and 15 asymptomatic persons were tested for WNV IgG avidity. Serological tests were performed using a commercial enzyme-linked immunosorbent assay (Euroimmun, Lübeck, Germany) and confirmed by virus neutralization test. Avidity index (AI) was calculated and expressed as percentage using the extinction values with and without urea treatment. The interpretation of AI results was determined as follows: AI < 40% = low avidity antibodies indicating acute primary infection; AI 40-60% = borderline avidity indicating recent (post-acute) infection; AI > 60% = high avidity antibodies indicating past WNV infection.

Results: WNV IgM antibodies were detected in 47/87.0% samples: 39/100% patients with neuroinvasive disease and 8/53.3% asymptomatic subjects. Recent WNV infection was documented by low/borderline AI in 44/93.6% IgM positive samples. In 3/6.4% IgM positive samples, high AI was detected indicating persisting IgM antibodies from a previous infection. All 7 IgM negative samples showed high AI. In 33/84.6% patients with WNV neuroinvasive disease tested within 30 days after onset of symptoms AI was low. Six patients (15.4%) tested 34-50 days after disease onset showed borderline AI (42-60%) indicating earlier maturation of WNV IgG antibodies.

Conclusion: The results of this study indicate that IgG avidity differentiates current/recent WNV infection from persistent IgM antibodies both in patients with WNV neuroinvasive disease and asymptomatic persons. Since many patients showed rapid avidity maturation, a low IgG avidity is indicative of WNV infection within the previous month.
Recombinant Of PUUV Genome S segment Isolated From Rodents Captured In The Republic Of Tatarstan, Russia

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Purpose: Background: Puumala hantavirus (PUUV) is the main cause of hemorrhagic fever with renal syndrome (HFRS) in Russia. Although known to cause HFRS endemic in the Republic of Tatarstan (RT), little is known about the genetic diversity of PUUV in the Republic. Therefore, we sought to characterize variations in PUUV S-segment in bank voles in the RT.

Methods & Materials: Material and methods: Total of 121 bank voles were captured in the north-west regions of RT during the 2014-2015 HFRS outbreak. Total RNA was extracted from lung tissue and used for RT-PCR and sequence analysis.

Results: Comparison analysis of viral S segment sequences (564 bp) revealed 92.9-97.3% nucleotide identity between 29 RT PUUV strains and RUS lineage strains, while the lower similarity of 83.7-87.6% was found in strains from FIN lineage. Interestingly, sequence similarity of four RT PUUV strains and RUS strains was lower 85.8-87.6% than that found in the majority of isolates. Surprisingly, these strains had a higher identity (99.6-99.8%) with strain “Sotkamo” of FIN lineage. Phylogenetic analysis demonstrated that the four strains with low similarity to RUS lineage clustered with the FIN strains cluster. Further analysis of the full coding region of strain “032” S-segment, which had more similarity to FIN lineage, revealed that the partial sequence from 242 to 857 nt was 99.5% identical to “Sotkamo” strain, while the segment from 864 to 1341 nt showed 92.1-93.5% identical to RUS lineage strains.

Conclusion: Conclusion: Therefore, our data suggest that strain “032” could be the progeny of the recombination between RUS and FIN genetic lineages. It remains to be determined if recombinant PUUV strains could cause different clinical presentation of HFRS.

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Molecular Identification Of a Presumably Novel Hantavirus In Bronze Tube-nosed Bat (Murina aenea) In Malaysia

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Purpose: Hantaviruses (Hantaviridae) cause two types of life-threatening human diseases, hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. To date, as a consensus, wild rodents were believed as natural hosts of hantaviruses. However, recent studies described several novel hantaviruses in shrews, moles and bats, suggesting the dispersal of hantaviruses in several animal taxa during their evolution. Interestingly, the co-evolutionary analyses of most recent studies have raised the possibility of bats and/or soricomorphs may have served as the primordial mammalian host and harboured the ancestors of rodent-borne hantaviruses. The aim of our study was to investigate the presence of hantaviruses in bat lung tissue homogenates originally collected for taxonomic purposes in Malaysia, 2015.

Methods & Materials: Hantavirus specific nested PCR screening of 116 samples targeting the L segment of the virus have revealed the positivity of two lung tissue homogenates originating from Murina aenea bat species.

Results: The obtained results indicate the first molecular evidence for hantavirus in Murina eanae bat species.

Conclusion: Preliminary sequence analysis of the PCR amplicon suggest the identified virus may represents a novel species within Orthohantavirus genus. Furthermore, our results provide additional genomic data to help extend our knowledge about the evolution of these viruses and we present the first hantavirus sequence from Murina bat genus.
Canine Leishmaniosis and First Report of *Leishmania infantum* in the Blood of Equids in Kabylia (Algeria)

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**Purpose:** Canine leishmaniosis is a severe zoonotic disease that affects millions of dogs. Algeria is the most affected country in the Mediterranean basin. Both diseased and sub-clinically infected dogs are infectious to sand fly vectors, allowing transmission of the parasite to other dogs, animals or humans. That is why prompt diagnosis of infected dogs is essential. The occurrence *Leishmania infantum* was assessed in domestic dogs from Kabylia (Algeria), by means of PCR and serological analyses.

**Methods & Materials:** 214 dogs, a donkey and a horse were sampled, in June 2018, from three locations in the Kabylia region: Bouira, Tizi-Ouzou and Sétif. Sera were analyzed using rapid immunomigration test (Witness® Leishmania, Zoetis, France) followed by immunofluorescence test (IFAT) with 1/50 as positivity threshold. Blood samples were analyzed by a real-time PCR pan-*Leishmania* designed system, targeting 28S rRNA gene followed by qPCR targeting *L. infantum* kDNA. All dogs were examined and classed into one of the clinical scores (CS), from CS0 to CS4, per the frequency of their clinical signs.

**Results:** The total prevalence is of 34.5% (74/214) when at least one of the tests is positive. IFAT was more sensible and the prevalence collected scored a 31.8% (68/214), followed by Witness® Leishmania (29.9%, 64/214) and PCR (4.7%, 10/214). All positive samples on *Leishmania* spp. PCR 28S rRNA were positives for kDNA *L. infantum* specific PCR. The donkey and the horse were, also, positives by the two PCR systems. The equids lived near *Leishmania* infected dogs. Each infected dog where diagnosed in different stages of the disease: 74.3% (55/74) had at least one clinical sign and 25.7 % (19/74) were asymptomatics; 43.2% (32/74) had CS1, 21.6% (16/74) had CS2, 5.4% (4/74) had CS3 and 4% had CS4.

**Conclusion:** Kabylia remains an active focus for CanL with a high prevalence; this epidemiological situation requires the implementation of a national program against this protozoosis. This study highlights, for the first time, *L. infantum* in equids from Algeria which suggests the possible involvement of these animals in the epidemiological chain of *L. infantum* in high-transmission areas. This preliminary study deserves further investigation due to the lack of equids.
21.080
Phase 2 clinical results: Chikungunya vaccine based on measles vector (MV-CHIK) induces humoral and cellular responses in the presence of pre-existing anti measles immunity

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**Purpose:** We have developed a live recombinant measles vector-based vaccine to prevent Chikungunya disease (MV-CHIK). Previously, the safety and immunogenicity was demonstrated in a Phase 1 clinical trial (EudraCT: 2013-001084-23). Here, we will present the final data of a Phase 2 clinical trial in 263 healthy, volunteer adults (NCT02861586). Chikungunya is a rapidly spreading viral disease affecting significant parts of the Americas, India and South East Asia. In the majority of patients, the febrile acute disease turns into a chronic disease causing severe, debilitating arthritis that can last years post-infection. So far, no vaccine or treatment has been licensed.

**Methods & Materials:** The clinical trial was double blinded, randomized, active - and placebo-controlled, with the objective to evaluate the optimal dose of MV-CHIK vaccine in regard to immunogenicity, safety and tolerability in healthy adult volunteers. Immune responses were determined by induction of neutralizing antibodies by plaque reduction neutralization titer (PRNT).

**Results:** The vaccine presents with a safety profile in humans that is comparable to the approved control vaccine in the trial. Overall frequency of solicited and unsolicited adverse events (AEs) was low (not more than 30%) and all events were transient. No vaccine related Serious AEs (SAE) were observed. The vaccine induced neutralizing antibodies after a single immunization in up to 93% in the high dose group. A boost substantially increased the neutralizing antibody titers and seroconversion rate. In addition, CHIKV specific T-cell responses were induced following vaccination. Interestingly, the vaccine induced a functional immune response even in the presence of pre-existing anti-measles immunity as demonstrated in a subset of subjects who received a measles prime immunization prior to the Chikungunya vaccine.

**Conclusion:** The data show that the live recombinant MV-CHIK vaccine is a safe and tolerable vaccine that induces a robust immune response. The data clearly path the way for the preparation of a late stage clinical development program.
In vitro Survey Of Mosquito-related Viruses On C6/36 Aedes albopictus Cell Line

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Purpose: Several mosquito-transmitted pathogens pose general threat to human health (such as Zika virus, Dengue virus and Yellow Fever virus) while, in recent years, a novel group of viruses classified into different viral families had been identified, called mosquito specific viruses, with inability to replicate on vertebrate cell lines and showing large-scale distribution around the world. The aim of this study was to investigate the presence of mosquito-related viruses in field collected female mosquito homogenates via in vitro virus isolation procedures. We also conducted genomic characterization and phylogenetic studies on the detected strains. Our major goal was to generate an isolate bank of mosquito viruses that would serve as a basis for future experiments.

Methods & Materials: We inoculated 440 mosquito pools onto C6/36 cell line of which 42 showed cytopathic effect after the second passage.

Results: PCR screening of supernatants targeting different viral families has revealed the presence of West Nile virus (Flaviviridae) in one sample, Negev-like virus in 33 samples, Guaiaco Culex virus (Flaviviridae) in 2 samples, Koyama Hill virus (Reoviridae) in 5 samples and we found coinfection of Marisma Mosquito virus (Flaviviridae) and a sigmavirus (Rhabdoviridae) in one sample.

Conclusion: Results of recent study have revealed a huge geographic expansion of Guaiaco Culex virus compared to our recent knowledge and the presence of presumably human pathogenic Marisma Mosquito virus among Serbian mosquitoes. Furthermore, we successfully identified a tentatively new sigmavirus (Rhabdoviridae), previously described only in drosophila species, suggesting the evolution of this virus group in other dipterans and broader host range.
Emerging Human Alveolar Echinococcosis in Hungary. Early Experiences in Clinical Management in a Single Center Study from 2005-2018

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Purpose: Cystic echinococcosis is the most prevalent reportable helminthosis and also a neglected zoonotic disease in Hungary. Still nowadays the majority of cases are cystic echinococcosis (CE) caused by Echinococcus granulosus s.l. which is known to be endemic in central and Southeastern rural parts of Hungary. The appearance of Echinococcus multilocularis in wild definitive hosts, such as Red fox was first documented in Hungary in 2002. Untill now, based on national serological data, 15 possible human alveolar echinococcosis (AE) cases caused by this species have been detected. The first confirmed human AE and the first confirmed autochton human AE in Hungary was officially reported and published in 2008 and in 2016 respectively. The purpose of this study was to describe the clinical characteristics of AE cases in a single center which recently follows most of the Hungarian patients.

Methods & Materials: We collected clinical data about AE patients under follow-up in our infectological center, encompassing the time period between 05.07.2005 and 01.07.2018. Data were retrieved from database management system (Medsol) of Dél-Pesti Centrum Kórház, Országos Hematológiai és Infektológiai Intézet, Budapest. For the evaluation of clinical features regarding diagnosis and treatment we applied the international guideline proposed by the WHO-IWGE expert consensus.

Results: A total of seven patients under follow-up in our center received the diagnosis of probable (n=3) and confirmed (n=4) AE. In the four histopathologically confirmed cases additional supporting methods such as immunohistochemistry (n=2) and polymerase chain reaction (n=1) were applied. Curative liver resection could be done in two cases. In four unresectable cases progression was stopped by continuous parasitostatic albendazol therapy. In one unresectable case albendazol therapy had to be ceased because of drug-toxicity and allergic reactions. In this case pulmonary propagation is suspected. AE related liver insufficiency or death has not occured.

Conclusion: Echinococcus multilocularis is an emerging parasite in Hungary. It causes alveolar echinococcosis which is a potentially lethal zoonosis. Because of its still low incidence differential diagnosis and therapy of AE is a new challenge in clinical practice in Hungary.
Purpose: Leishmaniasis is mandatory to notify by law in Sri Lanka in National communicable disease surveillance system since 2008. Current study conducted to further identify and describe local epidemiology of the disease and to identify possible risk exposures of Leishmaniasis in Sri Lanka.

Methods & Materials: Countrywide descriptive cross sectional study was conducted among a random sample of 500 Leishmaniasis patients selected based on the probability proportionate to size of reported cases from each district during the year 2017.

Results: Almost all reported cases were Cutaneous Leishmaniasis (CL) and no visceral Leishmaniasis. Majority (65%) of patients were in the age group of 21-60 years: 30.4% among 21 – 40 years and 34.6% among 41 – 60 years (age range: 1 year to 97 years). Out of the sample, majority (64%) was males and most of them (62%) were engaged in outdoor activities as their occupation such as farming, defence forces, masons and laborers. Most of the affected females were housewives and students (55%). All patients have taken medical care mainly from government institutions (96.3%) and others from private healthcare institutions (3.6%) and native treatment centers (0.2%), in varying time intervals (from 1-30 days). Considering the housing conditions, few patients (9.2%) had sand and dung floors in the house, almost 1/4th (26%) had houses without plastered walls while only 16% had houses with temporary made roofs. The possible risk exposures of surrounding paddy fields (21.2%), agricultural plantation (35%) and shrub jungles (29%) around their houses gave some directions for possible vector contacts in and around households. When considering possible animal contacts at household level 64.4% patients had dogs, 45% had cats, 12.4% had birds and 7% had monkeys as domestic pets while 13% knew that rats were infested in and around houses.

Conclusion: Majority reported living in an environment with possible vector contact of poor housing, agricultural risk exposures or domestic animal contacts. However, possible unidentified risk exposures for Leishmaniasis transmission in Sri Lanka needs to be further studied to formulate effective country specific preventive measures.
Seroprevalence and determinants of *Echinococcus granulosus* infection in owned dogs in Ibadan, Oyo state, Nigeria

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**Purpose:** Echinococcosis, is a parasitic zoonosis of worldwide distribution that has been recently termed emerging/re-emerging and has dog as a definitive host. It constitutes severe financial burden derived from human health costs and livestock production losses. The factors associated with *Echinococcus granulosus* (EG) seropositivity in dogs in Nigeria is unknown. This study, therefore, aimed at determining EG seropositivity and its determinants in owned dogs in Ibadan, Nigeria.

**Methods & Materials:** Sera from 185 dog blood samples (5mls) obtained via the cephalic vein were analysed for the presence of EG antibodies using the direct ELISA technique. Structured interviewer-administered questionnaire was used to obtain data on demography, management and environmental factors from participating dog owners. Data were analysed using descriptive statistics, univariate analysis and logistic regression at α = 0.05.

**Results:** The mean age of the respondents was 35.7 ± 11.3 years; while median age of the dogs was 20 months (range 2 – 96). The seroprevalence of EG infection was 33.51%. Factors: low educational level of dog owners (OR: 2.8; 95% CI: 1.3, 5.8); local breed of dog (OR: 3.3; 95% CI: 1.7, 6.3); confinement (OR: 0.4; 95% CI: 0.2, 0.8); interaction with other dogs (OR: 3.2; 95% CI: 1.4, 7.3) and self-deworming of dogs (OR: 2.8; 95% CI: 1.3, 6.4) were associated with exposure to EG. On logistic regression, being a local breed of dog (AOR: 2.5; 95% CI: 1.2, 5.1) and self-deworming of dogs (OR: 2.8; 95% CI: 1.1, 6.9) remained predictors of EG seropositivity.

**Conclusion:** Exposure to EG is high among owned dogs in the study areas showing considerable risk infection to humans. Owners who self-medicate their dogs against worms were more likely to have dogs with EG infection compared to those who seek veterinary services. Dog owners are advised to seek veterinary service in treating their dogs against tapeworm infection.
21.085
Investigation of Genetic Diversity of *Echinococcus granulosus* From Human Host and The First Report of *Echinococcus canadensis* From Central Anatolia Turkey

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**Purpose:** Cystic Echinococcosis (CE) is a zoonotic disease caused by larval forms of parasites of the genus *Echinococcus*. *Echinococcus granulosus* sensu lato (s.l.) is divided into 5 main species as *Echinococcus granulosus* sensu stricto (s.s.) (G1-G3 genotypes), *Echinococcus felidis* (lion strain), *Echinococcus equinus* (G4 genotype), *Echinococcus ortleppi* (G5 genotype), and *Echinococcus canadensis* (G6/7, G8, G10 genotypes). Molecular epidemiological data on *E. granulosus* of human isolates in Turkey remains limited and there is no information about their clinical characteristics. The purpose of this study was to investigate the genetic diversity of *E. granulosus* and provide well defined clinical features of patients.

**Methods & Materials:** Hydatid cyst materials were collected from 12 patients who received percutaneous or surgical treatment for CE. Genomic DNA was extracted from cyst material using a commercial kit (Thermo, GeneJet Genomic DNA Purification Kit) and a fragment within the cytochrome c oxidase subunit 1 (*cox 1*) mitochondrial gene was amplified as previously described (Nakao et al 2000). A PCR result was evaluated positive at gel electrophoresis when the band size of ~875 bp was obtained. All positive isolates were identified via sequencing.

**Results:** 16 hydatid cysts were taken from 12 patients (7 female, 5 male, mean age ± 34). According to WHO-IWGE expert consensus classification system, 4 of the 16 hydatid cysts were classified as CE1, 4 as CE2, 3 as CE3a and 5 as CE3b. Majority of the hydatid cysts were located in liver right lobe (11/16) and average cyst size was 7.5 cm. Total 16 isolates were confirmed by BLAST algorithm as *E. granulosus* sensu lato. The majority of isolates (12/16) belonged to *E. granulosus* G1 whereas three of them were identified as G3 genotype and one isolate was determined as *Echinococcus canadensis* (G7 genotype). On the other hand, haplotype analyses of 15 isolates showed the presence of, 11 haplotypes with a high genetic diversity (Hd: 0.956±)

**Conclusion:** In this study, we investigated the genetic variation of *E. granulosus* sensu lato and provided well defined clinical characteristics. This is the first report of *Echinococcus canadensis* (Genotype G7) in human host from Central Anatolia Turkey.
21.086

Acute Anteroseptal ST-Elevation Myocardial Infarction (STEMI) in the West Nile Virus Infection

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Purpose: To present a case of acute anteroseptal ST-elevation myocardial infarction in the West Nile virus (WNV) meningoencephalitis.

Methods & Materials: A 77-year-old patient was hospitalized in the late summer of 2017 on the second day of the illness manifested by a fever up to 38.6°C and diarrhea. The patient did not report recent travel but recalled mosquito bites. Past medical history included hypertension. At admission, routine laboratory tests, electrocardiogram (ECG) and chest x-ray were performed. In addition, cerebrospinal fluid (CSF), urine and blood samples were collected for a virological analysis.

Results: At admission, WBC count was 24.6 (reference range 3.4-9.7x109/L) with neutrophilia (92%, range 44-72%) and very high levels of cardiac enzymes: creatinine phosphokinase 1856 (range 17-153 U/L), lactate dehydrogenase 433 (range 2-241 U/L), myoglobin 3116 (range 20-80 ug/L) and troponin I 17.640 (range 0.000-0.056 ug/L). ECG showed ST elevation. In the cardiac intensive care unit, an emergency coronary angiography was performed which confirmed the coronary artery stenosis. The patient's condition complicated on the 4th day of the illness by an altered level of consciousness with progression to coma, accompanied by neck stiffness and positive meningeal signs. Computed tomography of the brain was normal. Cerebrospinal fluid (CSF) showed pleocytosis with 26 cells/mm3, predominantly mononuclears (73%) and elevated protein level (1.151, range 0.170-0.370 g/L). Both CSF and urine were positive for WNV RNA by real-time and nested RT-PCR. Phylogenetic analysis showed WNV lineage 2. The patient was initially treated with acyclovir, ampicillin and cefepime parenterally with supportive therapy (antiedematous, antiaggregation and antihypertensive therapy). On the 8th day of the illness a respiratory insufficiency developed. The patient was intubated and mechanically ventilated, but developed hypotension and low oxygen saturation in spite of an adequate respiratory support. Despite the cardiopulmonary resuscitation, the patient died due to cardiopulmonary arrest.

Conclusion: Although cardiac involvement is not frequently reported in the course of a WNV infection, physicians should be aware of the possibility of a WNV-related myocardial infection.
Isolation Of *Brucella Melitensis* In Azerbaijan In 2014

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**Purpose:** Republican Anti-Plague Station (RAPS) in Azerbaijan provides confirmatory tests for especially dangerous pathogens (e.g. *Yersinia pestis*, *Bacillus anthracis*, *Brucella* spp., *Francisella tularensis*). In Azerbaijan RAPS and its regional Anti-Plague Divisions (APDs) are responsible for testing human samples for abovementioned pathogens. RAPS receives samples for confirmatory test from patients referred by Baku, regional hospitals and APDs. The aim of this study was to isolate and identify *Brucella* cultures from human blood samples.

**Methods & Materials:** In 2014, 1,137 blood samples were collected by RAPS from patients with clinical symptoms such as high temperature, perspiration, chills, myalgia, arthralgia, headache, fatigue, lack of appetite. All samples were tested using the Azerbaijan Ministry of Health (EDPs Laboratory Guidelines, 2013) algorithm via bacteriology and serology tests. Blood serum was tested for the presence of the antibodies of *Brucella* spp. via Huddleson reaction, Rose Bengal test and Wright's reaction. Samples positive for *Brucella* spp. per Huddleson and Rose-Bengal reactions further were tested by Wright's reaction. Positive blood samples by Wright's reaction with titers 1/200 and higher were then cultured. Isolated pure cultures were examined by biochemical (Urease, Oxidase, Catalase, TSI/H₂S, Dye sensitivities) and serology (Trypaflavine, Agglutination with specific polyvalent serum, anti-A and anti-M monospecific sera) tests.

**Results:** Data showed that 54.3% from total number of serum samples were positive by Huddleson reaction; 35.6% from total were positive by Rose-Bengal reaction and 38.7% from total samples were positive by Wright's reaction. Positive blood samples with titer 1/200 and higher from first diagnosed patients (n=89) were cultured. All isolates were identified as *Brucella melitensis* (an overall isolation rate was 16.9%). The reports about positive results are sent to the Ministry of Health and State Veterinary Control Service (SVCS) and entered into the Electronic Integrated Disease Surveillance System (EIDSS) database of the Ministry of Health of Azerbaijan. Data from the system allows tracking the incidence of brucellosis and making operational decisions.

**Conclusion:** Future genetic analyses of these isolates can help identify subtypes of *Brucella melitensis* and establish the origin of *B. melitensis* circulating in the Azerbaijan territory and compare the results with those of neighboring countries.
Generation, Lyophilisation and Epitope Modification of High Titre Filovirus Pseudotyped Lentiviruses for use in Antibody Neutralisation Assays

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Purpose: Filoviruses, such as Ebolavirus, are zoonotic pathogens causing disease outbreaks with high mortality rates, requiring scarce high containment facilities for research. Nevertheless, pseudotyped viruses (PV), consisting of a lentiviral core (plus luciferase reporter) and the envelope glycoprotein (GP), allow basic and translational virology to be conducted under low containment. Consequently, filovirus PVs were generated and viability assessed after lyophilisation and long-term storage. Next, antibody neutralisation tests were performed using native and hybrid GPs to assess differentiation between genera and species.

