

# Prevalence and risk factors for seropositivity to *C. burnetii* infection in dairy farms and farmers in Chiang-Mai, Thailand 2015



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# Q fever in Thailand

- Zoonotic disease, caused by gram negative intracellular bacteria, *Coxiella burnetii*
- First reported in Thailand in 1968
- Lack of information on Q fever
- A one year prospective study of Q fever among dairy cattle farms and farmers (Jun 2015 – Aug 2016) to determine the incidence and risk factors of *C. burnetii* infection
  - Sub aims: to describe prevalence and factors associated with *C. burnetii* infection in dairy cattle farms at baseline using BTM as a proxy for infection



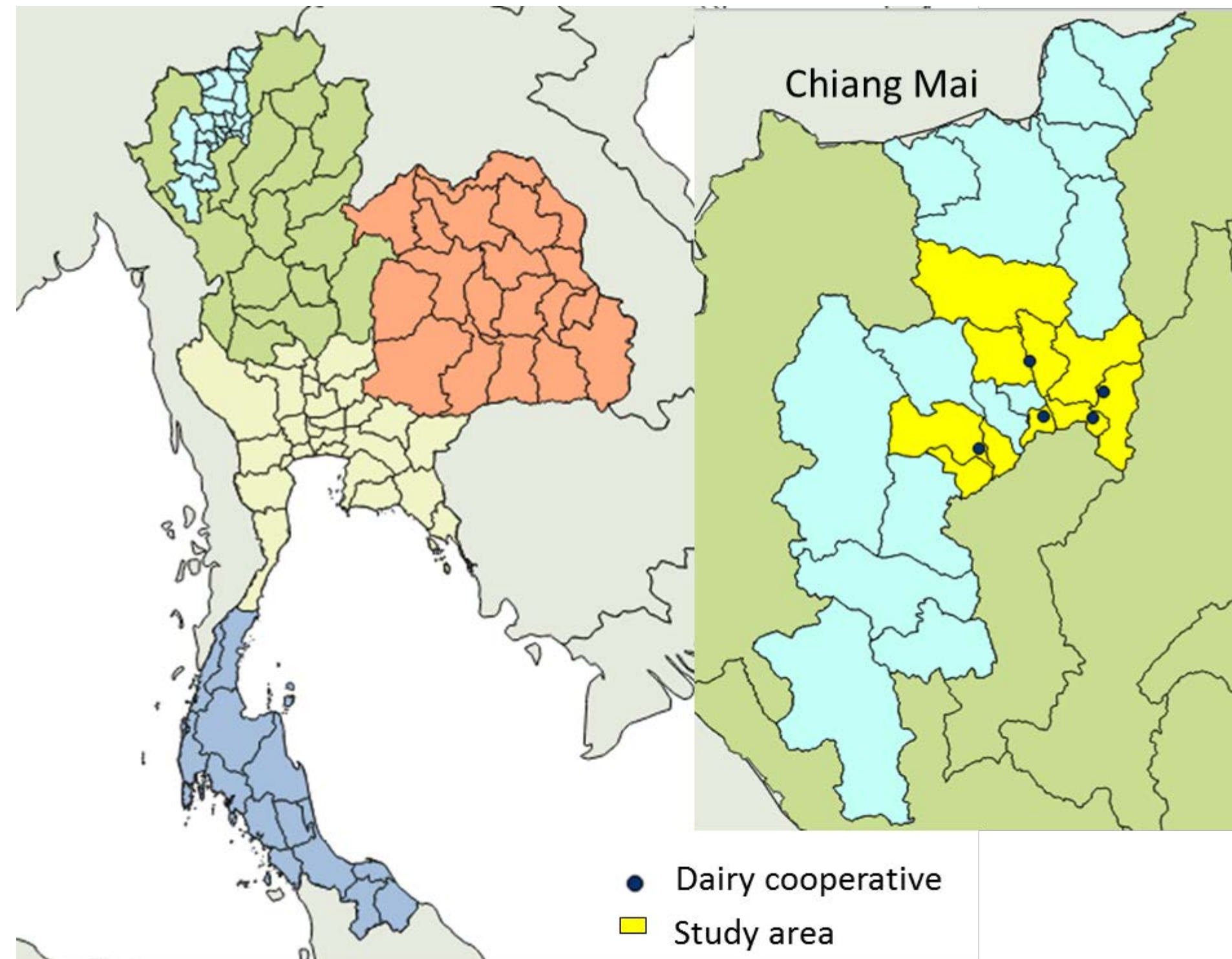
# Methods: study area and study population