Methods & Materials: PVs were produced using a 3-plasmid transfection system (representing core, reporter and envelope) in HEK293T/17 cells, and supernatant titrated. Supernatants were then lyophilised in sucrose cryoprotectant solution, stored under various conditions, reconstituted and titrated. For antibody neutralisation tests, serially diluted, polyclonal convalescent sera (NIBSC, UK) or anti-GP monoclonal antibodies (Xiangguo Qiu, PHA, Canada; Erica Saphire, Scripps, USA) were incubated with PV for 1h at 37°C, prior to titration. To create artificial GP antigens, EBOV neutralising epitopes were inserted into the GP of another genus (Cuevavirus; LLOV) by mutagenesis, PVs generated and infectivity and neutralisation assessed.

Results: High titre PVs were produced with titres between ~1x10⁸ RLU/mL (Ebolavirus/Cuevavirus) and ~1x10¹⁰ RLU/mL (Marburgvirus). Lyophilised PV titres remained constant stored at -20°C and 4°C for 12 months, while PVs kept at room temperature (22.5°C) demonstrated titre decreases of up to 3 orders of magnitude after 6 months. At 37°C, five log (Marburgvirus) or three log (Ebolavirus and Cuevavirus) decreases occurred after one month. Zaire Ebolavirus (EBOV) antibodies showed no cross reactivity with native LLOV PVs. Furthermore, EBOV epitopes inserted into the LLOV GP and expressed on PVs had no significant impact on PV infectivity, and EBOV neutralising epitopes were successfully reconstituted in these chimeric antigens.

Conclusion: In this study, high titre PVs were generated and found to be amenable to lyophilisation and long-term storage. Reconstituted PVs retained their function in neutralisation assays suggesting their structure is not compromised during freeze-drying. Insertion of epitopes in heterologous GPs did not impact infectivity or functionality. This data suggests a PV-based serological kit could be utilisable in resource-limited countries for serological studies, after simple refrigeration storage.
Tula Virus Phylogeography

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**Purpose:** Tula hantavirus (TULV) is zoonotic virus widespread across Eurasia, where numerous small mammal species have been shown to be its reservoirs. In the Balkans, Serbia is the first country where TULV was detected, in European pine vole, *M. subterraneus* trapped in 1987. Although TULV is not considered pathogenic for humans, cases of human infection have been reported on several occasions so far. Previously, we have shown the evidence of recombinantion events in studied TULV lineages from Serbia. In this study we applied Bayesian phylogeography framework to reconstruct the spatial and temporal dynamics of TULV based on sequences isolated from different geographical areas.

**Methods & Materials:** The analyzed dataset was made of 137 TULV S segment sequences existing in the database, including two sequences from Serbia; in total, 70 isolation sites within Europe and Asia, covering time span of isolation of 28 years (1987-2015) were included. Sequences were aligned using CLUSTAL W implemented in MEGA 6 and then manually edited. The best fit nucleotide substitution model was determined by jModeltest 0.1.1 using all 88 proposed models. TreePuzzle was employed to investigate the phylogenetic signal of each sequence in the dataset. To explore temporal structure of the dataset, root–to-tip analysis was done by TempEst. The phylogeny, including phylogeographic distribution was co-estimated in a Bayesian framework using a Markov Chain Monte Carlo (MCMC) method implemented in the Beast package v 1.8.4

**Results:** Studied TULV strains formed three well supported clades matching the geographical origin: the clade closest to the tree root consisted of sequences from Russia and Kazakhstan; the second clade contained strains originating from western and central Europe; the third clade consisted of sequences from western and southeast Europe. The place of origin was assessed to be in Kazakhstan, with posterior probability of 1. The routes of viral spread included local distribution across Kazakhstan and Russia but also Europe, with the complex pattern of local viral migration further on.

**Conclusion:** The place of TULV origin was assessed to be in Kazakhstan, with westward spread leading to single introduction of TULV to Europe.
Purpose: Leptospirosis is the most common zoonosis in the world and is considered as an emerging human disease. In French Guiana, recent epidemiological data indicate a significant increase in human cases since 2012, with an annual incidence in 2014 of 39 cases per 100,000 inhabitants, making it one of the major hotspots in the world. Considering this situation, the main goal of the present study was to investigate the incidence of the infection in dogs which are possible reservoirs or maintenance hosts for leptospires.

Methods & Materials: A serological survey was conducted in Guiana in 2016 on 95 dogs from Cayenne and Kourou. Location, race, sex, age, health and vaccination status were recorded for each dog. Sera obtained were tested for 27 serovars of pathogenic *Leptospira* species by the microscopic agglutination test. The results were interpreted according to the decision tree used in the VetAgro Sup leptospire laboratory, Lyon, France.

Results: Among the 95 samples, 59 showed agglutination at the cut-off point (1:20) for one or more pathogenic leptospiral serovars. Focusing on high titres (≥1:160), seroprevalence was 11.6%. No statistically significant difference of prevalence due respectively to sex and age was observed (p > 0.05). *Icterohaemorragiae* (40%), *Australis* (33.3%) and *Canicola* (27%) were the most frequently observed serogroups.

Conclusion: Dogs are not usually considered as a reservoir for *Leptospira*, except for *Canicola*, thus, the high prevalence found in this study in unvaccinated dogs probably results from a heavy exposure. However, the cut-off points selected and the absence of kinetic serology do not allow, in most cases, to conclude in favor of a current active infection. Dogs are highly exposed to pathogenic leptospires and humans living in the same environment are also at risk of infection. Thus, dogs could be considered as sentinels for human exposure to this zoonotic pathogen. In French Guiana, 98% of which is covered by equatorial rainforest, all the conditions are in place for the development of leptospirosis, particularly the climate which is characterized by abundant rainfalls and high temperatures all year round, the presence of numerous streams, and close contact with domestic animals and wildlife.
Purpose: Purpose
Owing to the recent emergence of Zika virus (ZIKV) as a global pandemic threat, the role of innate T cells response during ZIKV infection is as yet unclear. An expansion of Vδ2 T cells showing a terminally differentiated profile was observed during acute ZIKV infection. Vδ2 T cells were enriched of Granzyme B and preserved the ability to produce IFNγ after a specific stimulation. The aim of this work was to define the antiviral potential of Vδ2 T cells against ZIKV.

Methods & Materials: A549 cells were infected with two different strains of ZIKV (MR766 and INMI 1) for 24h/48h and the frequency of ZIKV-infected A549 cells was analyzed by flow cytometry using a specific mAb anti-Panflavirus. To test Vδ2 T cell expansion, peripheral blood mononuclear cells (PBMC) were co-cultured with ZIKV-infected or with uninfected A549 cells for 7 days and Vδ2 T cell frequency was analyzed by flow cytometry. The supernatants of activated Vδ2 T cell lines were tested for their anti-ZIKV activity. The antiviral capability of Vδ2 T cells were performed by co-culturing Vδ2 T cell lines with ZIKV-infected and uninfected A549 cells for 24h. A549 cell death was analyzed by Propidium Iodide/Annexin V staining and flow cytometry.

Results: After 48 h of infection, the frequency of MR766-infected A549 cells was higher than the frequency of INMI-1-infected A549 cells (37.1% vs. 4.1%). ZIKV-infected A549 cells were able to induce a specific Vδ2 T cells expansion when compared with uninfected A549 cells (index of expansion median: 9.7 vs. 5.0). Moreover, soluble factors (SF) released by activated Vδ2 T cells were able to inhibit ZIKV replication. Finally, Vδ2 T cells were able to recognize and kill ZIKV-infected cells.

Conclusion: Conclusions
We showed that Vδ2 T cells expand after recognition of ZIKV-infected cells and are able to exert anti-ZIKV activity thought cytolitic and non cytolitic mechanisms. Further data are necessary to identify the molecular pathways involved in the recognition of ZIKV-infected cells in order to clarify the role of Vδ2 T cells in the pathogenesis/protection of ZIKV infection and to identify new possible target for immune-therapy.
Seroprevalence of *Coxiella burnetii* Infection in Dogs from Southeastern France

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**Purpose:** *Coxiella burnetii* is the causative agent of Q fever, a major zoonosis. Humans are generally infected by aerosols or contacts with ruminants which often remain an asymptomatic source of infection. Dogs also can be infected and generally remain asymptomatic. To now, few studies have been carried out in France to investigate the role of dogs in the epidemiological cycle of the disease. The aim of this survey was to assess the seroprevalence of *C. burnetii* infection of apparently healthy dogs living in southeastern France.

**Methods & Materials:** In 2014, serum was collected from 264 dogs living in a dog shelter in Marseille. In the same way, from 2014 to 2017, 264 additional dogs, including military working dogs, were sampled, in the oriental coast of Corsica, mostly around the village of Ghisonaccia. The detection of anti-*C. burnetii* (phases I and II) antibodies was achieved using an immunoenzymatic (ELISA) commercial test (PrioCHECK™ Ruminant Q Fever Ab Plate Kit, Thermo Fisher Scientific – Courtaboeuf). In this test, the specific conjugate of ruminant antibodies was replaced by a protein A/G, which has a strong affinity to dog antibodies according to the manufacturer (Pierce™). Sera were considered positive when the optical density was over 20%.

**Results:** The overall *C. burnetii* infection seroprevalence was 31.2% (95% confidence interval: 27.3-35.2) among the 528 dogs tested. In Marseille, 25% (66/264) of the dogs were seropositive while 37.5% (99/264) of the dogs were positive in Corsica. The seroprevalence was significantly higher in Corsica compared to Marseille (Chi² test, p≤0.01). This difference is even stronger for highly positive sera: 1.5% (4/264) and 6.4% (17/264) sera with optical density over 80%, respectively in Marseille and Corsica.

**Conclusion:** In this survey, the overall seroprevalence is high but not exceptional. The increased seroprevalence in the oriental coast of Corsica could be explained by the important population of sheep in this area and the frequent infestation of dogs by ticks. Dogs could represent a secondary reservoir but they more probably reflect the environmental presence of *C. burnetii* and thus can be considered as sentinels of the infection.
Clinical And Laboratory Characteristics Of Neuroinvasive Viral Zoonoses Detected In Continental Croatian Regions, 2017-2018

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Purpose: To analyze clinical and laboratory characteristics of neuroinvasive viral zoonoses detected in Croatia during 2017-2018.

Methods & Materials: From April 2017 to June 2018, a total of 120 patients with neuroinvasive disease from continental Croatian regions were tested for the presence of neuroinvasive zoonotic viruses: tick-borne encephalitis virus (TBEV), West Nile virus (WNV), Usutu virus (USUV), Toscana virus (TOSV), Tahyna virus (TAHV) and lymphocytic choriomeningitis virus (LCMV). Cerebrospinal fluid (CSF) and urine samples were tested for the presence of viral RNA using a real-time RT-PCR and/or nested RT-PCR. Serological tests of serum/CSF samples (IgM/IgG antibodies, IgG avidity) were performed using ELISA (TBEV, WNV, USUV), IFA (TOSV, LCMV) and virus neutralization test (WNV).

Results: Etiology was confirmed in 28/23.3% patients: TBEV in 20/16.7% and WNV in 8/6.6% patients by detection of IgM and IgG antibodies of low avidity and/or detection of viral RNA in CSF and urine. Majority of patients with TBEV infection were males (15/75.0%). Although infections were detected in all age groups, 15/75.0% patients were less than 60 years of age. The main clinical symptoms were headache (18/90.0%), weakness (18/90.0%), nausea (12/60.0%) and vomiting (8/40.0%). Fever >39°C was noted in 16/80.0% patients. CSF leukocyte count ranged from 41-3520/mm³ with mononuclear cell predominance in 15/75.0% patients. All but one patient fully recovered. WNV infection was reported in 5/62.5% males and 3/37.5% females. All but one patient (7/87.5%) were older than 60 years. Majority of patients reported underlying diseases: hypertension (3/37.5%) and cerebrovascular disease (3/37.5%). The main clinical symptoms were headache (5/62.5%) and weakness (5/62.5%), while fever >39°C was noted in 4/50.0% patients. CSF leukocyte count ranged from 56-1096/mm³ with mononuclear cell predominance in 4/50.0% patients. One patient died. USUV, TOSV, TAHV and LCMV infections were not detected during the tested period.

Conclusion: TBEV infections were more common in patients less than 60 years of age, while WNV infections were most common in elderly. High fever was noted in 80.0% TBEV cases compared to 50.0% WNV cases. CSF pleocytosis was higher in TBEV infection.
Severe Undifferentiated Febrile Illness Outbreaks in the Federal Republic of Sudan – A Retrospective Epidemiological and Diagnostic Study

Purpose: The Federal Republic of Sudan has experienced 13 outbreaks of undifferentiated febrile illness (UFI) associated with haemorrhage and high case fatality rates (CFR) since 2012. As a range of high consequence pathogens is endemic in Sudan, these outbreaks are of significant concern. We investigated a 2015-16 UFI outbreak in Darfur (469 cases, CFR 25.6%) to explore aetiologies, inform public health interventions and promote development of diagnostic capacity.

Methods & Materials: We extracted clinical and epidemiological data from Sudan Ministry of Health databases and matched available legacy samples at the National Public Health Laboratory. Serum samples were tested further at the Rare and Imported Pathogens Laboratory, Public Health England, using a bespoke panel of molecular and serological assays covering a broad range of likely pathogens, including haemorrhagic fever viruses, arboviruses, leptospirosis and rickettsiae.

Results: Sixty-five samples were tested—51% from males and 48% from persons aged <15 years, that were collected a median 4 days (IQR 2-7) after onset of symptoms. The most common symptoms were fever (85%), bleeding (69%, including haematemesis in 49% and epistaxis in 43%), headache (49%), joint pain (43%), and loss of appetite (31%). There were no epidemiological patterns indicative of person-to-person transmission and no cases among healthcare workers. Of the 47 cases for whom an outcome was recorded, the case fatality rate was 6%. Seven (11%) of the 65 samples were positive for Congo Crimean haemorrhagic fever (CCHF) by both RT-PCR and IgG ELISA. Six of these were also ELISA IgM positive. An additional three of the PCR/IgM-negative samples were CCHF IgG positive. All CCHF-positive cases were men aged 21 to 30 years, except for one 5-year-old child. Four were farmers. All other samples tested negative on all assays.

Conclusion: Comprehensive diagnostic evaluation demonstrated CCHF as one important cause, but not the sole major aetiology of UFI in the Darfur outbreak. We are presently undertaking unbiased sequencing of the legacy samples to explore other aetiologies, including novel pathogens. A UFI prospective study protocol and trained study team is now in place to investigate future outbreaks.
**Gene Polymorphism IL17A (RS8193036; RS2275913) In The Brucellosis**

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**Purpose:** The study of association of polymorphisms IL17A gene (rs8193036; rs2275913) with brucellosis.

**Methods & Materials:** Study Design: Case-control. Group of cases - 89 patients with brucellosis, and the control group was 414 people who did not suffer from brucellosis. Genotyping of IL17A (rs8193036; rs2275913) was carried out by the Real-time PCR method in the Organic synthesis laboratory of the “National Center of Biotechnologies”, Astana, Kazakhstan. Statistical calculations were carried out on a calculator for genetic calculations under the Gene Expert program. (http://gen-exp.ru/calculator_or.php).

**Results:** It has been established that the factors predisposing to brucellosis can be the C allele and the CC genotype (OR (95% CI) = 4.42 (3.00-6.51) and 7.32 (4.39-12.20), respectively) , and T allele, CT and TT genotypes of polymorphism rs8193036 of the gene IL17A (OR 95% CI) = 0.23 (0.15-0.33), OR (95% CI) = 0.43 (0.26-0.72) and 0.19 (0.09-0.43), respectively) - the factors of resistance.

Studies on the association of polymorphism rs2275913 of the IL17A gene revealed that the factors of predisposition to brucellosis are G and GG (OR (95% CI) = 2.26 (1.51-3.38) and 2.25 (1.40-3.61) ), respectively, while the resistance factors may be the allele A (OR (95% CI) = 0.44 (0.30-0.66)) and the AA genotype (OR 95% CI) = 0.08 (0.01-0.61)).

**Conclusion:** The results of our study indicate that in brucellosis there is a possible association of brucellosis with polymorphisms of the IL17A gene (rs8193036, rs2275913).
Purpose: In order to better understand contributing factors to the high incidence rate of leptospirosis in Ukraine, this study was designed to analyze the seasonality, and how it is associated with severity and case fatality rate of the diseases.

Methods & Materials: We performed a retrospective analysis of 395 case studies of patients with leptospirosis that had been treated in Lviv Oblast Hospital for Infectious Disease during 2002-2016. Diagnosis was confirmed using the microagglutination test. Statistical analysis was performed using Fisher test.

Results: The majority of cases (318; 80.5%) were recorded during the summer and autumn. The highest number of cases was registered in August – 97 (24.6%) that is significantly higher than the numbers in other months (p <0.05). In September, 62 (15.7%) patients with leptospirosis were admitted to the hospital; in October – 73 (18.5%); in November – 33 (8.4%). During 6 months of the winter and spring, only 77 (19.5%) admissions were reported, which is significantly lower than during June-November (p<0.01). In other months, 8 to 18 cases per month (2.0-4.6%) were registered. Among 395 patients, 362 (91.7%) recovered and 33 patients died; the case fatality rate was 8.3%. Comparing the number of recovered and fatal cases, the probable difference in seasons was recorded during the summer months (39.5% of recovered cases against 21.2% of fatal cases, p <0.05).

Conclusion: The highest number of leptospirosis cases was found during June-November with a peak in August. The number of patients who were hospitalized during these months is 4.1 times higher than the respective number in January-May (318 versus 97, p <0.01). Taking into account the highest incidence rate of leptospirosis that was reported during the summer and autumn, it is necessary to bring authorities’ attention to compliance with sanitary regulations for water reservoirs that are open for bathing during the summer and to provide agricultural workers with waterproof protective equipment.
Heterogeneities in Vaccine Coverage and their Influence on Measles Incidence and Outbreak Sizes in the United States

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**Purpose:** The United States achieved measles elimination in 2000, but external importations are a continued source of local outbreaks. In 2015, the state of California experienced an outbreak of 131 confirmed cases. Analysis of the data showed a highly subcritical effective reproductive number, suggesting the occurrence of such outbreaks to be extremely rare. Using detailed computer simulations we investigated the influence of a range of vaccine coverages on the annual incidence and size of outbreaks due to external importations in the United States.

**Methods & Materials:** We used a detailed compartmental model for measles that incorporates maternal immunity, the vaccination programme adopted in the United States and takes into account vaccine efficacy. A range of vaccine coverages were generated using an algorithm controlled by two parameters that specify the size and depth of heterogeneities. Transmission dynamics were performed on a highly resolved spatial description of the human geographical distribution at a resolution of 5km which included individual mobility.

**Results:** We found that, assuming an average vaccine coverage of 92%, corresponding to the seroprevalence measured in the United States in 2015, the number of annual cases and outbreak sizes reported in recent years is compatible with the number of estimated importations combined with a highly subcritical reproductive number and a moderately heterogeneous vaccine coverage. Under these conditions, outbreaks of the size of the 2015 California outbreak are not rare. We also show that localized drops in vaccine coverage may lead to a catastrophic failure of herd immunity if they exceed a heterogeneity dependent threshold.

**Conclusion:** Given the current average level of vaccine coverage, outbreaks with sizes similar to the 2015 California one may occur in the future. However, the fact that the effective reproductive number is highly subcritical confirms the high level of control over measles currently enforced in the United States.
Risk Management and Preparedness: Use of Stochastic Modeling and Risk Analytics to Estimate Frequency and Severity of Filovirus Epidemics

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Purpose: Epidemics pose a significant risk for human and economic losses, yet this risk is challenging to quantify. Current methods for assessing epidemic risk are typically based on observational data, which are often sparse and incomplete. Global simulation models of infectious disease spread allow us to simulate spatiotemporal dynamics of hypothetical epidemic scenarios. Sovereign nations in Africa are at risk of experiencing filovirus epidemics, which can have devastating financial impacts. These models can help fill in the gaps in observational data and provide a clearer picture of epidemic risk.

Methods & Materials: We used a stochastic epidemic model to simulate over 200,000 filovirus epidemics, with varying initial parameters and differing availability and efficacy of intervention measures. Probability distributions of model parameters such as spark location, transmissibility, and case-fatality ratio were derived from the primary literature and historic case data. We constructed an event catalog by selecting simulated events using a sampling algorithm that accounted for the joint probability of each parameter combination for each scenario and the inter-arrival time distribution between filovirus epidemics. The event catalog was then used to generate exceedance probability metrics, which estimate the likelihood of observing an event of a given severity (e.g., expected deaths), or worse, in any given year.

Results: Based on our analysis, there is a 1.5% probability that a filovirus epidemic similar to or worse than the 2014 West Africa Ebola epidemic (based on global deaths) will occur in any given year. The countries that are predicted to most commonly experience filovirus epidemics (at least one infection that either originated in the country or was imported) were Democratic Republic of the Congo, Guinea, Liberia, and Uganda. We estimate that Democratic Republic of the Congo has a 31% annual probability of having at least one filovirus-infected individual.

Conclusion: Probabilistic modeling and stochastic simulations are powerful tools to quantify epidemic risk. With these risk metrics, organizations such as the African Risk Capacity, a specialized agency of the African Union, can work with sovereign nations on coordinated preparedness assessment, contingency planning, and risk modeling to equip them with the financial resources needed to respond to an epidemic.
Socio-demographic and Exposure Factors associated with Confirmed Cases of Monkeypox infection in Bayelsa State, Nigeria – 2017

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Purpose: An outbreak of Monkeypox, a rare viral zoonotic disease caused by an Orthopoxvirus, was reported in Bayelsa on September 22, 2017. We investigated the outbreak to characterize the cases and determine associated factors.

Methods & Materials: We reviewed hospital records of Yenagoa and Ogbia Local Government Area for suspected or confirmed cases at Niger Delta University Teaching hospital (NDUTH) and extracted data on sociodemography and clinical features. We conducted an unmatched case-control study from September 25 to November 8, 2017. We defined a confirmed case as any person presenting with a history of sudden onset of fever, vesiculopustular rash occurring mostly on the face, palms, and soles of the feet with laboratory confirmation of monkeypox virus (any of a positive IgM antibody, PCR or virus isolation). Controls were healthy family members and neighbors randomly selected. All 10 confirmed cases and 30 controls were recruited. A bivariate analysis was performed to determine associated factors, using Fisher’s exact test, and p<0.05 was considered significant. We also carried out an environmental assessment for the houses of cases.

Results: The median age of the cases was 28.5 years (Range: 6 - 43 years), while that of the controls was 31 years (Range: 16 – 68 years). One of the cases committed suicide on admission at NDUTH. About 80% of the cases were male and single. Being single (OR= 7.6; 95% CI: 1.4–76.1) and attending churches and burial ceremonies (OR: 0.02; 95% CI: 0.01–0.86) were associated with the risk of developing monkeypox infection. All cases and 26 (86.7%) controls were found to be living in rodent infested houses.

Conclusion: Being single and attending churches and burial ceremonies were found to be risk factors in acquiring monkeypox. Public awareness on avoidance of body contacts during social gatherings and strict environmental sanitation were recommended.
Measles epidemic in Brazil in the post-elimination period: Coordinated response and containment strategies

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Purpose: The measles virus circulation was halted in Brazil in 2001 and the country has a routine vaccination coverage against measles, mumps and rubella higher than 95%. In Ceará, the last confirmed case was in 1999. This abstract describes the strategies adopted and the effectiveness of the surveillance and control measures implemented during the epidemic in an attempt to understand the challenges posed by the regional context of post-measles elimination and explain the actions taken to prevent the re-establishment of endemic transmission when the virus is imported.

Methods & Materials: A descriptive study was conducted to examine immunization strategies, epidemiological and laboratory surveillance, and communication to contain and halt the chains of measles transmission during an epidemic between December 2013 and October 2015.

Results: The epidemic started in December 2013 and lasted 20 months, reaching 38 cities and 1,052 confirmed cases. The D8 genotype was identified. More than 50,000 samples were tested for measles and 86.4% of the confirmed cases had a laboratory diagnosis. The beginning of an campaign vaccination was delayed in part by the availability of vaccine of vaccine. The classic control measures were not enough to control the epidemic. The creation of a committee of experts, the agreement signed between managers of the three spheres of government, the conducting of an institutional active search of suspected cases, vaccination door to door at alternative times, the use of micro planning, a broad advertising campaign at local media and technical operative support contributed to containing the epidemic.

Conclusion: It is important to recognize the possibility of epidemics at this stage of post-elimination and prepare a sensitive surveillance system for timely response. The point in the epidemic when the number of cases began to decrease clearly coincided with the point at which the activities of epidemiological surveillance, the immunization sector, coordinated communication actions, laboratory surveillance and primary care were integrated. This coordinated response reduces the chances of spreading the virus and thus the harm and the impact of a measles epidemic.
Clinical and Molecular Epidemiology of the current Venezuelan Diphtheria Epidemic. A hospital-based experience

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Purpose: Since July 2016, Venezuela has experienced a large diphtheria outbreak with over 1,710 accumulated cases (1,086 of them confirmed), and 160 deaths, as of May 2018. Available clinical and epidemiological information is very limited. Response to this emergency has been hampered by a profound ongoing healthcare crisis

Methods & Materials: In cooperation with the Bernhard-Nocht Institute for Tropical Medicine in Hamburg and the Instituto Carlos III in Spain, a multicenter network of medical professionals from various regions of the country with disease activity was recently assembled, in order to accomplish a prospective, observational investigation, aimed to describe the clinical and molecular epidemiologic patterns of hospitalized cases and their direct family contacts

Results: Until now, 37 suspected or confirmed diphtheria patients and 10 close contacts have been followed (37% males, 63% females). Mean age of onset was 17 years, median 11 years (age range: 1 to 66 years). The most frequent location of lesions was pharyngeal-tonsillar combined (35.1%); however, other location such as tonsillar (29.7%), pharyngeal (21.6%) laryngotracheal (10.8%) and nasal (2.7%) were also observed. All patients received antibiotics, but only 67% of them diphtheric antitoxin. Delay in antitoxin administration was the rule (average: 2.4 days). Only 23% of the patients had a complete basic diphtheria immunization schedule and 36% of them developed clinical complications. Overall the case fatality rate (CFR) was 16.21%. Three bacterial isolates were characterized as toxigenic C. diphtheria biotype mitis, multilocus sequence type ST 174

Conclusion: The diphtheria outbreak in Venezuela remains active, reflecting the inadequacy of the strategies of outbreak contention implemented. The high CFR observed in this series may be related to the delay in antitoxin administration and the use of suboptimal doses. The marked predominance of adolescents and adults suggests the need of vaccine boosters doses among the non-pediatric population. Available data indicates that the ST 174 sequence identified has not been circulating in the region in recent years.
Outbreak of Infestation with Body Lice in a Home for Assisted Living, Austria, 2018

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Purpose: On 29 May 2018, the Austrian Agency of Health (AGES) was contacted by the head of a home for assisted living, which had 11 residents. She reported significant pest infestation, occurrence of vermin for three days throughout the whole facility. Vermin extermination (gassing by a professional) had no effect: vermin were still present on the residents in their groins, navels, and armpits and the pests still continued to multiply “by leaps and bounds, traveling throughout the house”. Residents suffered from “red skin rashes” and ambulance drivers refused to take the vermin infested patients to hospital.

Methods & Materials: Preliminary diagnosis of body lice infestation was made, based on outbreak history and a smart phone picture. A dozen body lice were collected and shipped to AGES for PCR confirmation. Species identification was also confirmed by Prof. Sally Cutler of University of East London, UK.

Results: Body lice spread through direct person-to-person contact or through contact of clothing, beds, bed linens, or towels that have been in contact with someone who has body lice. The bites during lice feedings cause itching at the feeding sites, which may look like small, red dots on the skin; scratching can break the skin and lead to distinct skin rash. The institution was advised to wash clothing, bedding, and towels using hot water and to dry with high heat; mattresses were discarded. Personal hygiene for the infested persons was improved by daily assisted showering using medicated lotions available for head lice on the body as well.

Conclusion: In contrast to head lice or pubic lice, body lice can cause real “outbreaks” of pest infestation. Body lice live and reproduce in clothing worn by humans; they feed on human blood and can survive only on people. The eggs (“nits”) hatch after about 1 week then pass through 3 stages (over 1 to 2 weeks) before becoming full-sized adults. In this case, a recently admitted resident from the homeless milieu four weeks earlier must be considered the source of introduction of body lice into the facility. This outbreak underlines that body lice are still endemic in Austria in 2018.
21.103
Real-time Coverage Surveys and Monitoring During Emergency Vaccination Campaigns in Insecure Humanitarian Contexts of DRC and Somalia

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Purpose: Mass vaccination campaigns in low-resource settings traditionally rely on ground coordination and post-campaign activities to assess coverage and programmatic performance. However, complex humanitarian crises in highly insecure settings present increased limitations on informational and operational access, suppressing accurate measurement and achievement of target coverage rates. To mitigate these contextual constraints, a real-time monitoring strategy implemented concurrently with immunisation activities may quickly identify and address low coverage causes before the campaign concludes.

Methods & Materials: Independent monitoring approaches were implemented during two recent emergency outbreak vaccination campaigns for yellow fever in the Democratic Republic of Congo (DRC) and cholera in Somalia. Enumerators conducted post-vaccination exit interviews and focus group discussions (nDRC = 591, nSomalia = 3025) with children and adults at clinical sites, and a two-stage, stratified clustered community household coverage survey (mDRC = 300, mSomalia = 1684). Daily coverage rates, barriers to uptake, and indicators of community awareness, mobilisation and communications strategies, and service delivery were captured.

Results: DRC beneficiaries indicated high levels of service satisfaction while also highlighting a lack of visual communication materials and insufficient instruction from clinical staff. Respondents in Somalia experienced good service delivery and emphasised lower understanding around adverse events reporting. Knowledge of cholera disease and prevention strategies centred around hand washing and clean drinking water, varying by age group. Primary barriers of vaccination were incompatible working hours (DRC) and distance to clinical sites (Somalia). Daily coverage indicators from the household survey identified low coverage areas and demonstrated final rates of 93.1% (DRC) [87.4, 98.8] and 99.08% (Somalia) [98.64, 99.41].

Conclusion: Collective results from the monitoring activities regularly informed coordinators in real-time, allowing them to refine outreach strategies during the campaign. Changes included identifying new communications and information sources, modifying campaign messaging to correct erroneous community perceptions, and targeting geographical areas. The resultant increasing daily coverage trends demonstrate that this monitoring framework can act as a vital source of independent evaluation during emergency vaccination campaigns. It enables immediate feedback and operational modification to increase coverage. This is crucial when pre-campaign information is incomplete and post-campaign activities may be limited or delayed.
Purpose: Infectious diseases are a major cause of morbidity and mortality worldwide. It is essential that healthcare professionals are able to correctly diagnose and report patients with serious infectious diseases without delays. This is so that, if required, patients will receive immediate treatment, referral, and isolation. To improve the capacity of healthcare professionals in the diagnosis of infectious diseases, BMJ has implemented the BMJ Clinical Decision Support (CDS) Initiative in Azerbaijan, Georgia, Ukraine, Jordan, Iraq and Vietnam. The Initiative provides access to primary care physicians and hospital specialists to infectious disease content on BMJ Best Practice (an online clinical decision support resource) and BMJ Learning (an online interactive and multimedia learning resource). The purpose of this paper is to demonstrate the impact of the initiative so far.

Methods & Materials: We conducted a quantitative and qualitative analysis of clinicians’ engagement with BMJ Learning and BMJ Best Practice over a period of one year to assess the impact of BMJ resources in education and clinical practice. We evaluated the number of clinicians who engaged with the resources, their performance on knowledge assessments embedded in the resources, and the sections of the resources that were most used. We also evaluated feedback on the resources to look for qualitative evidence of impact on clinical practice.

Results: Since launching, the CDS Initiative has trained more than 9,000 infectious diseases specialists and primary care doctors across 900 institutions in the 6 countries. Physicians improved their performance on knowledge assessments in infectious diseases from 51% in the pre-test to 82% in the post-test. The most used sections of the resources covered practical and actionable content on diagnosis and management - suggesting that the resources are being adopted to improve clinical care. Qualitative analysis of feedback on the content suggests that users find it helpful and use the content to improve their clinical care in infectious diseases.

Conclusion: To conclude, online learning and clinical decision support are useful resources to support healthcare professionals in improving the diagnosis and treatment of infectious diseases.
**21.105**
Detection and Response Timeliness Of Crimean Congo Hemorrhagic Fever And Hemorrhagic Fever With Renal Syndrome In Southeast European Region.

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**Purpose:** Timeliness is the key feature of detection and response of emerging infectious diseases outbreaks and especially hemorrhagic fevers. To better understand gaps, underlining reasons and propose improvements of surveillance systems a multi-country analysis and evaluation of Time to Detect and Time to Respond (TDTR) to haemorrhagic fever outbreaks has been carried out by Southeast European Center for Surveillance and Control of Infectious Diseases (SECID).

**Methods & Materials:** A standardized spreadsheet template has been developed and used by Albania, Kosovo* and Bulgaria to collect in a structured format the surveillance and response timelines data. The analysis of time lags between detection, confirmation, reporting and responding was estimated through descriptive methods based on measures of central tendency and accompanied by related standard deviations and complemented with graphical representations through bar charts and box plots.

**Results:** The data and surveillance process analysis show that the existing surveillance activities present notable gaps. We measured three main days interval groups:
1. Day intervals related to disease onset to progress of CCHF and HFRS;
2. Day intervals related to CCHF and HFRS surveillance such as reporting.
3. Day intervals related to CCHF and HFRS laboratory diagnosis.
4. Day interval related to disease control measures and outcomes.

Anyhow there was a considerable timeliness gap from first district visit to hospitalization and further clinical suspected cases and laboratory diagnosis respectively mean (+/-): 2,6; 4,3; 6,3; 8,0 days and maximum: 10; 44, 45 and 24 days.

Also considerable gaps were seen related to reporting and control measures.

**Conclusion:** The current system does not always allow analysis of disease, event or outbreak timeliness of detection and response especially when the activities are part of different institutions. During the analysis it was clear that response systems are not well described and documented to allow their timeliness and analysis of timeliness of laboratory diagnosis and its interpretation is the key feature to organize further prevention and control measures.
21.106

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**Purpose:** The 2013-2016 West African Ebola epidemic has been the largest to date with more than 11,000 deaths in the affected countries. The data collected have provided more insight than ever before into the case fatality ratio (CFR) and how it varies with age and other characteristics. However, the accuracy and precision of naïve CFR are limited because 44% of survival outcomes were unreported. We impute unreported outcomes and combine the imputed and reported outcomes to provide improved CFR estimates.

**Methods & Materials:** We imputed for unreported survival outcomes (i.e. recovery or death) using a Boosted Regression Tree (BRT) model and calculated the average out-of-sample performance of the simplified model used for imputation.

**Results:** The average out-of-sample performance measures were as follows: sensitivity=64·3% (95% CI: 53·8-73·0), specificity=64·3% (95% CI: 53·8-73·0), percentage correctly classified=64·2% (95% CI: 54·4-73·1) and area under the ROC curve=70·2% (95% CI: 59·3-78·1). The CFR estimates obtained with imputation for the 2013-2016 West African epidemic were 66·5% (95% CI 61·8%-71·1%) overall and 68·9% (95% CI 62·1%-74·5%), 65·7% (95% CI 61·4%-69·5%) and 61·4% (95% CI 55·9%-67·3%) for Sierra Leone, Guinea, and Liberia, respectively.

**Conclusion:** We achieved appreciable out-of-sample performance and CFR estimates with imputation were an improvement to CFR estimates obtained without imputation. Updated CFR estimates including imputed outcomes can be used to inform public health contingency planning for future Ebola epidemics. Improved baseline CFR estimates are essential for allocating resources and evaluating the effectiveness of future inventions.
Purpose: Accurate diagnosis in an outbreak scenario is crucial for the provision of appropriate healthcare and treatments. For emerging pathogens, the assays used for the diagnosis are either absent or poorly developed. A vital tool is the provision of appropriate reference standards, which allows for harmonisation of results from laboratories worldwide. Use of killed virus will be ideal, but for high containment level pathogens (BSL4) proving inactivation may be challenging, and their distribution globally hampered by bureaucratic requirements and legislation. As an alternative we evaluated a lentiviral vector platform to package RNA of the pathogen of interest within an HIV-like particle to act as reference material in nucleic acid amplification technology (NAT)-based tests.

Methods & Materials: During the Ebola outbreak in Western Africa in 2013-15, we developed a chimeric virus by incorporating Ebola virus RNA into virus-like particles for use with diagnostic kit or in house quantitative PCR and digital PCR. Ebola virus Makona strain sequences were synthesised in vitro and incorporated within a lentiviral vector plasmid. This was transfected into 293T cells together with a packaging plasmid containing HIV-1 gag and pol. The chimeric particles were harvested, purified and formulated in a universal buffer before lyophilisation.

Results: The freeze-dried preparations were evaluated in an WHO-sponsored International Collaborative study in which participants were asked to test the candidate material using assays routinely used in their laboratories. The HIV-Ebola chimeric particles were able to harmonise data received from the participants and reduce the inter-laboratory variation. Based on these results, the WHO Expert committee on Biological Standardisation established the material as an International Reference Reagent.

Conclusion: The lentiviral packaging system represent an enabling technology to assist the development and calibration of diagnostic kits. It is a safe alternative to handling a high containment level pathogen and obviates the issues related to bioterrorism law. Furthermore, this type of reference material can be prepared contemporaneously or in advance of any viral outbreak to assist calibration of sensitive and specific assays and their harmonisation through standardisation at the time of the emergency when they are most urgently needed.
Detection of Unforeseen Source of Botulism Outbreak, Egypt July 2017

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Purpose: Botulinum toxins are neurotoxin derived from Clostridium botulinum bacteria that produce enterotoxin (A-G). Botulinum toxins are used in dermatology and neurosurgery clinics for treatment of muscular hypertrophy. Botulism outbreak can occur by food borne or Botox injection such as food borne botulism outbreak Egypt April 1991. Global mortality rate of botulism around 5-10%. On July 2017, Botulism cases were reported from Giza in Egypt to the Ministry of Health and Population (MOHP). Reported symptoms were compatible with botulism after Botox injection. The objective was to evaluate the presence of Botulism outbreak after Botox injection.

Methods & Materials: Field investigation done to evaluate the outbreak, we made a communication with patient’s families using Questionnaire to collect Epidemiological and Contact data. Efforts were concentrated on active-case finding, standardized case definition (difficulty in breathing, swallowing, walk, flaccid paralysis, diarrhea, fever) used to collect data from centers of clinical & environmental toxicology. Serum samples sent to central public health laboratories to identify toxin using mouse assay.

Results: Nine cases were detected during 5-18/7/2017 from Cairo and Giza governorate, all cases were hospitalized for (2-4) days and treated using 17 bottle of botulism antitoxin, all cases cured, five serum samples collected. Median age was four years (2-31 years). Median incubation period was five days. All cases suffered from muscle pain. Seven cases had cerebral palsy, which affects their movement. Two cases had facial palsy, six difficulty in breathing, five difficult in swallowing and four dry throat. Investigation revealed that Neurosurgeons used unrecoumpensed type of Neuroxin for Botox injection that leded outbreak occurrence.

Conclusion: Botulism outbreak occurred due to Botox injection. We controlled the outbreak through preventive control measure.
Evaluation of main infectious diseases outbreak detection and response timeliness in Southeast European Region


Purpose: The objective of this study is to evaluate the timeliness of outbreak detection and response of most important infectious diseases occurring in five countries of South Eastern European Region, namely Albania, Bosnia and Herzegovina, Bulgaria, Macedonia and Kosovo*. This multicounty study was performed to demonstrate how public health institutions can conduct surveillance and response evaluation in relation to timeliness and to identify areas of improvement.

Methods & Materials: Diseases prioritization exercise was carried out and three of most important diseases for each country have been shortlisted. A spreadsheet tool was developed to collect in a structured format the surveillance timeliness data for the last five years. The completeness was calculated and compared for all diseases and all countries. The analysis of time lags between detection, confirmation, responding and reporting was estimated through descriptive methods based on measures of central tendency and complemented with graphical representations. Outbreak locations have been identified for all diseases and spatial distribution have been explored.

Results: Eleven diseases were identified as most burdensome: Crimean Congo Hemorrhagic Fever; Hemorrhagic Fever with Renal Syndrome; West Nile Fever; Anthrax; Tularemia; Measles; Hepatitis A; Listeria; Influenza; Brucellosis; and foodborne infections. In general data to estimate time lags were complete, however missing information rate was estimated quite high for date of disease detection with 51.4%, date of disease treatment with 57.8% and date of patient hospitalization with 66.6%. In general time from disease start to disease detection has a median of 1.0 days, time to laboratory diagnosis has a median of 8.0 days, time to disease treatment has a median of 4.0 days, and time to patient hospitalization has a median of 3.0 days. Disease reporting to national public health authorities is estimated with a median time of 4.0 days.

Conclusion: All countries have surveillance systems based mainly on paper-based disease reporting and this poses a major concern related to timeliness measures. Electronic communication and training of clinicians, epidemiologists, and strengthening the collaboration between countries are necessary steps to be followed in order to mitigate the untimely reporting, enable early disease notification and subsequently improve the response time to cross-border disease outbreaks.
Purpose: Aspergillus species are omnipresent organisms that can be found in every region. Invasive aspergillosis infections are often found to be associated with hospital construction or renovation, which can increase the amount of airborne aspergillus, causing in high-risk patients. The severity of aspergillosis is determined by various factors. The goal of this study was to (1) examine fungal hospital-acquired infections that were detected in our facility and (2) to evaluate infection prevention and control measures in our hospital based on previous experiences, evidence and guidelines.

Methods & Materials: We surveyed aspergillosis for these 10 years in our facility. From January 2008 to December 2017, diagnosis, treatment, clinical courses were surveyed using medical records in our facility. We showed 5 cases with hematological malignancies in clean room. They were treated just empirically without definite diagnosis.

Results: Five patients with leukemia were infected with aspergillosis in a clean room at hematology ward. Three cases of them were infected in the same room within 8 months. Invasive aspergillosis was highly suspected. They could not take bronchoscopic examination. Intervention by ICT did not detect definitive findings. Air condition and the facility around the room were not contaminated. That room was closed, however, another patients with AML was diagnosed as aspergillus sinusitis. All the patients were compromised hosts. In hematological ward, once aspergillosis prevails, it is not easy to terminate them because of the limitation to eliminate particles including aspergillus spore completely. In our survey of aspergillosis, 209 aspergillosis including 104 cases with hematological malignancies and 35 other kinds of malignancies, were treated by antifungal drugs. Inspection implementation rates for β-D-glucan and Aspergillus antigen in 30 cases of chronic pulmonary diseases were higher than average of all cases (63.2%, 55.5%), however, those of cases with hematological malignancies were lower. Empirical treatments were common in hematological diseases. As treatment, MCFG iv (54%), CPFG iv (25%) and VRCZ per os (43%), ITCZ per os (43%) were occupied in all patients.

Conclusion: Performing infection control risk assessments and implementing the reliable control measures is essential to prevent healthcare-associated fungal outbreaks.
21.111
A Disease Investigation On High Mortality Cases In Layer Chickens In Purbalingga, Jawa Tengah

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Purpose: The purpose of this research is to identify the cause of reported mortality event in layer chickens in Purbalingga, Jawa Tengah.

Methods & Materials: Disease investigation was carried out using interview, collecting samples, and laboratory testing.

Results: The result of investigation shows that the case involving sudden death in layer chickens have been occurred since 3 weeks with consecutive mortality event on about 20–30 chickens per day. The chickens were kept at 40 weeks of age in 2800 population and 80 weeks old in 1800 population. The flocks have actually been AI vaccinated. Necropsy of the carcasses shows congestions in lungs, regression of the ovaries, encephalitis and congestion in brain, and peritonitis. Serology test shows high antibody titers (> 24) and molecular testing confirmed H5 sub-type of Avian Influenza virus.

Conclusion: In summary, the reported cases in chickens layer was infected by H5 sub-type of Avian Influenza virus. It is recommended to respond the cases to prevent the AI transmission.
Epidemiological Investigation Of An Acute Viral Hepatitis Outbreak In An Urbanized Rural Area In A North Indian Union Territory.

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**Purpose:** A waterborne outbreak of acute viral hepatitis (AVH) was reported in an congested and overcrowded urbanized rural area, known as Burail, in Chandigarh, during January to December, 2017. This outbreak was investigated with an objective of describing its epidemiological features, identify the source of contamination and implement the containment measures.

**Methods & Materials:** A house-to-house survey was conducted to identify symptomatic cases, defined as per WHO’s standard case definition for acute viral hepatitis, using standard case record form. Laboratory investigation of all the suspected cases was done for Hepatitis A and E virus (HAV & HEV) by enzyme-linked immunosorbent assay method. Environmental investigation included water testing of all the tube wells supplying water in the study area for chlorination and faecal contamination. The outbreak was described in terms of time, person, and place analysis to generate the hypothesis and implement control measures to contain the outbreak.

**Results:** A total of 34,542 individuals were contacted and screened for symptoms of acute viral hepatitis. Hundred fifty four cases of AVH were laboratory confirmed. Out of these, 128 (83.1%) cases were positive for HEV, 21 (13.7%) for HAV, and 5 (3.2%) for both HAV & HEV IgM antibodies. Overall attack rate (AR) was 0.49%. It was highest among the age group of 16-20 years (0.98%), followed by 11-15 years (0.79%). However, all the age groups were affected. The AR among males (0.52%) was higher as compared to the females (0.43%). There were no deaths reported. Environmental investigation confirmed the sewage contamination of drinking water in the distribution system of one of the tube well, where clustering of cases was observed.

**Conclusion:** Dual HAV and HEV outbreak was confirmed that was transmitted by contaminated drinking water in the study area. HEV was the major etiological agent. The recognition of early warning signals, timely investigation, and surveillance of water quality can contain the outbreak.
Investigation of an outbreak of dengue fever in Punnakayal, a coastal area of Thoothukudi district, Tamil Nadu-India, 2017-2018.

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Purpose: Thoothukudi district is one of the dengue endemic areas in India. During the month of September 2017, an increased in number of fever cases were reported from Punnakayal, a coastal area of Thoothukudi district, Tamil Nadu, India. We investigated that outbreak with the objectives: to describe the distribution of cases, to find out the source of infection and to propose recommendations.

Methods & Materials: We did active case search based on the IDSP case definition and did stimulated and passive surveillance among residence of Punnakayal during September 2017. We searched for suspected cases in health care facilities and collected blood specimens. An environmental analysis by collecting water samples and also an entomological survey was studied. We analysed the outbreak by time, place and person and computed entomological indices.

Results: We identified 124 suspected cases (Attack rate: 15/1000, highest among 0-4 years age group 145/1000, followed by 5-9 years age group 38/1000, among males 28/1000, and no deaths. Out of 124 cases, 7 (6%) were confirmed by IgM Capture ELISA (MAC-ELISA). The outbreak occurred from September 2017 to January 2018 and peaked between November and December and lasted till 20 January, 2018. Suspected cases clustered in Savariyarpuram street (Attack rate: 24/1000), followed by South and Pon -manickam Streets (Attack rate: 16/1000). All patients had fever and headache, 99 (80%) had myalgia and 47 (38%) had retro orbital pain. Breteau index of the three streets were: Savariyarpuram-1.4, South Street-5.4 and Pon-manickam Street-3.8. We detected breeding of Aedes aegypti mosquitoes in domestic and peri-domestic stored water. They were receiving water from public water distribution system once in three weeks. No other water sources found. But, few people bought water from tanker Lorries. Water specimens showed chlorination nil.

Conclusion: The outbreak was of dengue fever due to increased density of Aedes aegypti mosquitoes both in domestic and peri domestic stored water. We found unavailability of water supplied on a day today basis by public water distribution system. So, we educated the public to close the domestic and peri domestic water storage containers and advised water board to supply water frequently at least once in a week after chlorination. Advised chlorination to tanker lorry water also.
Communication, Information, And Education Of *Avian Influenza* Disease On Poultry Farmers And Live Poultry Traders In Purbalingga, Central Java.

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**Purpose:** The purpose of this activity is to increase knowledge and awareness of poultry farmers and live poultry traders to reduce the risk of *Avian Influenza* transmission and increase productivity of poultry farm in endemic areas in Purbalingga.

**Methods & Materials:** Methodology carried out by conducting counseling and field practice on poultry farmers and live poultry traders conducted by DIC Wates, Directorate of Animal Health Ministry of Agriculture, and FAO ECTAD.

**Results:** The results of this activity is the application of biosecurity conducted by poultry farmers and traders in poultry farms and live birds markets.

**Conclusion:** In summary, this activity will create cooperation and communication among stakeholders in the poultry industry for the dissemination of poultry health information and its transmission.
Purpose: Hand, foot, and mouth disease (HFMD) is an acute febrile illness characterized by fever, sore throat, and vesicular eruptions on the hands, feet, and oral mucosa. Outbreaks of HFMD in children aged <5 years have been reported worldwide and the major causative agents are Coxsackievirus (CV)-A16, enterovirus (EV)-A71 and recently CV-A6, within the genus Enterovirus (EVs) and members of the family Picornaviridae. Other EVs including coxsackievirus type A (CV-A) and B (CV-B) as well as echovirus (E) could either cause HFMD, but rarely cause large outbreaks. The aim of this study was to report the causative agent of the HFMD outbreak of 211 suspected cases among children schools in São Paulo State, Brazil, between March and June of 2018.

Methods & Materials: HFMD is not a reportable disease in Brazil, but notification of outbreaks is mandatory. In 2018, between March and June, 237 outbreaks of HFMD were reported in the São Paulo State, by System for Notifiable Diseases (SinanNet), Ministry of Health, with 2,235 related cases. The highest concentration of cases occurred in the age group of 1 to 4 years (82%), followed by the age group of less than one year old (6.4%). Specimens (stool samples or oropharyngeal swab) of 211 children were sent to the Enteric Diseases Laboratory at the Adolfo Lutz Institute, the Regional Reference Health Center for São Paulo State for Enterovirus Surveillance, to conduct the diagnostic evaluation. Viral RNA was extracted directly from each specimen using a QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) and tested by RT-qPCR; 175 (83.0%) were enterovirus positive. Enterovirus-positive samples were inoculated in tissue-culture cell line—human rhabdomyosarcoma (RD, ATCC CCL-136) and reverse transcription polymerase chain reaction RT-PCR was applied to amplify a partial region of viral protein 1 (VP1) followed by sequencing for genotyping.

Results: Based on sequence alignment, the most frequent genotype was CV-A16 and CV-A6. Other enteroviruses were also identified, including CV-A1, CV-A5 and E-11.

Conclusion: This finding indicated the emergence of CV-A6 and CV-A16 and its predominance in HFMD, in São Paulo State, Brazil.
Purpose: Ciguatera fish poisoning (CFP) is the most common fish intoxication worldwide caused by ciguatoxin (CTX)-containing tropical reef fish. CFP is dominated by long-lasting neurologic symptoms, whereas gastro-intestinal symptoms may be present in the acute phase, only. In continental Europe, CFP may occur when CTX-contaminated fish is consumed during or after trips to endemic areas, or when it is exported to non-endemic countries. At the subtropical European Canary Islands and Madeira, CFP has been reported since the early 2000s. CTX-producing microalgae have been recently found in the Mediterranean Sea. In Germany, a CFP outbreak occurred in each of the years 2012 to 2017 caused by snappers imported from India, Vietnam and Indonesia. As outbreak investigations showed, German physicians are not aware of CFP, and patients were unsatisfied with medical care. Clinical and epidemiological characteristics of this series of CFP outbreaks are demonstrated here. Channels to report CFP cases and outbreaks to Public Health authorities without an established notification system will be shown.

Methods & Materials: Reports by physicians according to §16e of the German Chemicals Act were analyzed in order to retrieve CFPs based on the case definition of the EuroCigua-project. The degree of severity was evaluated on the bases of the Poison Severity Score (PSS). CFP cases were clustered according to the place where the fish was purchased.

Results: Between 2012 and 2017, six outbreaks with 65 CFP cases were registered in Germany. All patients suffered from neurologic disorders. Three patients showed severe symptoms like bradycardia <40 min⁻¹ or morphine resistant abdominal cramps. Nearly 20% of the patients were hospitalized. All outbreak cases were caused by snappers from India, Indonesia and Vietnam. Lutjanus bohar was the fish species present in all German CFP outbreaks.

Conclusion: Ciguatera caused by imported tropical fish is an emerging disease in Germany. Physicians should acquaint knowledge of CFP symptoms and therapeutic options, and are requested to report CFP to the German Federal Institute for Risk Assessment (BfR). CFP patients should be reminded to provide fish leftovers to local Public Health authorities for laboratory investigations. DRG code T 61.0 should be used when the CFP case definition of the EuroCigua project is applicable.
21.117
Hepatitis A outbreak in a semi urban area-Nathersha pallivasal, Tiruchirappalli Corporation, Tamil Nadu-India, 2016.

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Purpose: Hepatitis A (HAV) is one of the most frequent causes of waterborne infections in India. With improvement in economic and living conditions, the age of acquiring infection is shifting from early childhood to adolescence and young adulthood. On 17th June 2016, Natharsha palli vasal, ward no 12, (population=4589) of Tiruchirappalli corporation reported 74 cases of jaundice. We investigated this outbreak to confirm diagnosis, to identify source and to initiate control measures, and to propose recommendations.

Methods & Materials: We compared recent incidencies of acute hepatitis cases with baseline information. We defined a case as acute onset of jaundice in age less than 25 years among residence of ward 17, Natharsha palli vasal between June and July, 2016. We conducted a retrospective cohort study. We actively searched cases from door to door and also did simulated passive surveillance. We searched for cases in health care facilities and collected 5 sera for testing for leptospirosis, Hepatitis A Virus and Hepatitis E Virus. We interviewed for socio-demographic characteristics, food and water sources. An environmental analysis by collecting water samples for testing faecal coli. We analysed the outbreak by time, place and person and also calculated relative risk (RR) and 95% confidence interval (CI).

Results: We identified 75 cases (Attack rate 16/1000(74/4580), highest in 5-9 years age group (Attack rate: 21/1000(61/2912), among males(Attack rate: 17/1000(37/2190) and no deaths. All five blood samples were anti-HAV IgM positive. The outbreak occurred from May 2016 to July 2016 and peaked on 31June and 9July and lasted till 21July 2016. There was clustering of cases among Nathrasha palli vasal (Attack rate:21/1000(61/2912), followed by Valluvar street(Attack rate:9/1000(11/1239). All patients had fever and jaundice, 15(20%) had loss of appetite and 14(19%) had vomiting. Corporation engineering department found a breakage in main water supply line. Analytical study showed who is drinking water from damaged pipeline a significant risk factor. Water specimens showed chlorination nil.

Conclusion: This Hepatitis A outbreak was due to drinking contaminated piped water because of breakage in public water distribution system. Probably residual chlorine in affected areas almost nil and monitored irregularly. After immediate repairing of the broken pipe, increased amount of residual chlorine was ensured.
Purpose: In a digital world where the public’s voice is growing increasingly strong, how can health experts best exert influence to contain the global spread of infectious diseases? Digital media sites provide an important source of health information, however are also powerful platforms for the public to air personal experiences and concerns. This has led to a growing phenomenon of civil skepticism towards health issues including Emerging Infectious Diseases and epidemics.

Methods & Materials: Following the shift in the role of the public from recipients to a vocal entity, this presentation, based on my book titled Risk Communication and Infectious Diseases in an Age of Digital Media, published by Routledge Studies in Public Health, explores the different organizational strategies for communicating public health information and identifies common misconceptions that can inhibit effective communication with the public. Drawing on original research and a range of global case studies, this timely presentation offers an important assessment of the complex dynamics at play in managing risk and informing public health decisions, by providing thought-provoking analysis of the implications for future health communication policy and practice.

Results: In order to create a credible scientific discourse, certain patterns that are still ingrained in the health organizations must be overcome: (1) homogeneous executive committees; (2) public health experts versus “people with agendas”; (3) from certainty to uncertainty; (4) the preference for consensus; (5) conflicts of interest; (6) facts/rationality v. emotions/myths; and (7) the medicalization of public health.

Conclusion: In the digital age, organizations can deconstruct what Foucault called the field of knowledge/power by using the different narratives of diverse publics to create a more balanced democratic system that also has creative flexibility. For that to happen, the establishment must change its mode of thinking in its scientific discourse, while at the same time building digital and communication systems that use crowdsourcing to solve scientific problems, and involving and including the public in the discourse in the public sphere. These steps can contribute to a different risk management approach for epidemic crises, bringing organizations closer to their metaphorical potential of embracing the public sphere.
The Four R’s: A Community Engagement Framework for Disease Preparedness Research in Sierra Leone

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Purpose: Building trust and engaging the community were core components of the 2015 EBOVAC and 2018 PREVAC Ebola vaccine trials in Sierra Leone. Thus, it recruited a community liaison team (CLT) to engage with the community through public meetings, radio chat shows and other activities, and a social science team (SST) to assess community members’ and participants’ perceptions of the trial. Both teams provided regular updates the clinical team to adapt procedures. This study sought to understand barriers and facilitators to community engagement around biomedical research. Specifically, it aimed to determine the viability of rolling out the EBOVAC and PREVAC trials’ model in other disease preparedness programmes.

Methods & Materials: A field study was conducted to assess the model’s implementation in these trials during following the Ebola outbreak. EBOVAC and PREVAC team members attended 15 in-depth interviews, community members and local leaders attend 3 focus group discussions (FGDs) to discuss the Ebola epidemic, the current trials and community engagement activities.

Results: Based on initial findings, four main principles characterized the community engagement model used in the Ebola vaccine trials: reciprocal, relatable, relational and respectful. The trial incorporated two separate teams to ensure reciprocal communication between the trial and the community. The CLT delivered key messages from the trial, whilst the SST completed ethnographic research in the field to uncover trial rumours and perceptions in the community. These findings were brought back to the CLT and incorporated into targeted messaging. Trial workers approached the communities as equals by dressing modestly, speaking local dialects, and using relatable examples. Appreciation and understanding of the importance of interpersonal relationships and respect for the people, their customs and traditions also played a large role in the success of the community engagement programme.

Conclusion: These findings could provide a community engagement framework for other programmes occurring in “peace time” (disease preparedness) or during a disease outbreak. The framework should be tested in other settings where such interventions are ongoing, to ascertain whether these principles remain consistent across settings and diseases. If this is the case, this framework could be incorporated into international guidelines on community engagement.
Socioeconomic factors predict the increase of incidence rates of visceral leishmaniasis in highly endemic areas in Brazil

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Purpose: To evaluate the association between socioeconomic factors and the changes in the incidence of visceral leishmaniasis (VL) in highly endemic areas in Brazil.

Methods & Materials: Among the 2,136 Brazilian municipalities with at least one VL case from 2001 to 2013, we selected those 480 with an average of ≥1 VL case/year. VL cases for each municipality were obtained from the Brazilian System for Notifiable Diseases. Socioeconomic and demographic variables were obtained from the Brazilian Census (2010) and included income inequality (Gini index), % population living in rural areas, % population <5 years of age, % households with access to water supply, sewerage system, electric light, and garbage collection. Information on the annual coverage of the implementation of euthanasia of VL infected dogs was obtained from the Brazilian VL Control Programme (2005-2012). The outcome is the VL incidence rates from 2006 to 2013. Associations between variables were evaluated using a multivariate multilevel Poisson regression. Associations were expressed as incidence rate ratios and 95% confidence intervals.

Results: A total of 23,638 VL cases were registered in the 480 municipalities from 2006 to 2013. Results indicated an annual decrease in the incidence rates by 3% (p<0.001), with significant variations in trends among the municipalities. For every increase in 0.1 points in the Gini index, the annual VL incidence rates decreased by 48% (p<0.001). For every increase in 1% in the households with access to water supply plus sewerage system and electric light, the VL rates decreased by 2% (p=0.044 and p=0.034, respectively). For every increase in 1% of the rural population and of the population <5 years of age, the VL rates increased by 0.8% and 17%, respectively (p=0.005 and p<0.001). The implementation of euthanasia of infected dogs in a particular year was associated with an increase in 9% in the VL incidence in the following year, reflecting the widely accepted knowledge that control measures are usually implemented after VL cases identification.

Conclusion: Efforts for decreasing income inequality and providing essential urban services might be effective strategies for controlling the spread of VL in urban areas.
Social factors for severe hand, foot and mouth disease in Chengdu, China

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**Purpose:** This study was aimed to analyze the demographic characteristic, hospital visit behavior and health services of a group of pediatric HFMD patients, and analyze the risk factors for severe HFMD in children.

**Methods & Materials:** A self-administered questionnaire survey with cross-sectional design was administered among parents of HFMD inpatients in one children's hospital in Chengdu, China to identify social factors associated with the occurrence of severe HFMD. 249 patients have been included between September 2016 to June 2017.

**Results:** Multi-factorial logistic regression was used to analyze independent associations between potential influence factors and death from severe HFMD. We found that the floating migrants were more likely to progress to severe HFMD than resident population (OR=17.95, 95%CI: 4.52-69.92). The children who first visit village level clinic and not confirmed diagnosis at first visit to hospital had high risk of death from severe HFMD (OR=8.64, 95%CI: 2.49-16.78 and OR=5.87, 95%CI: 2.23-10.44). Analysis also revealed that younger parents had lower odds of their children progressing to severe HFMD, whereas the children whose parents reported availability of health related web sites were less likely to become severe HFMD. And children's health care counseling provision in community or school were associated with lower odds of severe occurrence.

**Conclusion:** To reduce the mortality from severe HFMD, health care providers need to pay attention to the floating migrants and the children with young parents which would associated with severe HFMD case. And health administrative departments should pay more attention to the optimization of health service systems. In addition, increase financial and manpower input into village level health institutions will be benefit to control the incidence and morbidity of HFMD.
Hepatitis E: Determinants of Severe Disease in Displaced Populations of South Sudan

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Purpose: Hepatitis E virus (HEV) has over the last three decades emerged as a cause of outbreaks in displaced populations. Effective deployment of an HEV intervention toolkit that includes HEV vaccines requires epidemiological characterization of HEV trends in vulnerable populations. The study purpose is to describe the epidemiology of HEV and identify factors for severe HEV disease in displaced populations of South Sudan.

Methods & Materials: The agent-host-environment model was used. A nested retrospective cohort study was used with a sample of 14,404 cases for the descriptive case-series and 4,810 cases for the retrospective cohort. Data analyses included cumulative incidence and mortality rates, SatScanÒ space-time analysis, correlation and simple linear regression, odds ratio, and logistic regression.

Results: Sustained HEV transmission occurred from 2012 to 2017 with rising transmission in the rainy season but no significant correlation between precipitation and HEV cases. The median outbreak duration was 1 year 11 months. The outbreaks were attributed to HEV genotype 1 subtype 1e with the risk of HEV disease and death (as cases and deaths per 10,000) being higher in males (591 versus (vs) 23), adults (18-59 years) (367 vs 14), and elderly (60+ years) (353 vs 22). The factors associated with severe HEV disease include (a) altered mental status (adjusted Odds Ratio [aOR] = 640.24, 95% CI: 209.35–1958.02), (b) death (aOR 28.06, 95% CI: 14.77-53.29), (c) pregnancy (aOR 16.90, 95% CI: 9.54-29.94), (d) illness onset in rainy season (aOR 0.33, 95% CI: 0.23-0.46).

Conclusion: HEV is a major public health problem in displaced populations in South Sudan with pregnancy being a major determinant of severe HEV disease. The implications for positive social change entail using present findings to guide clinical screening of HEV cases and to inform the effective deployment of the HEV intervention toolkit, including HEV vaccines that reduce the impact of HEV in displaced populations.
The increasing importance of non-culture methods in diagnosis and serotyping of invasive pediatric pneumococcal infections revealed the continuous dominance of vaccine serotypes in the era of PCV13 vaccination.

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**Purpose:** We evaluated the role of non-culture methods for diagnosis in evaluating the impact of continued 13-valent pneumococcal conjugate vaccine (PCV13) use in the private market in pediatric invasive pneumococcal disease (pIPD) in Portugal (2012-2015).

**Methods & Materials:** The pIPD cases were identified and isolates were submitted to a central laboratory for further characterization. In possible pIPD cases where culture of pleural or cerebrospinal fluid was negative, real-time PCR reactions targeting conserved (lytA and cpsA) genes and capsule specific genes were used to detect and serotype pneumococci.

**Results:** The most frequently detected serotypes were: 3 (n=32, 13.8%), 14 (n=23, 9.9%), 1 (n=23, 9.9%), 7F (n=15, 6.4%), 19A (n=13, 5.6%), 6B and 15B/C (both n=12, 5.2%), and 24F, 10A and 12B (all with n=10, 4.3%). The use of PCR to detect and serotype pneumococci in both pleural and cerebrospinal fluid samples contributed to 20% (n=47) of all pIPD. Serotype 3 was mostly detected by PCR (n=21/32, 65.6%) and resulted from a relevant number of vaccine failures. The incidence of pIPD varied in the different age groups but without a clear trend. There were no obvious declines of the incidence of pIPD due to serotypes included in any of the PCVs, and PCV13 serotypes still accounted for the majority of pIPD (53%).

**Conclusion:** Our study indicates that a higher vaccination uptake may be necessary to realize the full benefits of PCVs, even after 15 years of use, and highlights the importance of using molecular methods in pIPD surveillance, since these can lead to substantially increased case ascertainment and the identification of particular serotypes as causes of pIPD.
Purpose: Over the past four decades, WHO and its partners have assumed greater responsibility for ensuring global stockpiles of life-saving vaccines, which are available to prevent, pre-empt and control epidemics and respond to humanitarian emergencies. Several WHO-supported emergency stockpiles currently exist. Their governance aims to ensure availability of, fair and consistent criteria for access to, and equitable allocation of scarce resources in response to multiple, potentially competing emergency requests. The operational framework for each stockpile is adapted to specific challenges and reflects request rules, allocation mechanisms, stockpile storage and management, and funding mechanism. Three stockpiles (meningitis, yellow fever and cholera) are managed by the International Coordinating Group on Vaccine Provision (ICG), a partnership of four founding agencies, which combines centralised decision-making with decentralised storage and distribution of vaccines. It reviews emergency vaccine requests by affected countries and makes decisions on allocation. We reviewed the governance and allocation mechanisms and evaluated the performance of the ICG mechanism in responding to vaccine requests.

Methods & Materials: We performed a descriptive analysis of requests for meningitis, yellow fever and cholera vaccines to the ICG in 2016–2017. Times for each part of the ICG process were compared against key time performance targets and challenges in effective implementation of timely vaccine allocation were identified.

Results: In 2017 the ICG oversaw and approved the distribution of over 16.5 million doses from the three stockpiles. All vaccine requests were circulated for decision-making within one working day. Decisions were reached within two working days for 95%, 94% of 88% of meningitis yellow fever and cholera requests. Gaps in laboratory, surveillance, logistics and outbreak response capacity contributed to delays between decision and reception of vaccines by affected countries.

Conclusion: Stockpile governance requires good partnership and is essential to overcoming a “first-come, first-served” approach which in the past had led to inefficient and inequitable vaccine allocation. All stockpiles face common challenges, particularly chronic vaccine shortages. Vaccine response requires comprehensive disease control strategies and access to healthcare at the country level to be truly effective.
21.125
Sustained Transmission Of Measles Virus Causing Outbreaks In The Philippines, 2013-2018

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Purpose: Emergence of measles virus continues to be a major public health concern despite improvements in vaccination programs and efforts for elimination. In 2013, a major outbreak of measles occurred starting second quarter of 2013, which peaked in first quarter of 2014. Likewise, outbreak occurred in late 2017, 3 years following a major outbreak. Molecular analysis of measles viruses (MV) will provide vital information on outbreak linkages and transmission pathways. This reports the sustained MV transmission causing subsequent outbreaks in the Philippines.

Methods & Materials: Emergence of measles virus continues to be a major public health concern despite improvements in vaccination programs and efforts for elimination. In the Philippines, measles virus outbreaks occurs every 2 to 3 years interval. In 2013, a major outbreak of measles occurred starting second quarter of 2013, which peaked in first quarter of 2014. Likewise, another outbreak occurred in late 2017, 3 years after a major outbreak. Molecular analysis of measles viruses (MV) will provide vital information on outbreak linkages and transmission pathways that can be helpful in the implementation of appropriate control programs. This reports the sustained MV transmission causing subsequent outbreaks in the Philippines.

Results: A total of 40,860 samples were received in 2013 while 3,048 in 2017 with 20,000 samples tested from both outbreaks. 80% of the samples positive for Measles anti-IgM. Of 1,405 swabs received, 33% were positive and further tested for partial N gene. In 2013, majority of the isolates were B3 with one case of D9, while only B3 was isolated from 2017 outbreak. Phylogenetic analysis revealed that both 2013 and 2017 B3 strains from a single clade with highest homology from isolates found in Ibadan, Nigeria.

Conclusion: Measles virus activity in the country remains high despite global efforts towards elimination. The same wild-type strains (B3) were found during both 2013 and 2017 indicating authochthonous transmission. Low vaccination coverage and high birth rate prevented the complete interruption of Measles virus in the country. Significant efforts should be made to improve routine immunization program and continue molecular surveillance of MV to track transmission pathways and epidemic links of MV.
Knowledge, Attitudes, and Practice Regarding Influenza Vaccination in Pregnant Women in Albania

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Purpose: Pregnant women are among the priority group to receive influenza vaccines under government program in Albania. Our study purpose was to assess pregnant women’s knowledge of influenza, and influenza vaccine during pregnancy and their attitudes and practices.

Methods & Materials: A cross-sectional survey of 555 pregnant women was performed during the influenza season in 2017-2018 in five districts of the country. Questionnaires were distributed in the waiting room for pregnant women in primary care services and maternity hospital. Questions included demographic and social data and KAP regarding influenza and influenza vaccination. Data were analyzed using SPSS 20.0 software for windows.

Results: Only 83.3% of them had ever heard of influenza vaccine. Media (54.4%) and healthcare workers (HCW) (39.3%) were the main sources of information. Most women (77.8%) believed that influenza is more dangerous for pregnant women than non-pregnant women but 58.6% of them agreed that influenza vaccine helps to protect pregnant women against influenza. Only 32.6% agreed that women should receive influenza vaccine during each pregnancy. About half of the women (52.8%) agreed that when a pregnant woman gets the influenza vaccine, it helps protect her unborn baby. 67% of women trusted the advice of the health care providers but only 17.1% referred that HCW recommended the influenza vaccine during this pregnancy. 29.2% of women had heard/read reports that would make them hesitate to be vaccinated with influenza vaccine whereas 9.5% were discouraged from receiving the influenza vaccine. 24.7% of the women incorrectly believed that the vaccine is not safe for pregnant women and 31.2% for the unborn baby. Significant predictors of influenza vaccination in multivariate model were “if vaccine was offered free” (β=4.07 p<0.001) and higher/postgraduate education (β=1.3 p=0.02).

Conclusion: Pregnant women’s knowledge about influenza and influenza vaccine during pregnancy is still poor. Education and the way how vaccine is offered play an important role as well as increasing knowledge and practices among health care providers. There is substantial room for improvement among prenatal care providers in both patient education and offering the vaccine.
21.127
Pertussis epidemiology in the Czech Republic: Steady increase of pertussis since 1990th
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Purpose: In the last two decades pertussis has re-emerged in the Czech Republic despite high and stable vaccine coverage. Several measures, i.e. introduction of an aP booster for adolescent and recommendation for vaccination of adults and during pregnancy were implemented to decrease disease burden. The main objective was to describe the impact of the measure and the incidence and trends of pertussis.

Methods & Materials: Pertussis data collected by the national surveillance programme were analysed. The period 1984 -2015 was divided in 7-years-stages to assess the impact of the measures taken.

Results: Incidence declined to <1 case/100,000 inhabitants in 1989. Since 1993, an upward trend has been observed in all age groups, with the highest incidence rates repeatedly found in group 10-14-year. Since the introduction of the aP in 2007 (5 doses), there has been a significant increase in reported cases. After introduction of an aP booster in 2009 (for adolescent in 10-11 year) the peak of incidence moved to the older age groups, mainly 15-19 year. Furthermore, since 2015 the most of cases has been reported in adults, with the proportion increasing from 16% in 2010 to 64% in 2015. Infant less than 1 year of age as well as the whole population have shown a steady upward trend in reported cases since the 1990s. The incidence was the lowest in 1998 (1.1/100,000 population) and the highest in 2014 (73.9/100,000). Most of infant (79%) developed pertussis within the first four months of life, 77% were not vaccinated before the onset of the disease and 75% needed hospitalization.

Conclusion: After introduction of an aP vaccine and an adolescence booster dose there has seen the shift in pertussis morbidity to older age groups. The high proportion of fully vaccinated cases in older children suggests waning immunity 5-10 years after primary immunisation. An upward trend was observed in pertussis cases in children under one year of age. Most children developed the disease within the first four months of life while not vaccinated against pertussis. This fact unambiguously supports the maternal vaccination and an introduction of booster dose at 25 years of age.
Descriptive analysis of Measles cases presented to Allied Hospital Faisalabad Pakistan January-June 2018.

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Purpose: An acute viral, contagious and vaccine-preventable disease. Outbreaks are common in Pakistan despite well planned national immunization program activities at gross root levels. Allied hospital faisalabad Punjab is a tertiary care hospital catering the whole district. Children presenting with measles during January to June were enrolled to evaluate clinical presentation, vaccination status and its outcome.

Methods & Materials: In this cross-sectional study, patients presented with measles were recruited. Using semi-structured questionnaire, demographic details, vaccination history, and clinical details, were obtained after Informed consent. Children were followed till recovery/outcome. The data obtained were grouped and tabulated. Variables were tested for association using the chi-square test with level of significance at \( p < 0.05 \) employing SPSS for Windows v12.0.

Results: Out of 205 cases of Measles evaluated in this study, 111 (54\%) were males, mean age was 34 months while median age was 12 months. Median duration (days) of hospitalization was 4days. 43\% were fully, 40\% partially vaccinated and 17\% were not vaccinated. Most of the cases (73\%) were between 1 to 36 months where 36\% were less than 9months. Cases presented during April to June were 90\%. Almost 100 \% cases had fever, conjunctivitis and flue, 68\% had oral ulcer, 52\% vomiting, 51\% cough, and 23 loose stool. Complications; 29\% Pneumonia, 24\% Gastro-enteritis, 16\% Otitis Media, and 2\% Encephalitis. 205(100\%) of the patients were given antibiotics for treatment, all i.e 205(100\%) patient was treated with antivirals and 205(100\%) patients were given vitamin-A supplementation. The outcome was categorized as recovered, Left Against Medical Advice (LAMA) and death; 74\% recovered, 20\% LAMA and 6 died of complicated Measles. The analysis indicates a protective association between vaccination, outcome and complication.

Conclusion: Measles incidence is still high among vaccinated and unvaccinated children. Fully vaccinated children are relatively protected. Mothers’ antibodies provide protection for early 2.6 months, since, the first dose is administered at 9 months, leaving the child susceptible for first 3-4 months of life. Proper monitoring of vaccine supply chain, efficient surveillance, enhanced vaccination coverage, parents awareness, and optimal time for the first dose are recommended. Limitations include convenient sampling technique and no assessment of nutritional status.
Purpose: Pneumococcal conjugate vaccination (PCV) has been introduced into India’s universal immunization programme (UIP) recently but on a limited scale. This study evaluates cost effectiveness of nationwide scale up of two available vaccines, PCV10 and PCV 13 compared to no PCV vaccination.

Methods & Materials: The incremental cost-effectiveness (ICER) of introducing either PCV 10 or PCV 13 into India’s UIP compared to no vaccination was conducted using an age-stratified static cohort model. This model assesses the risk of different clinical presentations of pneumococcal disease at different ages, in both vaccinated and unvaccinated individuals. Besides the direct impact of PCV on vaccinated individuals, the model also considered potential indirect effects of PCV vaccination. The ICER for PCV-10 or PCV-13 introduction was estimated in terms of the incremental cost per DALY averted.

Results: The ICER for PCV-10 and PCV-13 are US$ 295 and US$ 224 respectively from a societal perspective, and US$ 527 and US$ 470 from healthcare provider perspective. Adding either PCV-10 or PCV-13 to India’s UIP would avert around 62-78 thousand deaths and 681-827 thousand hospitalisations over thirty-year period. The potential reduction in pneumococcal disease through the vaccination programme would also save around US$ 979-1,227 million in healthcare costs and around US$ 3,748-3,659 million in societal costs over thirty year period.

Conclusion: Introducing PCV10 or PCV13 in India’s UIP appears to be highly cost effective compared to no vaccination both from health provider and societal perspective. The ICERs remains robust not only for the base case scenario but also for other conservative scenarios considered.
Seroprevalence of Tick-Borne Encephalitis and Lyme Borreliosis in a Defined Czech Population

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Purpose: The South Bohemian Region of the Czech Republic is an endemic area of tick-borne encephalitis (TBE). In 2017, the inhabitants of Římov municipality developed a serious course of TBE and Lyme borreliosis (LB). This led experts to research and serology examinations of the inhabitants of Římov in order to detect the seroprevalence of TBE and LB.

Methods & Materials: Římov is situated on the Malše river. A water dam has been built on the river, there are extensive deciduous and mixed forests around the waterways and the biotope allows for the development of ticks. A questionnaire was distributed to all the inhabitants of the municipality to get the basic information (life span, occupation, experience with TBE and LB, vaccinations against TBE, number of tick bites per year and the interest in detection of antibodies of TBE and LB). The questionnaires have been evaluated and participants, who gave their consent, were blood tested and serological tests have been done. A total of 114 people participated in the study.

Results: From the questionnaires and the serological examination, it appeared that 4 persons (3.5%) experienced the manifest form of TBE and 5 people (4.4%) experienced the latent form of TBE. In total, 7.9% of the sample experienced the manifest or the latent form of TBE. 80% of the sample confirmed sufficient antibody levels after vaccination. 12.1% of the population remain vulnerable. 32.5% of the sample experienced Lyme disease (9 people, i.e. 7.9% manifested the disease, 22 people (19.3%) had latent disease, early phase of the infection was confirmed in 6 people (5.3%). 77 people (67.5%) remain vulnerable.

Conclusion: A serological examination of the population's research sample revealed that of the people living in the focus point of TBE in Římov, approximately 8% have developed antibodies after being diagnosed the disease or having experienced the latent form and 80% have created antibodies after vaccination. Anamnestic antibodies after being diagnosed or having experienced the latent form of LB, or the early phase of the infection was demonstrated in 32.5% of the study sample. Further research will be focused on testing the ticks of the given site.
Efficiency of Immunization in protection of different serotypes of Rota virus among immunized children with acute diarrhea in Khartoum state

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Purpose: Rotavirus remains a leading cause of severe diarrhea in children worldwide, especially in developing countries. The Global Alliance for Immunizations has ranked rotavirus as a priority for vaccine. To ensure the success of this, it is important to document the local strain(s) of rotavirus in circulation in various regions. This study aimed to Serotype and genotype rotavirus that cause gastroenteritis among children below 5 years of age in Khartoum state.

Methods & Materials: A cross-sectional hospital based study was conducted and a total of 200 Rota virus positive fecal specimen (150 from immunized and 50 non immunized children) were collected and analyzed during the year 2014 from pediatric hospitals in Khartoum state. The samples were screened for rotavirus strains using antigen based enzyme immune-sorbent assay (ELISA), genotyping was done by RT-PCR to determine rotavirus genotypes using genotype-specific primer sets targeting VP4 and VP7 genes.

Results: Results from this study emphasize that rotavirus infection is common in children of Age of 2 months till one year. This study found out that rotavirus was in circulation throughout the year however, with more cases were detected during dry and cold months of November and December. Prevalence was high among children aged one and two years. The common globally distributed strains G1, G2, G3 and G9 accounted for 3.76%, 44.7%, 2% and 12% respectively in immunized children, while control group of non-immunized children shows elevating incidence of G1 66%. G1[P8] was the common genotypic combination in children, accounting for 53.1% infection in non-immunized children and 21.1% in immunized children indicating the value of vaccination to minimize such serotype. G2[P4] still circulating among immunized population. Uncommon combination of genotype G3P [6], G2P [6], G1P [6] and G12P [8] was seen after children's immunization.

Conclusion: This study concludes that there is strain diversity in rotavirus circulating in Khartoum state. In addition, decreasing incidence of G1 serotype after immunization indicated positive impact of Rota Vaccine. But the presence of uncommon serotypes circulating after immunization is considered failure of vaccine and may lead to epidemics if not controlled.

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Purpose: On 16th February 2017, District Health officer, Kachhi district, reported 14 suspected measles cases from village Pir Salman Shah to provincial disease surveillance & response unit (PDSRU) Quetta and requested for investigation. On 17th February 2017, a FELTP fellow was deputed to affected area to confirm outbreak, evaluate risk factors and recommend control measures.

Methods & Materials: Investigation was carried out from 18th to 19th February 2017. A Case was defined as “sudden onset of non-vesicular maculopapular rash and fever along with one of symptoms of cough, coryza and conjunctivitis in a resident of Pir Salman Shah Village from February 16th to 19th, February 2017. Descriptive study was conducted, active case finding was done along with routine immunization status assessment. Five blood samples were sent to NIH Islamabad.

Results: 14 cases (attack rate = 0.90%) including 4 deaths (Case Fatality Rates = 28.57%) were identified. Mean age of children was 47 months (range = 4-132 Months). Age-group 0-4 years was most affected (n=14, 71.43%). Coryza (n=52, 95%), conjunctivitis (n=14, 92%) and pneumonia (n=14, 77%) were most predominant symptoms. Routine Immunization (RI) status assessment of 152 showed that none of these children had been immunized in affected village. 56.36% of the respondents were unaware about RI, 16.36% mentioned that vaccinator had not visited their homes, 14.54% reported that health facility is far away and 12.72% reported that even if they could get access to a health facility, the vaccines were not available. All 5 samples were lab positive.

Conclusion: Most probable cause of outbreak was index case having traveling history to nearby district for treatment of diarrhea one week earlier. Functionalization of EPI center, vaccinations of all children with Outreach activity and mass awareness among community regarding routine immunization was strongly recommended. Surveillance system for vaccine preventable diseases (VPDs) should be strengthened to prevent such outbreaks.
Utilization trend of paediatric vaccines in the private sector in India (2010 and 2015): A descriptive time series analysis

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Purpose: This research was carried to generate evidence on utilization trend of pediatric vaccines in the private sector in India and to estimate private sector vaccine share in overall immunization coverage in the country.

Methods & Materials: We developed innovative methodology to estimate private sector vaccine share in immunization coverage. First, we calculated children fully vaccinated in the private sector by dividing selected vaccine doses sold in the retail market with the number of doses required to complete the respective vaccination course (1 dose for BCG, 1 dose for measles, 3 doses for DPT3 and 4 doses for OPV) (equation 1). Next, we calculated number of children vaccinated in the country (both public and private) by applying vaccine specific immunization coverage rates estimated from National Family Health Survey-4 (equation 2). Finally, we calculated private-sector vaccine share by estimating ratio of children vaccinated in the private sector (equation 1) and children fully vaccinated in both public and private sectors (equation 2) multiplied by 100.

Results: Over 265 individual vaccines formulation were available in the private sector market in the India during study period. The market share of those vaccines that are not part of universal immunization program (UIP) has increased significantly in the private sector as compared to vaccines that are available through UIP. In addition, private sector remains major contributor to immunization services in India, the private sector vaccine share for BCG was 22.5%, Hepatitis B (8.46%), OPV3 (6.92%), DPT3 (2.99%) and for Measles (2.38%) in overall vaccine coverage in India.

Conclusion: The private sector remains important access point for immunization services for significant proportion of the Indian population. Universal immunization program is required to develop strategies to engage private sector to enhance vaccine access and coverage.
Purpose: Rotavirus (RV) is one of the most common causes of acute gastroenteritis worldwide. RV vaccine is used to protect against severe forms of the disease. RV vaccine RotaTeq® has been implemented in National Vaccination Program of Republic of Armenia since 2012. However, disease can occur in vaccinated children as well. Aim of the study is to describe clinical and epidemiological characteristics of RV infection among vaccinated children.

Methods & Materials: During the period January-June 2014 423 children were admitted to the Infectious Clinical Hospital in Yerevan with the primary diagnosis “Acute intestinal infection”. Enzyme linked immunosorbent assay was used to detect RV antigen in stool. The SPSS program was used to analyze the data.

Results: Among all hospitalized children 87 (20.6%) were vaccinated against RV infection. RV antigen was detected in 24 (27.6%) of vaccinated children, of those 62.5% were males. Mean age of infected children was 14.9±6 months. They were admitted to the hospital on the 3±1.4 days of the diseases, 12.5% of them were admitted to intensive care unit due to severe course of the disease. Mean duration of hospitalization was 6±2.5 days. Majority (79.2%) had gastroenteritic variant of the diseases, however, gastritic (4.2%), gastroenterocolitic (8.3%) and enterocolitic (8.3%) variants were reported as well. Results of the stool culture revealed that 20.8% of children had mixed RV infection, combined with pathogenic and facultative pathogenic microflora (Shigella, Salmonella, Staphylococcus aureus, etc.).

Conclusion: Despite of the fact of vaccination some children develop severe form of RV infection and need to be treated in intensive care units. High percentage of detection of pathogenic and facultative pathogenic microflora with RV in stool can consider additional methods of treatment.
Purpose: Secondary bacterial infections are commonly identified in ulcers of cutaneous leishmaniasis (CL), thus, antibiotics are often empirically prescribed prior to anti-Leishmania therapy. Here, we aim to identify the spectrum of bacterial pathogens present in CL ulcers to better enhance antimicrobial stewardship and targeted antimicrobial therapy.

Methods & Materials: DNA was extracted from filter paper lesion impressions (FPLIs) of 6 CL ulcers and amplified by real-time PCR (qPCR) and end-point PCR targeting organisms implicated in skin-and soft-tissue infections. Targeted species included: Staphylococcus aureus, Enterobacter cloacae, Enterococcus spp., Citrobacter freundii, Klebsiella pneumoniae, and 16S rDNA. Amplified products were Sanger sequenced for bacterial species confirmation. Samples were then sent for Whole-Genome Sequencing (WGS). The severe CL phenotype was defined as: intercurrent mucosal involvement; multifocal disease (ulcers ≥4 in number in ≥2 anatomic locations); purulent/exudative ulcers with associated erythema and pain (“secondarily infected” appearance); or ulcers with lymphatic involvement.

Results: Of 6 FPLIs, 4 (68%) were identified as Leishmania Viannia braziliensis, 1 (16%) was L. V. peruviana, and 1 (16%) was L. tropica. Based on single assay detection, the following common potentially pathogenic flora were detected in all 6 FPLIs: S. aureus (100%), C. freundii (100%), Enterobacter spp. (67%), and Klebsiella pneumoniae (67%). Whole Genome Sequencing yielded identification of many environmental and skin contaminants; however, Brevundimonas nasdae was detected in 3 out of the 6 FPLIs (50%), in addition to all of the aforementioned flora using the single assay detection protocol. Overall, the distribution of flora in ulcers of severe (n=2) and non-severe phenotypes (n=4) did not differ (p=1.000).

Conclusion: CL ulcers with a secondarily-infected phenotype are universally treated with broad-spectrum antibiotics, often without microbiological confirmation of presence of bacterial pathogens. Therefore, understanding the presence and complement of bacterial potential pathogens in ulcers of CL has implications for antimicrobial stewardship and evidence-based management strategies. WGS enables detection and identification of the full range of organisms present in the microbiome of the CL ulcer. Further prospective analysis, including additional WGS studies of CL ulcers, is necessary to determine the role of empiric antibiotic therapy in CL ulcers with an inflammatory phenotype.
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Risk factors for Crimean-Congo Hemorrhagic Fever Virus Infection in Livestock in Pakistan

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Purpose: Crimean-Congo hemorrhagic fever (CCHF) is a severe, often lethal viral disease in humans caused by the arbovirus Crimean-Congo hemorrhagic fever virus (CCHFV). It is mainly transmitted to humans and animals by Ixodid ticks of the genus Hyalomma. The epidemiological situation regarding CCHFV in livestock is important for assessing the potential risk of exposure of the human population to this virus.

Methods & Materials: A cross-sectional study was conducted to study potential risk factors for CCHFV infections in livestock animals of the region Balochistan, Pakistan. Blood samples were collected from 1600 sheep and goats in Quetta, Sibi and Zhob divisions of Balochistan from July to September 2016. Farm and animal-related information was collected in interviews using a standardized questionnaire. Pools containing five serum samples each were tested for CCHFV genome fragments by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). For serological analysis, two indirect CCHFV-IgG-Enzyme-Linked Immunosorbent Assays (ELISA) were used. In case of divergent results, an adapted commercial immunofluorescence assay (IFA) was also performed.

Results: RT-qPCR identified 8 (5%, CI: 2%-10%) out of 160 sheep serum pools positive for the CCHFV genome fragments, while all goat serum pools (0%) were negative in this assay. In the serological analysis, 149 (19%, CI: 16%-21%) out of 800 sheep serum samples and 37 (5%, CI: 3%-6%) out of 800 goat serum samples were positive for CCHFV-specific IgG antibodies. The open type of housing (OR= 3.76, CI: 1.57-9.56, p= 0.003), grazing (OR= 4.18, CI: 1.79-10.37, p=0.001), presence of vegetation in/around the farm (OR= 3.13, CI: 1.07-10.15, p=0.043), lack of treatment against ticks (OR=3.31, CI: 1.16-10.21, p=0.029), absence of rural poultry (OR=2.93, CI: 1.41-6.29, p-value=0.004), sheep with age > 2 years (OR=2.72, CI: 1.36-6.22, p=0.008), sheep infested with ticks (OR=2.11, CI: 1.38-3.29, p=0.001), and goats infested with ticks (OR=2.68, CI: 1.23-6.72, p=0.02) were identified as statistically significant risk factors associated with the occurrence of CCHFV infections in livestock.

Conclusion: Risk factors identified in this study might elevate the probability of CCHFV infection in livestock. Humans who are in close contact with these animals may consequently also at a higher risk of infection with this virus.
Purpose: The purpose of this work was to characterize the population of ticks that attack humans in the Eastern Siberia and analyze the dynamics of tick-borne infections in the region.

Methods & Materials: Ticks were delivered to the laboratory by bitten people between 25.03.2007 and 17.10.2017. The developmental stage and species of ticks were identified morphologically using appropriate key guides. Four tick-borne pathogens, i.e. tick-borne encephalitis virus (TBEV), Borrelia burgdorferi sensu lato, Anaplasma phagocytophylum, Ehrlichia muris, E. chaffeens were detected in ticks using the range of laboratory diagnostic methods, including direct light microscopy, ELISA and real-time PCR.

Results: Total of 46357 tick specimens detached from bitten people were identified and tested individually during 10 consecutive years. The majority (85.4 %) of victims was bitten by I. persulcatus; 14.55% of attacks on humans were caused by D. nuttalli and D. silvarum; whereas H. concinna was documented only in 25 cases (0.05%). Among all studied ticks, several unconventional species i.e. Rhipicephalus sanguineus, D. reticulatus and Amblyomma americanum were identified. In 2017, besides two cases of R. sanguineus bites, the bite of A. testudinarium was registered. Analysis of tick bite histories indicates that five cases have local origin whereas other five ticks are imported from endemic regions.

Mean prevalence of infection in I. persulcatus comprised 12±6.5 %, 7.8±2.7%, 4.6±1.5% and 1±0.7% for B. burgdorferi sensu lato, A. phagocytophilum, Ehrlichia sp. and TBEV respectively. The dynamics of the prevalence of TBEV and B. burgdorferi s.l. in I. persulcatus indicate the significant increase of infection rate during 2014 – 2017. In Dermacentor sp., the prevalence of every infection was below 5%, with the highest rate for A. phagocytophilum (4.2±3.7%). No pathogens detected in H. concinna.

Conclusion: I. persulcatus remains the most important vector of tick-borne diseases to humans in Eastern Siberia with highest attack rate and with about 25% of ticks being infected with at least one of four pathogens. The H. concinna does not play any significant role as a disease vector. The regular events of invasion of non-endemic tick species are discovered.
**Purpose:** West Nile Disease (WND) is a zoonotic infection with a complicated transmission cycle, involving pathogen, human and animal hosts, vectors and ecosystem. Public Health Authorities are encouraging interaction between multiple disciplines to implement a comprehensive surveillance system. The aim of this work is to present the “One Health approach” enforced in Northern Italy, focusing on Piemonte, Liguria and Valle d’Aosta Regions (Northwestern Italy).

**Methods & Materials:** Since 2015, Veterinary and Human Public Health Authorities of some Northern Italian Regions enforced an integrated Surveillance Plan aiming to early detect the West Nile Virus (WNV) spread during the transmission season and to activate control measures to reduce the risk of human transmission.

The working group established throughout the whole Po river valley a standardized protocol of human, entomologic and veterinary surveillance activities. This allowed to connect the surveillance of human clinical cases with entomological surveillance and veterinary monitoring, giving an accurate assessment of the epidemiological situation, and implementing extensive control measures in case of outbreak.

In order to apply the guidelines, in Piemonte, Liguria and Valle d’Aosta Regions an information network was activated between Veterinary Services and local Human Health Authority.

**Results:** The first local WNV detection (animals, vectors or humans) in a Province during the transmission season was identified as trigger criteria to activate the information flows between Public Health Authorities. Immediately WNV Nucleic Acid Amplification Test (NAT) screening of all blood donations was introduced in affected areas and blood donors having been for at least one night in the affected Provinces were deferred from donations for 28 days. The integrated surveillance system allowed to detect WNV circulation in Piemonte Region in vectors, animal and human hosts.

To date, more than 80,000 blood bags were screened, and no one tested positive.

**Conclusion:** The described multidisciplinary approach allows to early detect WNV appearance during the transmission season. The network has been demonstrated a key point to timely implement control measures against the spread of the infection and the risk of human transmission. This approach was adopted at National level in 2016 where the Ministry of Health published the National integrated WNV Surveillance Plan, actually unique in Europe.
Novel Cell Line Derived From Natural Host *Apodemus Peninsulæ* For *In-Vitro* Studies Of Tick-Borne Encephalitis Virus

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**Purpose:** The purpose of the research was to develop a natural host-derived cell line for the study of the host specificity of tick-borne encephalitis virus and evaluate the reproduction of virus in this cell line.

**Methods & Materials:** The cell line was established from trypsinized homogenates of kidneys of female *Apodemus peninsulæ* (Korean field mouse). Cell line was characterized by cariotyping and by nucleotide sequence analysis of the cytochrome B gene and the D-loop region of mitochondrial genome. Cells were infected with the tick-borne encephalitis virus (TBEV) isolate 92M belonging to Siberian subtype. Tissue cultures were sampled at 0, 4, 8, 12, 16, 20 and 24 hours (hpi) as well as after 4 and 7 days post infection (dpi). TBEV reproduction dynamics was evaluated by plaque titration of supernatant medium from infected cells. Replication of intracellular RNA was measured by quantitative PCR. Production of TBEV antigen was evaluated using ELISA test. Porcine embryo kidney cell line SPEV was used in parallel as reference. All experiments were performed in triplicates.

**Results:** The adhesive diploid cell line was obtained by serial passages of primary culture and designated as ApnK. Sequencing confirmed the species origin of the cells. This cell line was used for experiments at passage 30. The growth of TBEV infectivity started at 12 hpi and concentration of infectious virus gradually increased up to 4 dpi. Afterwards it stabilized with titres of 7 lg PFU/ml. The synthesis of intracellular genomic RNA was firstly confirmed at 8 hpi and the dynamics was similar to the infectivity growth reaching the plateau at 4 dpi with concentration of 8 lg copies/mkl. TBEV antigen was detected in cell supernatants at 20 hpi and the dynamics corresponded to those of infectivity growth and RNA replication. In all experiments the reproduction of TBEV in ApnK cells was slowly than in SPEV cells up to 4 dpi when they equilibrated.

**Conclusion:** The stable cell line of natural host of TBEV, *A. peninsulæ*, was established. This cell line susceptible for TBEV infection, however the reproduction of the virus is restricted in comparison to convenient *in vitro* model, porcine embryo kidney cell line SPEV
Purpose: Yellow fever is a reemergent infectious disease considered as an important public health threat by the WHO International Health Regulations. The State of São Paulo (BR) has been observing a sylvatic yellow fever since April 2016 when the first case was confirmed, after seven years without registering cases. The objective of this study is to describe the human autochthonous cases of sylvatic yellow fever in the State of São Paulo between April 1, 2016 and June 15, 2018.

Methods & Materials: This is a descriptive, cross-sectional study that uses secondary data from the zoonosis division of the Center for Epidemiological Surveillance. The frequency, incidence and central tendency were calculated by the program EpiInfo™ vs 7.2.0.1.

Results: During the period of study, 560 cases of human yellow fever were confirmed in 76 municipalities, mostly located in the northeast and southeast regions of the State. The incidence rate per 100,000 inhabitants was 1.24 cases. Regarding the clinical outcome, 343 (61.2%) had a cure, 215 (38.4%) died and two (0.3%) are still hospitalized up now. The lethality rate was 38.4%. The median age was 44 years (1-90); 455 (81.2%) cases were men and 78.4% of cases were not vaccinated. Twelve cases underwent liver transplantation, nine of which died (case fatality rate 75%). The calculated median of the results for laboratory tests at the time of notification to sanitary authorities were 2395 U / L for aspartate aminotransferase; 1906 U / L for alanine aminotransferase; 2.4 mg / dL for total bilirubin and 2.05 mg / dL for direct bilirubin.

Conclusion: The follow-up and monitoring of the notified cases of yellow fever are necessary to decide on the measures of prevention in public health and to evaluate their effectiveness. Reinforce a mass vaccination for yellow fever, with a greater coverage, need to be achieved in order to avoid new cases during the next period of seasonality of the disease in the State of São Paulo, Brazil.
Body lice of homeless people reveal the presence of several emerging bacterial pathogens in northern Algeria

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Purpose: Human lice, Pediculus humanus, are obligate blood-sucking parasites. Body lice, Pediculus h. humanus, occur in two divergent mitochondrial clades (A and D) each exhibiting a particular geographic distribution. Currently, the body louse is recognized as the only vector for louse-borne diseases. In this study, we aimed to study the genetic diversity of body lice collected from homeless populations in three localities of northern Algeria, and to investigate louse-borne pathogens in these lice.

Methods & Materials: In this study, 524 body lice specimens were collected from 44 homeless people in three localities: Algiers, Tizi Ouzou and Boumerdès located in northern Algeria. Duplex clade specific real-time PCRs (qPCR) and Cytochrome b (cytb) mitochondrial DNA (mtDNA) analysis were performed in order to identify the mitochondrial clade. Screening of louse-borne pathogens bacteria was based on targeting specific genes for each pathogen using qPCR supplemented by sequencing.

Results: All body lice belong to clade A. Through amplification and sequencing of the cytb gene we confirmed the presence of three haplotypes: A5, A9 and A63, which is novel. The molecular investigation of the 524 body lice samples revealed the presence of four human pathogens: Bartonella quintana (13.35%), Coxiella burnetii (10.52%), Anaplasma phagocytophilum (0.76%) and Acinetobacter species (A. baumannii, A. johnsonii, A. berezeniae, A. nosocomialis and A. variabilis) in total 46.94%.

Conclusion: To the best of our knowledge, our study is the first to show the genetic diversity and presence of several emerging pathogenic bacteria in homeless' body lice from Algeria. We also report for the first time, the presence of several species of Acinetobacter in human body lice. Our results highlight the fact that body lice may be suspected as being a much broader vector of several pathogenic agents than previously thought. Nevertheless, other studies are needed to encourage epidemiological investigations and surveys of louse-associated infections.
Protection against Canine Vector-Borne Diseases Using a Monthly Prevention of Dinotefuran-Permethrin-Pyriproxyfen and Milbemycine Oxime

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**Purpose:** Corsica, is a French island in the Mediterranean Sea between the European and the African continents. The ecological conditions of the site offer suitable environments for the canine vector-borne diseases (CVBD); which need to be further investigated to better implement targeted and cost-effective control programs. The objective of the study was the assessment of the preventive efficiency of a new prophylactic protocol against three major CVBD occurring in eastern coast of Corsica: monocytic ehrlichiosis, dirofilariosis and leishmaniosis.

**Methods & Materials:** A total of 80 dogs split into two groups, were enrolled into a cohort surveyed during one year. The first group consisted of 25 military working dogs from the Solaro area, which received monthly prophylactic treatment: per os administration of 1.5 tablet of milbemycine oxime-praziquantel (Milbactor®, Ceva, France), and a topical line-on application of a 3.6 mL solution of dinotefuran-permethrin-pyriproxyfen (Vectra®3D, Ceva, France). The second group consisted of 55 dogs from regions of Solaro and Aleria under various other preventive protocols. The efficiency of the treatment was assessed at Day 0, 6 and 12 months' post-treatment. The *Dirofilaria* spp. infection was investigated using two customized designed PCR methods: a pan-filarial real time qPCR targeting the 28S gene and a standard sequencing analysis of the 18S rRNA gene. Indirect immunofluorescence assay was used to diagnose leishmaniosis and ehrlichiosis.

**Results:** By the end of the investigation, no new cases of CVBD were recorded within the first group; while five new cases of CVBD were recorded (9%, n=55) including three canine filariosis (two with *Dirofilaria immitis* and one with *D. repens*), and two serological cases of ehrlichiosis. Noteworthy, no new cases of leishmaniosis were detected in both groups.

**Conclusion:** The preliminary data obtained during this investigation showed that monthly prevention using Vectra®3D and Milbactor® protect dogs against the most common CVBD circulating in the Mediterranean basin. In the meantime, the other protocols failed to protect at least 9% of the dogs in the same endemic area.
Epidemiology Of Skin Infection In Homeless Population In Marseille
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Purpose: To describe homeless demographics, chronic medical conditions, ectoparasite and vector-borne disease prevalence over a 19 year-period of time.

Methods & Materials: Single-day cross sectional surveys were performed from 2000-2018 using standardized questionnaires and clinical examination of 2387 participants. Samples were collected including:

- **Human skin** (2014) from hair, neck, arm pits, pelvic belt: *Acinetobacter* spp., *Acinetobacter baumannii*.
- **Blood** (2014): *Acinetobacter* spp. (qPCR, culture)

Detection of *Sarcoptes scabiei* (2018): physical examination, dermatoscopy, qPCR (skin scrapings)

Results: The population was characterized by a majority of males (95.6%) from North African (49.0%) with a relatively high prevalence of chronic homelessness (44.0%). Pruritus (26.5%) and scratch lesions (17.4%) were recorded. The prevalence of body lice (10.2%) significantly decreased overtime (14.4% in 2000; 1% in 2018). Positive associations were reported between body lice infestations and older age, duration of stays in France for migrants, frequent consumption of alcohol, and tobacco smoking during the 2000-2017 time period. During 2013-2018, we observed *Bartonella quintana* (1%), *Rickettsia* spp. (0%) and *Borrelia* spp. (0%) among 588 body lice specimens. In 2014, *Acinetobacter baumannii* was found in crushed body lice (42.3%). Homeless infested with body lice were at a greater risk of carrying *A. baumannii* on the skin (OR=13, p<0.004); all blood samples were negative for *Acinetobacter* spp.. In 2018 we found no scabies in homeless suffering pruritus.

Conclusion: The decreasing prevalence of body louse and louse-borne infection in recent years could possibly be due to the changes in the characteristics of the population but also to the effect of delousing interventions conducted in the shelters by our team or by the shelter staff following our recommendations (providing showers, clothes, laundry and ivermectin treatment). For *A. baumannii*, we found no evidence that lice can transmit the bacteria through blood circulation, suggesting that infection of lice is due to direct contact with the skin rather than blood feeding. Several risk factors for body lice were identified, allowing a better identification of individuals at higher risk on whom to base targeted preventive interventions.
Vector Borne Diseases GAP Analysis And Training Needs Assessment Study In Pakistan.

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Purpose: Vector borne diseases (VBDs) share around 22% of all infectious diseases and 29% of emerging diseases globally. More than half of the Pakistan’s population is at risk. Likewise more than one 10 million people are infected and many die of VBDs every year. Prevalence of these diseases is high in the country and besides affecting the pocket of common people; these are a matter of concern for health care system as well leaving negative effects on productivity. Objectives: To determine gaps with respect to surveillance, prevention and management of VBDs, to identify the perceptions of health professionals and community and to assess training needs of health staff.

Methods & Materials: Both quantitative and qualitative approach of data collection, analysis and report formation was used. Separate questionnaires were designed for GAP Analysis and training need assessment at provincial, district and facility levels, along with assessment of community perception at health facilities.

Results: Despite for commonalities in diagonos, management and response, the preparedness of vector borne diseases varied from one type of diseases to other. Like for dengue and malaria some adequate measures exist at various administrative levels, but other VBD lack such backup support - like for leishmania, Congo hammorhagic fever and trachoma. Thre existed tremendous compartmentalizations to address this issue. Overall, less than 50% health facilities staff in pilot districts was reported to attend any training on Vector Borne Disease Prevention or control- specially on preparedness or outbreaks response. While around 60% respondents told about non-availability of any regular training plan on VBDs. 95 % & 100% people knew about malaria and dengue respectively but only 5 % people knew about Leishmania. There were discrepancies observed definition of vector borne diseases. No explicit on the job training program was documented.

Conclusion: To comoliment efforts and resources there is an urgent need for a 'One Health based Integrated VBD Program Study revealed that some adequate work has been done for malaria and dengue but diseases like Leishmania and Chikungunya remain neglected. The gains made under dengue and malaria control could form the basis of integrated management of these diseases.
Usutu Virus Detection in Abruzzo Region, Italy: the Entomological Surveillance as Key Tool for the Mosquito-Borne Disease Prevention

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Purpose: Usutu (USUV) and West Nile (WNV) viruses (genus Flavivirus, family Flaviviridae) are emerging zoonotic agents whose natural transmission cycles usually involve ornithophilic mosquitoes and avian hosts. USUV and WNV appeared in Italy in 1996 and 1998, respectively. Aiming to early detect arbovirus circulation and to reduce the risk of infection in humans, several National surveillance programs have been put in place by the Italian Ministry of Health since 2002. The one health philosophy has been adopted in designing all these plans in which synergism, collaboration and an increasing degree of integration between human and animal health sectors are clearly evident. In these surveillance plans, humans, animals and mosquitoes are monitored. This report described the first evidence of USUV circulation in Abruzzo region (Italy), as result of the integrated surveillance activities.

Methods & Materials: Between June 2016 and May 2018, 56 mosquito collections were performed in the “Ospedale Civile Santo Spirito”, Pescara municipality, Abruzzo region, in collaboration with the local health authorities. Adult mosquitoes, collected by using Bg-Sentinel traps, were identified by morphology, the females sorted in 412 pools and tested for Chikungunya, Usutu, West Nile and Zika viruses by Real Time RT-PCR assays.

Results: Overall, 19,066 mosquitoes were collected. Four genera were identified and Culex pipiens was the most abundant species (n. 6,505). When tested for the presence of arboviruses, 3 pools of Cx. pipiens resulted positive to USUV.

Conclusion: Although its presence has been demonstrated in several Italian regions, this is the first report of USUV circulation in Abruzzo. Being probably introduced by migratory birds which may act as reservoirs of many zoonotic mosquito-borne flaviviruses including USUV and WNV, the repetitive positivities found in the mosquito pools stand for the establishment of an enzootic cycle between native birds and local bird feeding mosquitoes. Culex pipiens can feed both on humans and birds and is known to be the main vector of flaviviruses in Italy. So far no circulation of USUV has been reported among humans and animals, however following the USUV detection the entomological monitoring was strengthened, moreover vector control measures and monitoring in humans and animals were set up to prevent human infection.
Innovative approach to control Zoonotic cutaneous leishmaniasis: An Early warning system to predict ZCL outbreaks in Tunisia.

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Purpose: Tunisia is one of the most exposed countries to climate change. The increase in temperature and degree of moisture is a favorable condition for the development of vectors of several diseases. Thus, the zoonotic cutaneous leishmaniasis (ZCL), vector-borne disease highly sensitive to climatic conditions, has seen a dramatic resurgence in parts of the country as Sidi Bouzid, taking advantage of the warming in recent decades. The aim of this work was to build a regression model of ZCL cases to identify the relationship between ZCL occurrence and possible risk factors, and to develop a predicting model for ZCL’s control and prevention purposes.

Methods & Materials: We used monthly reported ZCL cases from an active surveillance system which was implemented in three rural areas. Bioclimatic variables were monthly data between July 2009 and June 2015, recorded from a private station implemented. We used a generalized additive model (GAM) and a generalized additive mixed model (GAMM) to assess the relation between ZCL incidence and climate factors (temperature, rainfall, relative humidity, wind speed and rodents’ density). Coefficients from the best fit model were used to predict monthly ZCL number for the next season and were compared to observed ones.

Results: There were a total of 1019 notified ZCL cases from July 2009 to June 2015. The best-fit model showed significant associations between ZCL incidence and accumulated rainfall lagged 1 month, average temperature lagged 4 months, relative humidity with 4 months lag and rodents’ density lagged 2 months. Prediction from GAMM approach gives a good prediction accuracy. The Pearson correlation coefficient value was 0.81.

Conclusion: Understanding the role of the environmental and bioclimatic factors in ZCL occurrence can help to guide government policy-makers towards the creation and implementation of more effective policies to tackle the disease, and has important implications for prevention measures.
Molecular Detection of Rickettsial Infection in *Rhipicephalus sanguineus* Ticks Ectoparasitized on Dogs in Kaohsiung City of Southern Taiwan

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**Purpose:** *Rhipicephalus sanguineus* ticks are known vector for various causative agents of human and zoonotic pathogens. Thus, the prevalence of rickettsial infection in these ticks was determined and the genetic identity of detected *Rickettsia* was clarified.

**Methods & Materials:** *R. sanguineus* ticks ectoparasitized on dogs were collected from three districts of Kaohsiung City of Southern Taiwan. We performed a nested polymerase chain reaction (PCR) assay targeting a specific fragment of citrate synthase gene (*gltA*) of *Rickettsia* spp. to detect the *Rickettsia* infection in *R. sanguineus* ticks in Kaohsiung City.

**Results:** Results indicate that the prevalence of *Rickettsia* infection was observed in an average of 2.2% (25/1153) in examined *R. sanguineus* ticks. The higher seasonal prevalence of *Rickettsia* infection in these ticks was observed on June, July, October, January, and February with an infection rate of 4.9%, 6.0%, 3.4%, 3.1% and 2.5%, respectively. Sequence and phylogenetic analyses based on the comparison of *gltA* gene indicate that all these *Rickettsia* species detected in *R. sanguineus* ticks are genetically affiliated to the genospecies of *R. felis* and *R. honei*.

**Conclusion:** This study provides the first molecular evidence of *Rickettsia* infection detected and identified in *R. sanguineus* ticks in Southern Taiwan.
Detection of Q-fever in Samarkand Region of Uzbekistan: Mite Surveillance Study

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Purpose: The worldwide increase in incidence of Q-fever, significant changes in epidemiologic patterns and close proximity of potential endemic areas such as North Asia has led to raised attention to this disease in Uzbekistan. Some clinical cases of patients hospitalized to Samarkand regional hospital with acute fever of unknown origin were diagnosed with Coxiella burnetii using of immune-enzyme analysis (IEA). True prevalence in the region is not clear, due to asymptomatic course of the disease thus making surveillance for C. burnetii in ticks a critical component of control and prevention of this disease. The aim of the study was to identify spread of C. burnetii in ticks collected in Samarkand region of Uzbekistan.

Methods & Materials: In 2015, 1200 ticks were collected from domestic animals in the following districts of Samarkand region: Jambay, Taylak, Ishtikhan, Narpay, and Urgut. The collected ticks were divided by species and pooled for grinded suspensions. DNA from supernatant of each pool was extracted using "Ample Prime ®RIBO Sorb" for further testing by PCR on Qiagen Rotor-Gene. Simultaneously, a PCR detection was conducted among patients with acute fever for more than 5 days that were admitted to the Samarkand regional hospital.

Results: The results of the study showed that the most common imago mites in Samarkand region were Hyalomma a. anatolicum and H. as. asiaticum. The DNA of C. burnetii was detected in 9.1% of total mite samples in Samarkand region. 155 of Argas persicus specimens (13 pools) and 308 H. asiaticum specimens (14 pools) collected in Narpay district were positive for Q-fever. From Urgut region 96 specimens was Q-fever positive in H. asiaticum mites. In the Ishtikhan region C. burnetii was detected in 96 specimens of H. a. anatolicum mites and in 54 specimens of H. detritum mites. Simultaneously, out of 56 blood serums, the Q-fever was established in 21.4% of cases.

Conclusion: Application of Real Time PCR diagnostics shows first detection of C. burnetii among mite population in Uzbekistan that proves their significant role in spread of Q-fever disease and will help increase the effectiveness of epidemiological surveillance of this pathology in the Republic of Uzbekistan.
Seroprevalences for Zika, Chikungunya and Dengue in a Major Urban Center and in a Rural Village in Bahia State, Brazil

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Purpose: Dengue virus (DENV) have been circulating in Brazil for decades and several outbreaks have occurred. Since the introduction of Chikungunya virus (CHIKV) in 2014 and Zika virus (ZIKV) in 2015 new concerns raised about the emergence of these arboviruses. An unprecedented and concurrent outbreak of DENV, CHIKV, and ZIKV happened in Brazil in 2015 and 2016, especially in major cities in the northeastern region. Since then, approximately 1.8 million, 500,000 and 300,000 cases of DENV, CHIK, and ZIKV were notified in the country. However, since there is a large amount of asymptomatic infection, the real burden of these infections is not well understood.

Methods & Materials: In 2016 and 2017, we performed two cross-sectional studies, in the city of Salvador, capital of Bahia state to verify the seroprevalence of DENV, CHIK, and ZIKV in puerperal women admitted in a maternity hospital. Besides that, in 2018, we performed a cross-sectional study in a rural area, 170km from Salvador. Sociodemographic data and sera samples were obtained. Samples were tested by ELISA IgG for DENV, CHIKV, and ZIKV.

Results: In Salvador, 159 and 301 women were enrolled in 2016 and 2017 respectively and the seropositivity for DENV, CHIKV, and ZIKV were 92.4%, 29.5% and 88.8% in 2016 and 91.3% and 56.8% for DENV and ZIKV in 2017. On the other hand, 175 residents in a rural community were enrolled with 26.4%, 0% and 2.3% seropositivity for DENV, CHIKV, and ZIKV respectively.

Conclusion: With this, we state that the previous exposition to arboviruses was intense in a population from a major urban center, but the majority of individuals from the study in this rural environment is still susceptible to future infections with these arboviruses. The identification of susceptible populations is important for the understanding of the dynamic of circulation of these arboviruses and for the awareness of future outbreaks.
The burden of imported Malaria cases in Albania during 2010-2016

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Purpose: The incidence of malaria is declining worldwide whereas cases reported in Europe related to travel in endemic countries have remained steady in recent years. Albania is not malaria endemic since 1967 and the incidence presented a stable trend until 2010 when an increase of imported cases was reported.

Methods & Materials: A retrospective study was conducted including all smear-positive imported malaria cases presented to University Hospital Centre in Tirana during 2010-2016. Imported malaria has been described as “an infection that was acquired in an endemic area by an individual (either a tourist or indigenous native) but diagnosed in a non-endemic country after development of the clinical disease”. The diagnosis was made by light microscopy of Giemsa stained thick and thin blood smear (on separate slides each) and was confirmed at the Institute of Public Health (IPH).

Results: A total of 43 confirmed malaria cases were reported in Albania during the study period among Albanian workers in malaria endemic countries. The incidence of malaria cases after 2010 is 1.5 cases/10⁵ inhabitants. Country of origin of imported cases was Equatorial Guinea (72%), followed by Greece (9%), 4.6% Ghana (4.6%), Nigeria (4.6%) and 2.3% from Cameroon, Cad, Liberia and Uganda respectively. All patients were males with a median age of 34 years with a range 17 – 67 years. According to species-specific diagnosis, 58.1% of cases were caused by Plasmodium falciparum and 25.6% by Plasmodium ovale. Coinfection (Plasmodium ovale and falciparum) was detected in one (2.3%) of the patients. A fatal outcome due to cerebral involvement occurred in one patient, representing a case fatality rate (CFR) of 2.3% (95%CI 0.056 – 12.48). The rest of the patients recovered fully without any complications.

Conclusion: Workers in African countries constitute the group of malaria importation in Albania. Timely laboratory diagnosis and increased awareness by doctors and patients had a positive impact on the treatment. The traveler's counseling process has been strengthened in terms of protection against mosquito bites and use of proper prophylactic measures.
The Role of Arbovirus Genome Untranslated Regions on Neurotropism

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Purpose: Arboviruses are a group of viruses transmitted by arthropods, mostly ticks, and mosquitoes. Among the members of this group are the families Togaviridae and Flaviviridae, (+)ssRNA viruses, such as Chikungunya, Dengue, and Zika. Multiple of these viruses have demonstrated their ability to cause neuropathology in humans.

In Zika, a protein binding motif known as Musashi binding element (MBE) has been attributed to promoting replication, neurotropism, and pathology. Musashi-1 (MSI1) is an RNA-binding protein involved in the maintenance and self-renewal of stem cells and a translational regulator in many biological systems. MSI1 predominantly binds single-stranded UAG motifs in the 3' untranslated region (UTR) of RNA.

We have recently analyzed Musashi binding elements (MBEs) in the 3'UTR of flaviviruses (FV) in silico. In this study, we could show that MBEs in the 3'UTR of neurotropic viruses such as Zika, West Nile, and Powassan virus are highly accessible, and mostly occur in an unpaired structural context, which renders them optimal Musashi binding targets and corroborates previous experimental studies by a theoretical model.

Methods & Materials: In order to expand to other related viruses, we systematically analyzed the properties of Musashi binding elements (MBEs) in the 3'UTR of Alphaviruses (AV) based on a thermodynamic model for RNA folding and correlated to the currently described pathogenicity in literature.

Results: Our preliminary results indicate that MBEs in the 3'UTR of Chikungunya, Venezuelan Equine Encephalitis, and Eastern Equine Encephalitis viruses occur predominantly in paired, double-stranded structural context, suggesting that the mechanism to cause neurotropism in these AV must be a different one. Analyses in other AV are currently being performed to investigate the role of MBEs in a more broad dataset. Our initial analysis suggests that the MBE role in neuropathology may be an exclusive feature of FV.

Conclusion: Our study addresses the broader question whether other emerging arboviruses can cause similar neurotropic effects. We expanded our study from a previous Flavivirus dataset to all (+)ssRNA arboviruses to evaluate the link between MBE and currently described neuropathology. Moreover, our thermodynamic model can be the initial indicative for in vivo studies to evaluate the potential neurotropic effect of MBEs on different viral families.
Purpose: to determine the role of ticks in the existence of tularemia natural foci, including expansion of their boundaries and emergence of new enzootic areas.

Methods & Materials: During 2013-2017, 34,170 samples of ticks were collected by flagging and dragging; identified and tested using IHA and PCR methods.

Results: The most permanent focus of tularemia with the tendency of geographical distribution in Lviv Oblast is the Bystrytsia river floodplain near the Carpathian region. In samples collected from natural biotopes, *I. ricinus* was subdominant (43.0%) and *D. reticulatus* was a dominant species (57.0%). *D. reticulatus* (76.0%) was also a dominant species in samples collected from cattle. The density index (DI) of *D. reticulatus* varied from 1.5 to 5.1, and the specific weight (SW) was from 0.6 to 1%. DI of *I. ricinus* varied from 0.6 to 6.6, and SW was from 0.3 to 0.95%. Analysis of the data demonstrated a change of a dominant species.

In serological studies, *F. tularensis* antigen was detected in 8.0% of 34,170 ticks (358 samples), including *D. reticulatus* (57.0%) and *I. ricinus* (43.0%). *F. tularensis* DNA was detected in 4 (6.3%) samples (3 *D. reticulatus* and one *I. ricinus*) of 6,340 ticks (64 samples) using PCR. Most of the positive findings were obtained in the known enzootic areas. Positive results were sporadically detected in areas that had not been listed as enzootic, and that requires additional studies.

Conclusion: The results of entomological monitoring indicate the preservation of natural foci of tularemia in Lviv Oblast. Vector spread of the pathogen contributed to the formation of permanent natural foci of tularemia with an epicenter in the Bystrytsia floodplain in the Carpathian region, where since 1975, 134 cultures of *F. tularensis* have been isolated from ixodic ticks: 123 from *D. reticulatus* and 12 from *I. ricinus*. Based on the results of tick testing, a new territory that is located far from well-known foci was discovered. The change of the dominant tick species from *I. ricinus* to *D. reticulatus* was established.
Purpose: *Plasmodium falciparum* is the most fatal strain cause severe infection and death especially in children and pregnant women. 80% of malaria deaths in Africa occur in pregnant women and children below 5 years. Cerebral malaria (CM) in children is the most severe neurological complication of infection with *Plasmodium falciparum* and it is a leading cause of childhood neuro-disability in surviving children who have an increased risk of epilepsy with behavioral difficulties. Pregnancy associated malaria (PAM) is more severe and more fatal due to puerperal sepsis includes cerebral malaria, fever illness and mortality can occur from haemorrhage. There is a high risk of perinatal morbidity and low birth weight.

Methods & Materials: pRBC sequestration in cerebral microvasculature is major factor in CM pathogenesis. *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1) mediate binding to host intercellular adhesion molecule-1 (ICAM-1) is the most important and its expression is triggered by nuclear translocation of NF-κB from infected erythrocytes to cerebral endothelia. This binding mediates the release of inflammatory cytokines TNF-α, IFN-γ and IL-β which further up regulates expression of ICAM-1. In PAM cytoadhesion of pRBCs mediated by binding of PfEMP-1 to chondroitin sulfate A (CSA) receptor which promotes sequestration in placenta.

Results: unique domains of PfEMP-1 are involved in placenta and cerebral microvasculature cytoadhesion. var genes encode *P. falciparum* erythrocyte membrane protein (PfEMP)1 variants with distinct N-terminal domain types like domain cassette 8 except cysteine-rich interdomain region 1 bound to brain endothelium. The DBLγ domain is essential for CSPG recognition and is expressed by VAR2CSA.

Conclusion: These findings may provide important implications to design vaccine for PAM and CM.
Mass drug administration (MDA) to eliminate Lymphatic filariasis: Even 100% coverage would fail to achieve elimination of infection in community in 6 years

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**Purpose:** Introduction: Lymphatic filariasis is still endemic in many parts of India. Main strategy to implement mass drug administration (MDA) was with DEC and albendazole added in 2006 to ensure increased impact on the microfilaria (mf) rate in the community. However, effective coverage remained low in the community leading to lower than desired impact on the parasite population in human. This paper examines dynamics of different treatment strategies and coverage after 6 MDA rounds in 18 villages of Uttar Pradesh and measures incidence of infection.

**Methods & Materials:** Paper examines the impact of annual DEC+12 days additional dose to mf positive found at respective rounds. Sample of 20 CMM was taken after 7PM and tested for microfilaraemia positivity using standard procedure.

**Results:** Data revealed that mf rate declined by 91% in a cohort of 1063 persons ingested DEC for 6 years. A cohort participating for 5 annual rounds could bring down mf rate by 87.1%; after 4 rounds it declined by 83.9%, after 3 rounds by 78.2%, after 2 rounds about 70% and one round by 43.3%. At community level, it is seen that MDA coverage was around 60% at RO, reached to 65% at R3, declined to about 60% at R5 and further to 40% at R6 and mf rate declined by 74.2% (12 to 3.1) after 6 rounds. It is also seen that new infection is recorded all over this period and also in young children. 3.83% population never took DEC and 7.36% took every time for 6 years. New infection among children up to 5 years entered at various rounds suggested that about 2% get infected every year and increased cumulatively to 4-5%.

**Conclusion:** Annual MDA is although effective in clearing parasite from blood but effectiveness never reaches 100% as observed under several treatment regimens. Present one offering 12 days additional treatment to mf positive, effectiveness reached 91% among those who ingested DEC for 6 years. Inspite of best efforts at field level, MDA coverage in community never crossed 70% level. Therefore, programme claim of 90 to 100% coverage is doubtful and therefore MDA alone cannot achieve 100% clearance or elimination of infection.
Improving The Efficiency Of Reactive Screen-And-Treat For Malaria Elimination In Southern Zambia

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Purpose: Malaria screen-and-treat (called Step-D in Zambia) is a reactive case detection strategy in which cases detected at health centers trigger community health workers (CHWs) to screen for secondary malaria cases within a 140-meter radius of the index case household using PfHRP2 RDTs¹. Few studies evaluated whether an evidence-based strategy using environmental features that characterize the immediate surroundings of a household, can improve the efficiency of secondary case identification. As Zambia works towards eliminating malaria, more refined and targeted case detection strategies are required to find the untreated malaria cases that could serve as potentially asymptomatic sources of infection. This study utilized the Step-D program and extended the screening radius to 250-meters to assess which local environmental variable can guide CHWs in finding malaria-positive secondary households situated near index case houses more efficiently.

Methods & Materials: Demographic information, malaria diagnosis, bed-net use and ownership, cooking energy source, and household floor material were obtained from surveys. Households were stratified into malaria positive and negative secondary households using RDT and qPCR results. ArcGIS was used to generate the following local environmental variables: screening radius (140 vs. 250-meters), number of animal pens within 100-meters, distance to nearest animal pen, distance and elevation difference between index and secondary houses, as well as the following large scale environmental variables: distance to main road and nearest strahler stream category. Generalized estimating equations (GEE) estimated the cross-sectional effect for the difference in odds of a positive vs. negative secondary household for each predictor.

Results: Secondary households had an overall parasite prevalence of 1.9% which was higher by qPCR (1.8%) than RDT (0.7%). Key results revealed that if the nearest stream to a household was of a higher order stream (strahler category 3, 4, or 5), there was an increased odds of being a positive secondary household compared to a lower order stream (OR stream category 3: 1.5; p=0.03, OR stream category 4: 1.9; p<0.01, OR stream category 5: 1.5; p<0.01).

Conclusion: Screening for secondary households within low-transmission settings in Zambia could be optimized by using large-scale indicators such as streams as environmental guiding tools.
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Overproduction of IL-6 and Type I IFN in a Lethal Case of Chikungunya Virus Infection in an Elderly Man during the 2017 Italian Outbreak

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Purpose: Chikungunya fever is a mosquito-borne viral disease caused by Chikungunya virus (CHIKV) and is generally considered self-limiting non-fatal disease. However, severe clinical presentations with high mortality rate (48%) are reported associated with the presence of several underlying medical conditions. Recently, a CHIKV epidemic occurred in Italy, involving 270 confirmed cases in Lazio and Calabria regions. Here, we report the virus characterization and an abnormal pattern of circulating cytokines in a lethal case of CHIKV during the 2017 Lazio region outbreak.

Methods & Materials: In September 2017, a 77-year-old male with underlying cardiac diseases was admitted for acute neurological syndrome to the Santa Maria Goretti Hospital in Latina, Rome, and he died after 9 hour admission in sub-intensive unit care for acute cardiac arrest. Due to the ongoing CHIKV outbreak, RT-PCR and indirect immunofluorescence assays were performed to assess a possible infection. The virus was isolated on BHK-21 cells, and the near-complete genome sequencing was performed. Circulating IFNs and inflammatory cytokines were evaluated and quantified using ELISA, and the levels compared to those detected during the very early stages of the infection in four non-fatal cases belonging to the same outbreak.

Results: Laboratory tests showed CHIKV infection, based on positive RT-PCR test (Ct: 12), in the absence of CHIKV-specific antibodies. The sequence of the isolate (CHIKV/ITA/Lazio-INMI2-2017) showed only one nucleotide difference (synonymous substitution) as compared to the outbreak prototype strain (CHIKV/ITA/Lazio-INMI1-2017), and clustered with recent isolates of the Indian Ocean sublineage of ECSA in the maximum-likelihood phylogenetic tree. The evaluation of the inflammatory response showed that IFN-α, IFN-β and IL-6 levels were extremely high as compared to non-fatal cases, while IFN-γ and TNF-α levels were in the same range. The elevated levels of IFN-α, IFN-β and IL-6 seemed not directly correlated to elevated levels of CHIKV-RNA.

Conclusion: The analysis of inflammatory cytokines revealed a remarkable and strong increase of circulating type I IFN, as well as of the IL-6 pro-inflammatory cytokine, suggesting a possible role of type I IFN in cytokine storm, which may be correlated with unfavourable prognosis of CHIKV infection.
Prevalence of Lyme borreliosis in Istria County, Croatia

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Purpose: There are few data on the prevalence to Lyme borreliosis (LB) in Istria County. The aim of this study was to analyse, by two-tire serological strategy, the prevalence and epidemiological characteristics of LB in Istria, the largest Croatian peninsula located at the westernmost part of Croatia.

Methods & Materials: From January 2015 to October 2017, a total of 472 serum samples from patients aged one to 85 years were tested for the presence of antibodies to *Borrelia (B.) burgdorferi* sensu lato (sl). All samples were initially screened using VIDAS Lyme IgM and IgG (bioMérieux, Marcy l’Étoile, France). The test is a third generation enzyme-linked immunofluorescent assay (ELFA), coated with recombinant chimeric proteins DbpA, Osp C and VlsE. All IgM and IgG positive or equivocal samples were confirmed using Anti-Borrelia EUROLINE-WB (Euroimmun, Lübeck, Germany). The immunoblot test strip contains specific proteins from *B.afzelii* with an additional chip containing VlsE antigen from *B.burgdorferi* sensu stricto.

Results: There were 293 (62.1%) female and 179 (37.9%) male participants. In the screening test, the overall prevalence was 10.8% for IgG and 13.3% for IgM. Using immunoblot, prevalence of IgG and IgM antibodies was 9.9 % and 12.7%, respectively. The immunoblot overall agreed with ELFA in 92.2% for IgG and in 95.2% for IgM. According to age group, the prevalence rates varied from 0.0% to 18.6% for IgG and 0.0 to 22.0% for IgM. There were 13.5% IgG and 9.6% IgM positive children under the age of 10. In the following age groups, the seropositivity declined with the increase in persons older than 50 years (from 12.0% to 18.6%). Among the confirmed IgM immunoblot tests, the diagnoses of tick bite, dermatitis and LB were significantly represented (54/60; 90.0%). Using Fisher’s exact test, statistically significant differences were found in the IgG seropositivity between age groups (p=0.009) and diagnoses (p=0.001). LB cases showed seasonal distribution from March to September (47/60; 78.3%).

Conclusion: LB is present in Istria. Cases occurred in all age groups with seasonal distribution from spring to autumn.
Detection of trypanosomes in non-cyclical haematophagous arthropods in Kacia grazing reserve Nigeria

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Purpose: Haematophagous arthropods were collected from Kachia Grazing Reserve (KGR) to determine the occurrence and species composition and the detection of trypanosomes infection in them.

Methods & Materials: Eighteen bi-conical traps were set in strategic positions in the reserve for the collection of the insects in the peak of the rainy season of 2017. The arthropods collected were identified according to taxonomic keys to species level, with the aid of microscopy. The parous constituents of each insect identified were pooled and stored separately accordingly in RNAlater®. Trypanosomal DNA was extracted from each pool according to the protocol and the purity and concentrations of DNA extracted was quantified in a Nanodrop spectrophotometer. Amplification of the ITS-1 region of the 16S rDNA marker for the Trypanosoma species detection by PCR, using generic primers and specific primers. The successes of amplification were resolved in 1% Agarose Gel Electrophoresis and documented in a transilluminator.

Results: The 525 arthropods collected and identified consisted of Haematobia irritans (53.1%), Haematopota puniens 4(0.8%), Stomoxys calcitrans 176(33.5%) and Tabanus pertinens 6(1.1%). The apparent density for Haematobia irritans, Haematopota puniens, Stomoxys calcitrans and Tabanus pertinens were 2.58, 0.04, 1.63 and 0.06 flies/traps/day, respectively. The disparity in density may be related to trapping efficiency. The amplicons were characteristics showed infections of Trypanosoma simiae (850bp) detected from Haematobia irritans, Trypanosoma theileri (988bp) detected from Haematopota puniens, Trypanosoma vivax (611bp) and Trypanosoma simiae (850bp) detected from Stomoxys calcitrans. The four species of trypanosomes detected in the arthropods collected suggests their possible role as mechanical transmitters of trypanosomiasis in KGR. This may greatly underscore the transmission routes and constitute a risk to livestock production in KGR and elsewhere.

Conclusion: The study reported the occurrence of Haematobia irritans, Haematopota puniens, Stomoxys calcitrans and Tabanus pertinens at varied densities. Trypanosoma simiae was detected from Haematobia irritans, Trypanosoma theileri from Haematopota puniens, Trypanosoma vivax and Trypanosoma simiae were detected from Stomoxys calcitrans, during the investigation. The detection of these trypanosomes species might be responsible for the incidence of African Animal Trypanosomiasis (AAT) in the reserve and calls for more detailed study and control mitigations.
Purpose: Pathogens of the genera *Anaplasma*, *Borrelia* and *Rickettsia*, and Tick-borne Encephalitis virus (TBEV) may be associated to various tick species which are hematophagous ectoparasites potentially hosted by any terrestrial vertebrate, humans included. This peculiarity makes ticks potential horizontal vectors of zoonotic importance.

Liguria is characterized by mountains and an intense urbanization of the terminal part of the alluvial plains. The 12% of its surface is a protected natural area.

The aim of our study is to provide an overview of the infection rate of tick-borne agents by analyzing ticks collected between 2016 and 2017 from wild mammals in Liguria.

Methods & Materials: More than 200 ticks (124 *Ixodes*, 68 *Dermacentor*, 34 *Haemaphysalis* and 9 *Rhipicephalus* spp.) were collected from 49 roe deer (*Capreolus capreolus*), 41 wild boars (*Sus scrofa*), 7 chamois (*Rupicapra rupicapra*), 2 fallow deer (*Dama dama*) and 1 marten (*Martes foina*) and stored in 70% ethanol.

After ticks identification and total nucleic acid extraction, simplex PCR assays to detect *Anaplasma*, *Borrelia*, *Rickettsia* spp. and TBE virus were performed. By project’s design, all ticks belonging to a given species from the same animal and collection date were pooled and treated as a single sample.

Results: PCR results revealed 36 ticks positive for *Rickettsia* spp. and 3 for *Anaplasma* spp.; all samples were negative for *Borrelia* spp. and TBEV. *Rickettsia* spp. were found in 32 *Ixodes ricinus* and 4 *Dermacentor marginatus* ticks, while *Anaplasma* spp. were related to 2 *Haemaphysalis punctata* and 1 *Ixodes ricinus* (co-infected with *Rickettsia*). Regarding the host species, the 15% of wild boars and 61% of roe deer sampled were carrying ticks infected with *Rickettsia*, while the 29% of chamois ticks infected with *Anaplasma*.

Conclusion: Our study confirms the presence of *Rickettsia* and *Anaplasma* spp. in ticks in Liguria, giving proof of their circulation in wild mammals. This panorama suggests the importance of taking forward the health monitoring in order to ensure the early detection of potential zoonotic infections in this area.
Comparison Of Feeding Success Of Larval *Ixodes Scapularis* And *I. Ricinus*, The Main Vector Tick Species Of *Borrelia* spp. Using Mice As Host

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**Purpose:** Several ticks of the genus *Ixodes* such as *Ixodes scapularis* and *I. ricinus* are among the most important vectors of Lyme disease pathogens. Studies focused on tick-host-pathogen interactions in Lyme borreliosis requires robust animal models. Mice are used most frequently as hosts for this purpose because they are the natural biological reservoirs for *Borrelia* spp. The objective of this study was to develop a standardised and reproducible protocol for feeding *I. scapularis* and *I. ricinus* tick larvae on mice.

**Methods & Materials:** Twenty Swiss mice aged 10 weeks with a body weight >25g were allocated into two groups of 10 each. After sedation, mice from group 1 and 2 were infested with 100 pathogen-free *I. ricinus* and *I. scapularis* larvae each, respectively. The attachment success was assessed one hour after infestation while the feeding success was determined from the total number of engorged larvae that dropped off the mouse 48, 72, 96 and 120h after infestation.

**Results:** Attachment rate was 96.9% and 77.5% for *I. ricinus* and *I. scapularis*, respectively. Cumulative feeding success assessed 48, 72, 96 and 120h after infestation was 3.3, 34.6, 42.3 and 42.3% and 0, 5.7, 33.3 and 40.7% for *I. ricinus* and *I. scapularis*, respectively. There was no significant difference (X^2^ test, p>0.05) between *I. ricinus* and *I. scapularis* for both attachment and feeding rate success.

**Conclusion:** Our infestation procedures provide a simple protocol for feeding larval *Ixodes* spp. on mice. This tick infestation model was regarded suitable for potential future research on tick-host-pathogen interactions as well as to evaluate the effectiveness of host-targeted acaricides against immature *I. ricinus* and *I. scapularis* ticks.
A Metagenomic Survey of Ticks Reveals Pathogenic Rickettsia and Francisella/Coxiella-like Endosymbionts in Anatolia

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**Purpose:** We investigated tick-borne bacteria in field-collected ticks via a metagenomic approach using high throughput sequencing.

**Methods & Materials:** A total of 210 ticks, collected from various regions of Anatolia, were identified morphologically and processed in 39 pools. Homogenization, nucleic acid purification and library preparation in pools were performed using standard assays and protocols, followed by sequencing using the Illumina MiSeq system.

**Results:** A near-complete Rickettsial genome of 1,485,148 nucleotides with 89.5% identity to Rickettsia felis strain URRWXCal2 was assembled from a pool of Haemaphysalis parva ticks. The identical pool further provided a 5690-nucleotide sequence of the Rickettsial conjugative plasmid. Varying lengths of 16S, OmpA, Sca1 and gltA sequences were obtained from 7 tick pools (17.9%) comprising Haemaphysalis parva, Rhipicephalus bursa, Rhipicephalus sanguineus sensu lato and Hyalomma marginatum ticks. The sequences were characterized as Rickettsia felis and Rickettsia hoogstraali in 5 pools whereas strain identification remained obscure in two pools. 16S rRNA sequences of Coxiella-like endosymbionts were detected in 15 pools (38.4%) of Haemaphysalis parva, Rhipicephalus bursa, Rhipicephalus sanguineus s. l., Hyalomma marginatum, Hyalomma excavatum and Dermacentor marginatus ticks. These sequences formed three phylogenetically-distinct clusters with previously-characterized Coxiella-like endosymbionts from Rhipicephalus, Ixodes and Dermacentor species. Finally, Francisella-related 16S sequences were characterized in 9 pools (23%) of Rhipicephalus sanguineus s. l., Hyalomma marginatum, Dermacentor marginatus and Hyalomma aegyptium ticks. These sequences formed two distinct clusters and grouped with Francisella-like endosymbionts of Dermacentor ticks, sharing a common ancestor with Francisella percica.

**Conclusion:** Rickettsia of the spotted fever - Rickettsia akari group were characterized in this preliminary tick screening study, with detailed sequence information obtained for various strains. Widespread infection of ticks with Francisella- and Coxiella-like symbionts were also identified. The impact of these endosymbiotic bacteria on the circulation and detection of pathogenic strains in ticks requires further investigation.
Falciparum Predominant Malaria Outbreak in Hunkund taluk, Bagalkote District, Karnataka, India, 2015 to 2016.

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Purpose: India accounts for 58% of malaria cases in South East Asia region. Four villages of Hungund taluk, Bagalkote district in Karnataka state, India reported clustering of malaria cases on 15th of November, 2015. We investigated to describe the outbreak and to give recommendations.

Methods & Materials: Our study population included residents of all four villages, Hirekodagalli, Gugglemari, Gudur and Hanumnal. We surveyed fever cases in fever clinic, between 20th of November, 2015 and 31 of March, 2016. We defined a confirmed case of Malaria as any patient whose blood smear was positive for malarial parasites, Plasmodium falciparum and Plasmodium vivax. We collected line list of confirmed malaria cases from fever clinics in the four villages. We calculated attack rates by village, age groups and gender.

Results: There were 601 falciparum cases and 213 vivax cases. Sixteen cases had mixed infections. The epicurve showed that falciparum cases started on 26th October, 2015, with peak between 27th and 29th October and started coming down after 1st of December, 2015. Last case occurred on 18th of February, 2016. Overall attack rate of Falciparum cases was 15% (601/4111). Median age was 22 years (range: 1.5 – 81 years). Falciparum incidence was highest (24% in Gugglemari village). Vivax cases started on 26th of October peaked between 27th October and 16th of November and last case occurred on 21st of December, 2015. Median age was 22 years (3 to 80 years). Overall attack rate of vivax cases was 5% (213/4111). Attack rates among females (5%, (103/213)) and males (6%, (110/1978) were almost equal. Highest attack rate (7%) was in Gugglemari (69/959) and Gudur (45/668) while attack rate was 4% in Hirekodagalli (83/2024) and 3% in Hanumnal (15/460).

Conclusion: There was malaria outbreak between 26th October, 2015 and 18th February, 2016 in four villages of Hungund taluk. Falciparum accounted for majority of cases and Gugglemari village was most affected. We recommend identifying entomological and environmental factors for the outbreak.
Purpose: Highest burden of dengue is in India, accounting for 34% of global cases. Dengue cases started increasing in Namakkal district of Tamilnadu, India from July and peaked in October, 2017. District surveillance unit planned interventions to control the outbreak. After two months, we evaluated the impact of interventions.

Methods & Materials: We did an experimental study between October and December, 2017 in all the 15 blocks of Namakkal district. We defined a confirmed case of dengue as any patient with fever> 2 days and positive for IgM or NS1 antigen by ELISA (Enzyme linked immunosorbent assay). We started daily surveillance of fever cases in primary health centres, selected private hospitals and district hospital to increase the sensitivity of dengue surveillance. We diverted block level rapid response team (RRT) to the high risk areas. RRT conducted special medical camps, anti-larval works and fogging activities in resident area of the fever cases for consecutive three days in the affected areas. RRT also did active surveillance, collected clinical samples from the fever cases and sent samples to district public health laboratory. We analysed data on weekly basis for identifying high risk areas and risk population. We promoted community participation by conducting meetings at village level and involving local bodies. We educated to identify dengue larva and reduce breeding. We did paired t test to evaluate impact of interventions. We calculated mean differences (MD) in incidence of cases in different blocks between October and December, along with standard deviation (SD) and p value significant at <0.01.

Results: Dengue cases increased from 117 in July to 554 in October, 2017(196 cases in August and 270 in September). We started our interventions in third week of October. Cases decreased in November (222 cases) and December (28 cases). Paired t test showed that interventions were effective at one month interval (MD: -22, SD=0) which was statistically significant (p<0.01). At two months interval (December, 2017), mean difference of paired t test was -35 with SD=0(p<0.01).

Conclusion: Dengue cases decreased following interventions in Namakkal district. We recommend continued surveillance and maintain interventions to prevent future outbreaks.
Purpose: Wild birds represent the natural hosts and carriers of diverse zoonotic diseases, e.g. Avian FLU, zoonotic flavivirus encephalitis, chlamydiosis, deserving specific monitoring efforts from Public Health Institutions. Surveillance plans on wild animals are in place to early detect the occurrence of pathogens in the north western Italian region of Piemonte, including West Nile virus (WNV) and Usutu virus (USUV) findings.

Methods & Materials: Migratory and resident wild birds found dead were collected by passive surveillance (Wildlife Plan). The West Nile Disease (WND) active Surveillance Plan on culled black crows, magpies and jays was scheduled since 2015 from July to November by trapping animals in wet areas. Central nervous system and a pool of lung, heart, spleen and kidney were analyzed separately for each animal. Upon viral RNA purification, samples were tested with Real-time one step RT-PCRs: one able to discriminate between WNV Lineage 1 and 2 and one specific for USUV. All positive samples were sent for confirmation to the OIE National reference Centre for WND in Teramo.

Results: Between 2015 and March 2018, 2,666 wild birds were analyzed: 1,092 from the Wildlife Plan and 1,574 from the WND Plan. Positivities for WNV Lineage 2 were found in 13 wild birds: 1 eagle owl, 1 kestrel and three hooded crows in 2015, six hooded crows in 2016 and two in 2017. USUV was confirmed in two black birds in 2015, one from the Wildlife Plan and one from the WND Plan. From July to 20th August, 7 hooded crows, 2 magpies, one pheasant and a kestrel resulted infected with WNV Lineage 2. Moreover, 2 hooded crows, 3 magpies and a black bird were found positive to USUV.

Conclusion: WNV Lineage 2 circulates in wild birds in Italy since 2011 and is the most frequently detected Lineage in Italy. In 2018 an increase in the WNV cases was registered in the summer in six out of eight provinces of Piemonte. As a consequence, all the prevention and control measures required by the Italian WND integrated surveillance plan were promptly activated to reduce the risk of infection for humans.
The possibility of reintroduction of Malaria in Portugal: are surveillance systems doing enough?

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Purpose: Malaria was eliminated in Portugal in 1973. In 2014, an electronic Surveillance System (SS) was implemented but the disease is still believed to be underreported. With the increase in the number of locally acquired P. vivax cases in Europe, population mobility and climate change, the possibility of resurgence of autochthonous cases is real. This study aims to estimate the completeness of malaria notification using the capture-recapture (CRC) method.

Methods & Materials: Cross-sectional study of malaria cases identified either in the notification database of the SS or the Diagnosis-Related Group (DRG) database containing all public hospital admissions in 2016. The variables sex, date of birth and residence were used for matching the cases. Maximum likelihood population estimate and completeness of notification relative to hospital cases were calculated.

Results: 423 cases were identified in the DRG database and 197 cases in the SS, with 139 cases being reported by both databases. Completeness of notification by the SS relative to DRG was 32.9%. The maximum likelihood population estimate was 600 cases. Malaria cases were mainly caused by P. Falciparum, more frequently men, with a mean age of 40 years. Case fatality was less than 2.5%.

Conclusion: The study confirmed that malaria is underreported. However, the introduction of an electronic SS seems to have decreased sub-notification, since a previous study on paper-based notifications found a completeness notification of 21.2%. The likelihood population estimate indicates that the total amount of cases in 2016 was underestimated. This might be explained by the diagnosis of malaria in other clinical settings or ambulatory treatment of hospital cases. The results of this study highlight the need for effective notification strategies for malaria, especially when there is a risk of reintroduction of disease.
Clinical Presentation of Reported Lyme Disease Cases among Children from Quebec (Canada) between 2009-2017.

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Purpose: In southern Canada, Lyme disease (LD) is an emerging tick-borne disease caused by Borrelia burgdorferi. Cases have increased from 144 to 987 between 2009 and 2016. The infection is more frequent in children and older adults probably due to increased exposure to high risk areas during outdoor activities. The aim of this study is to describe the clinical presentation and course of illness among children reported in Monteregie, an emerging area from Quebec province located north of New York state.

Methods & Materials: This is a retrospective cohort study of all cases among children (0-18 years) reported to the Monteregie Public Health Directorate between January 2009 and December 2017. Public health records were reviewed. These records are based on information obtained from treating physician and patient at the time of reporting. Follow-up interviews were conducted with patients (or their parents) to assess outcome for 63 cases reported before December 2016. Variables studied included sociodemographic data, detailed clinical presentation and evolution, laboratory results, hospitalization and exposures.

Results: Eighty-five confirmed, probable or suspected cases were reported. Half occurred in the 0-6 age-group. Erythema migrans was mentioned by 73% of cases but only 53% of all cases were reported at the early localized stage. Neurological symptoms (18%) were as frequent as arthritis (16%). Onset of arthritis occurred more often between October and May (55%) compared to other presentations (10%, p<0.001). The follow-up interview was completed for 43 cases (68%) a mean of 3 years after the onset of the disease. Symptoms disappeared within 6 months after treatment in 38 cases (88%) and lasted between 6 and 12 months in 3 cases (7%). Two cases that initially presented with arthritis were still symptomatic respectively 2 and 4 years after treatment.

Conclusion: Lyme disease is emerging in southern parts of Canada. Younger children are at higher risk and patients can seek advice any month of the year. It is important to include LD in the differential diagnosis of cutaneous, neurological or musculoskeletal symptoms in children exposed to at-risk areas. Early diagnosis and treatment could decrease the occurrence of sequelae.
Purpose: Surat city observed a rising trend in dengue from 2011 to 2016. Our objectives were to estimate the incidence of dengue in terms of time, place and person, analyze the entomological indices pertaining to *Aedes aegypti* breeding and climatic factors for dengue transmission in the city.

Methods & Materials: We analyzed the surveillance data of vector-borne disease control (VBDC) department from 2011 to 2016. We calculated age, sex and location specific cumulative incidence of dengue. The data was plotted to understand the seasonal distribution of cases. Median House index (HI), Breteau index (BI) and container index (CI) were calculated. Temperature, humidity, rainfall and number of patients were correlated with vector density using spearman correlation coefficient ($r_s$).

Results: Overall 2446 (11%) of 22101 suspected cases were confirmed positive for dengue as per IgM or NS1 ELISA tests. Incidence increased from 1.5 (2011) to 17.5 (2016) per 100,000 population. Incidence was higher among males. Median age of dengue cases was 20 years (Inter quartile range: 14-28). Five (16%) of 32 VBDC units had incidence above the city average since 2012. Incidence was highest post monsoon. The median HI and BI was <1% and the median CI was <0.4% throughout the study period. Vector density was significantly correlated with humidity ($r_s$=0.556), rainfall ($r_s$= 0.644) and number of cases ($r_s$=0.708). Peak vector density followed peak number of rainy days.

Conclusion: Higher incidence in young males and low house index indicates the probable transmission away from the house. The reasons for high incidence in 5 VBDC units require further micro level evaluation to find out the risk factors. Climate is conducive for vector breeding.
Chikungunya Outbreak In Village Goth-Haji-Bachoo-Khan, District Lasbela, Balochistan, Pakistan March 2017.

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**Purpose:** On 24th March 2017, District Health Officer Lasbela reported Provincial Disease Surveillance & Response Unit Quetta about suspected 18 chikungunya cases from village Goth-Haji-Bachoo-Khan and requested for an epidemiological investigation. A FELTP fellow was assigned and was sent to confirm, investigate the outbreak and suggest control measures on 26th March 2017.

**Methods & Materials:** A case was defined as onset of fever with joints pain during the month of March 2017 in a resident of village Goth-Haji-Bachoo-Khan, District Lasbela. Active case finding was done from 26th to 31st March 2017. Public and private health facilities were visited to evaluate their records. Water & sanitation practices were observed in order to identify vector habitat. Blood samples collected and sent to NIH Islamabad.

**Results:** Total of 52 cases were identified (overall AR= 3.36%). Four out of four blood samples (100%) were Chikungunya positive. Females were more affected 56% (n=29). Mean age was 36 years (range=1 to 80 years). Most affected age group was 0 to 20 years (46%, n=24, attack rate = 1%) followed by 21 to 40 years (31%, n=16, AR= 0.81%), 41 to 60 years (17%, n=9, AR= 0.84%) and 61 to 80 years (6% n= 3, AR= 0.77%). Investigation revealed that most of healthcare providers and community was unaware about chikungunya and its prevention and control. Water storage & sanitation practices were not appropriate. During household survey vector larvae was identified.

**Conclusion:** Vector Presence, poor sanitation practices along with warmer temperature in area are most probable causes for this outbreak. Vector control and awareness sessions in community regarding sanitation were recommended. Two Awareness sessions also conducted in community through involvement of department of health and district administration. Chikungunya Surveillance system was established.
Novel screening ELISA for sensitive detection of Mayaro virus infected patients

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**Purpose:** Mayaro virus (MAYV) is an emerging alphavirus circulating in Central/South America. It is transmitted to humans by mosquito bites, causing a febrile illness often with prolonged joint inflammation which resembles other infections with co-circulating arboviruses e.g. Dengue (DENV), Chikungunya (CHIKV) or Oropouche virus (OROV). Serological differentiation from alphavirus infections is complicated by antibodies targeting homologous antigens from related viruses (Semliki Forest virus complex), primarily CHIKV. Still, antibody detection can expand the time for diagnostics of acute MAYV infections beyond the short viraemic period suitable for MAYV RNA detection. Here, serum samples of patients with MAYV or other arboviral infections have been analysed with a novel Anti-Mayaro-virus-ELISA for detection of specific IgM and IgG at the Instituto Evandro Chagas.

**Methods & Materials:** Serum samples originate from Brazilian patients with clinically and serologically characterized febrile infections drawn between day 11 and 117 post symptom onset. Pre-characterization included analyses for IgM and haemagglutination inhibition (IH) antibodies against MAYV, CHIKV, DENV, Yellow fever virus (YFV), Zika virus (ZIKV), OROV, Eastern and Western Equine Encephalomyelitis virus (EEEV and WEEV) and flaviviruses in general using in-house MAC ELISA and indirect haemagglutination inhibition assay (IHA).

The first collective encompassed 46 samples, including 25 negative and 21 positive for anti-MAYV IgM and IH antibodies. The second collective (n=12) consisted of 6 anti-MAYV IH antibody positive and 6 anti-MAYV IH antibody negative samples.

Samples were investigated with the Anti-Mayaro-virus-ELISA (Euroimmun, Germany) IgM (collective 1) and IgG (collective 2).

**Results:** The Anti-Mayaro-virus-ELISA IgM was 100% sensitive (21/21) and 76% specific (19/25). 6 samples with reactivity in the ELISA had been pre-characterized for CHIKV (n=5/11) or general flavivirus (n=1/3) infection.

Testing collective 2, the Anti-Mayaro-virus-ELISA IgG revealed a sensitivity of 100% (6/6) at 50% specificity (3/6). 3 samples with anti-CHIKV IH antibody positive but anti-MAYV IH antibody negative pre-characterization were also reactive in the ELISA.

**Conclusion:** The novel Anti-Mayaro-virus-ELISA IgM and IgG showed high sensitivity at moderate specificity. This specificity meets the expectations and, in the majority of cases, can be explained by the cross-reactivity with antibodies against related viruses, primarily CHIKV. Thus the ELISA are suitable as screening assays reliably detecting MAYV infected patients.
Purpose: Chikungunya caused by a mosquito-borne virus, chikungunya virus (CHIKV), is an acute febrile illness. This disease nowadays becomes an important health problem since its wide spread and many re-emerged outbreaks in over 60 countries. Among the epidemics area, Thailand has been reported for many previous outbreaks and sporadic cases during the last decade. Since the report of an outbreak of CHIKV infection in 1990s, the study on this neglected disease in Khon Kaen and the northeastern region of Thailand is still limited. Therefore, our study aims to investigate the prevalence of CHIKV infection in human population as well as in circulating Aedes mosquito vectors by both molecular and serological diagnosis.

Methods & Materials: A SYBR green-based real-time PCR targeting non-structural protein 1, which is the most conversed region in this viral genome, was used for the detection of CHIKV in 161 plasma samples obtained from acute febrile illness patients on dengue surveillance from June, 2016 till September, 2017 from 8 hospitals in 4 of 20 provinces in Northeastern Thailand – Khon Kaen, Roi Et, Kalasin and Mahasalakham and in 187 pools of the Aedes mosquitoes collected at the patients’ house and 100-meter surroundings. The positive viral RNA was confirmed by sequencing. Anti-CHIKV IgM and IgG antibodies in the plasma samples were determined using commercial enzyme-linked immunosorbent assays.

Results: CHIKV-RNA was found in 8 of 161 (4.9%) plasma samples. Meanwhile, the plasma samples were assessed for a seroprevalence, anti-CHIKV IgM and IgG-specific antibodies were detected in 6/161 (3.7%) and 17/161 (10.6%), respectively. The detection of CHIKV-RNA in 187 mosquito pools was performed, 6 pools (3.2%) were positive with CHIKV non-structural protein 1 gene.

Conclusion: Acute febrile illness in Northeastern Thailand may be caused by CHIKV transmitted by Aedes mosquitoes besides other arboviruses. Characterization of an existing genetic diversity should be performed for further surveillance.
Correlation Between Dengue Virus Serotypes in Dengue Patients and in Mosquitoes at Patients’ Houses and Surrounding in Northeastern Thailand

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**Purpose:** Dengue virus (DENV) is the most important causative agent of arthropod-borne viral disease in the tropical and subtropical regions of the world. DENV is transmitted between human to human via mosquitoes, Aedes aegypti and Aedes albopictus. By the late 2000s, the dengue was widespread among regions in Thailand and dengue hemorrhagic fever (DHF) had become a leading cause of hospitalization and death. Meanwhile dengue surveillance by mosquitoes control has been performed immediately after case report. To understand the role of dengue surveillance in Northeastern Thailand, DENV serotypes were investigated in mosquitoes at patients’ houses and surrounding area during 2016-2018.

**Methods & Materials:** One hundred and eighty-eight mosquito pooled samples were collected from 97 dengue fever-suspected patients’ houses in 4 provinces in Northeastern Thailand – Khon Kaen, Roi Et, Kalasin and Mahasalakham province. DENV and serotyping were investigated in all pooled mosquito samples by two step reverse transcription quantitative polymerase chain reaction (RT-qPCR) using 5 sets of primer. The plasma samples of dengue fever-suspected patients from 8 hospitals were determined for DENV serotype by RT-PCR

**Results:** DENV-RNA was found in 28.87% (28/97) of dengue-suspected patients and consisted of DENV-1 (15%), DENV-2 (7%), DENV-3 (17%), and DENV-4 (61%). Meanwhile, DENV-RNA was found in 34.04% (64/188) of mosquito pools and consisted of DENV-1 (43%), DENV-4 (33%), DENV-2 (16%) and DENV-3 (8%). Interestingly, DENV detection in mosquito pools was positive in both of dengue patients’ houses (71%) and DENV negative patients’ houses (93%). The matching of DENV serotype in dengue patients and mosquito pools collected from their houses and surrounding area were 57% whereas unmatching DENV serotype was found in 43% of dengue patients and mosquito pools. Most of these patients were students (70%) and workers (29%) who spend day time outside their houses.

**Conclusion:** The circulation of DENV was found in mosquitoes at patients’ houses and surrounding area and also from DENV negative patients’ houses. From the correlation of matching DENV serotype result might suggest that the source of DENV transmission is not only from mosquitoes in patient’s house but also from others regions.
Purpose: To map and evaluate the *Onchocerca volvulus* transmission using the epidemiologic units from basins of Marahoue and Bia rivers; To detect a suitable therapeutic strategy in this peculiar area.

Methods & Materials: A transversal prospective study of 2887 subjects from a total of 3537 residents settled in 22 GPS-recorded villages from the basins of Marahoue and Bia rivers has been conducted. The skin biopsy (snip) with a microscopic examination (early at 30 minutes and 24 hours later) has been performed. The detected microfilaria were collected and sent to a DNA laboratory elsewhere. An eyesight examination of all subjects over 5 years-old has carried out. The positive cases received Ivermectine (IVM) treatment and were referred for posttreatment monitoring to the local health centers.

Results: There were examined 2887 subjects (1778 from Marahoue river basin and 1109 from Bia one) of 3537 inhabitants from 22 settlements, with 81.6 % general participation rate. A number of 192 microfilaria-positive cases have been detected, mostly male (130 or 67.7%), adults from 30-40 year-old group (92 or 47.9%), but 40 cases (20.8%) children under 5 year-old. The highest general prevalence (35.4%) and 0.96 microfilaria load community (CMFL) have been noticed in Dioulabougou, Ketesso-Aboisso district, Bia river basin. Two blind subjects and 20 cases with decreased eye acuity have been identified, meaning a 0.07% cumulative prevalence of river cecity and 0.69% reduced eyesight prevalence, respectively.

Conclusion: The zonal epidemiologic profile asserts the low *Onchocerca volvulus* parasite transmission, implying a sustained surveillance and the community-directed treatment with IVM (CDTI) coverage. A prior activity to inform the targeted population assures a good and active participation for the onchocerciasis field-evaluations.
Differential Infection of Dengue Virus Serotypes in Primary Endothelial Cells and Effect on Barrier Integrity

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Purpose: Severe dengue mostly manifests with hemodynamic disturbances including vascular leakage and thrombocytopenia. The detailed mechanism for vascular permeability in dengue remains to be elucidated. Although the inflammatory response is implicated in disruption of endothelial barrier functions in dengue, the direct effect of virus infection of endothelial cells has not been explored thoroughly. In this study, we tested whether direct viral infection of endothelial cells (ECs) in vitro, has any effect on endothelial barrier functions and if the effect is serotype-dependent.

Methods & Materials: Primary endothelial cells (ECs) were infected with all the four serotypes of dengue virus (DENV 1-4). Viral titres in culture supernatants were measured by plaque assays, viral entry and replication was measured by qRT-PCR, viral protein translation was assessed by western blot analysis, endothelial barrier integrity in infected cells was measured by measuring electrical resistance across the monolayer, inflammatory mediators in the supernatant was measured by Luminex assays or ELISA.

Results: We report here that the infection efficiency, as measured by viral titers in the culture supernatants, in both primary endothelial cells and polarized epithelial cells is serotype-dependent whereas the same effect was absent in human hepatoma cells (Huh-7). This differential effect was due to differences in the kinetics of viral RNA replication or stages beyond this as there was no significant difference in the amount of viral particles entering the cell. DENV was capable of egress from both apical and basolateral surfaces without disrupting the barrier integrity of either epithelial or endothelial cells. We are further investigating the role of inflammatory mediators in endothelial and epithelial barrier functions in dengue infection.

Conclusion: Our data indicate that DENV serotypes infect endothelial and epithelial cells differentially and without disrupting the permeability barrier function of these cells suggesting that direct infection of these cell types by the virus may not play a role in pathogenesis observed in severe dengue infections.