## Chiang Mai (9 coops total)

- 5 studied coops:
  - 3 coops with previous evidence of *C. burnetii* seropositivity in the farms in 2012
  - 2 coops in the adjacent areas

## Study populations:

- All farms
- All cows in the participating farms
- Thai dairy farmers age  $\geq 18$  years who work in the participating farms
- Two stage random sampling – farms and farmers



# Study protocol

- Study among farmers
  - Face to face interview and collected blood sample
  - Indirect Immunofluorescence Assay (IFA) at the Thai National Institute of Health
- Screening of Bulk Tank Milk (BTM)
  - Collected BTM to test for antibodies to *C.burnetii* using ELISA
- Field investigation in the farms with positive BTM
  - Collected blood and vaginal samples from 8 cows/farm, samples from other animals, samples from environment e.g., soil
  - Cow's sera – tested for antibodies to *C.burnetii* using ELISA
  - Vaginal swab, samples from other animals, environment – tested using real time PCR



# Results

- Farms:
  - 282/306 (92%) randomly selected farms participated
  - 115/282 farms (41%) had positive result
  - 99/115 positive farms (87%) were visited
- Farmers:
  - 532/637 (84%) randomly selected farmers participated
  - 90/532 farmers (17%) had positive antibodies to *C. burnetii* (IFA cut-off value  $\geq 1:64$ )



# Multivariate analysis of factors associated with positive BTM

Covariates	OR	95% CI		chi2 p-value	type3 p-value
Dairy cooperative					0.0301
- coop5				Ref	
- coop1	2.40	0.85	6.78	0.10	
- coop2	1.33	0.48	3.71	0.59	
- coop3	1.24	0.41	3.71	0.70	
- coop4	0.52	0.15	1.77	0.29	
farm size					0.0085
- farm with 1 - 20 cows				Ref	
- farms with 21 - 40 cows	1.53	0.61	3.80	0.36	
- farms with > 40 cows	3.24	1.34	7.83	0.01	
Stall base					0.043
- concrete and soil				Ref	
- concrete	2.09	0.96	4.57	0.06	
- Concrete and rubber mat	3.93	1.32	11.65	0.01	
Distance from the positive farm					0.0162
- > 2 km				Ref	
- ≤ 1 km	2.88	1.17	7.06	0.02	
- > 1 to ≤ 2 km	1.23	0.39	3.86	0.73	
Proportion of cow age ≥ 2 year in the farm					
- ≥ 80% vs < 80%	2.34	1.09	5.06	0.03	0.0302
Ever cleaned cow birthing areas	0.23	0.08	0.86	0.03	0.0263
Ever quarantined newly purchased animals	0.54	0.30	0.97	0.04	0.0406

# Laboratory results from the farm investigation

Test results	Individual (N = 790)	Test results	Herd level (N = 99)
ELISA test (sera)		ELISA test (sera)	
<ul style="list-style-type: none"> <li>- <b>Positive</b></li> <li>- Suspect</li> <li>- Negative</li> </ul>	<p><b>224 (28.35%)</b></p> <p>32 (4.05%)</p> <p>534 (67.59%)</p>	<ul style="list-style-type: none"> <li>- <b>At least 1 cow positive</b></li> <li>- &gt;50% of the herd positive</li> <li>- Positive in all cows</li> <li>- Negative</li> </ul>	<p><b>91 (91.92%)</b></p> <p>5 (5.05%)</p> <p>1 (1.01%)</p> <p>8 (8.08%)</p>
PCR (vaginal swab)		PCR (vaginal swab)	
<ul style="list-style-type: none"> <li>- <b>Positive</b></li> <li>- Negative</li> </ul>	<p><b>146 (18.48%)</b></p> <p>644 (81.52%)</p>	<ul style="list-style-type: none"> <li>- <b>At least 1 cow positive</b></li> <li>- &gt;50% of the herd positive</li> <li>- Positive in all cows</li> <li>- Negative</li> </ul>	<p><b>39 (39.39%)</b></p> <p>13 (13.13%)</p> <p>7 (7.07%)</p> <p>60 (60.61%)</p>





# Environmental investigation

Samples	Total	Positive	Negative
Swab at the birthing areas	99	12 (12.12%)	87 (87.88%)
Soil at the end of sewage area	99	7 (7.07%)	92 (92.93%)
Ticks	52	1 (1.92%)	51 (98.08%)
Food samples	52	0	52
Blood from other animals (25 dogs, 4 chicken, and 2 cats)	31	0	31
Cow placenta	1	0	1



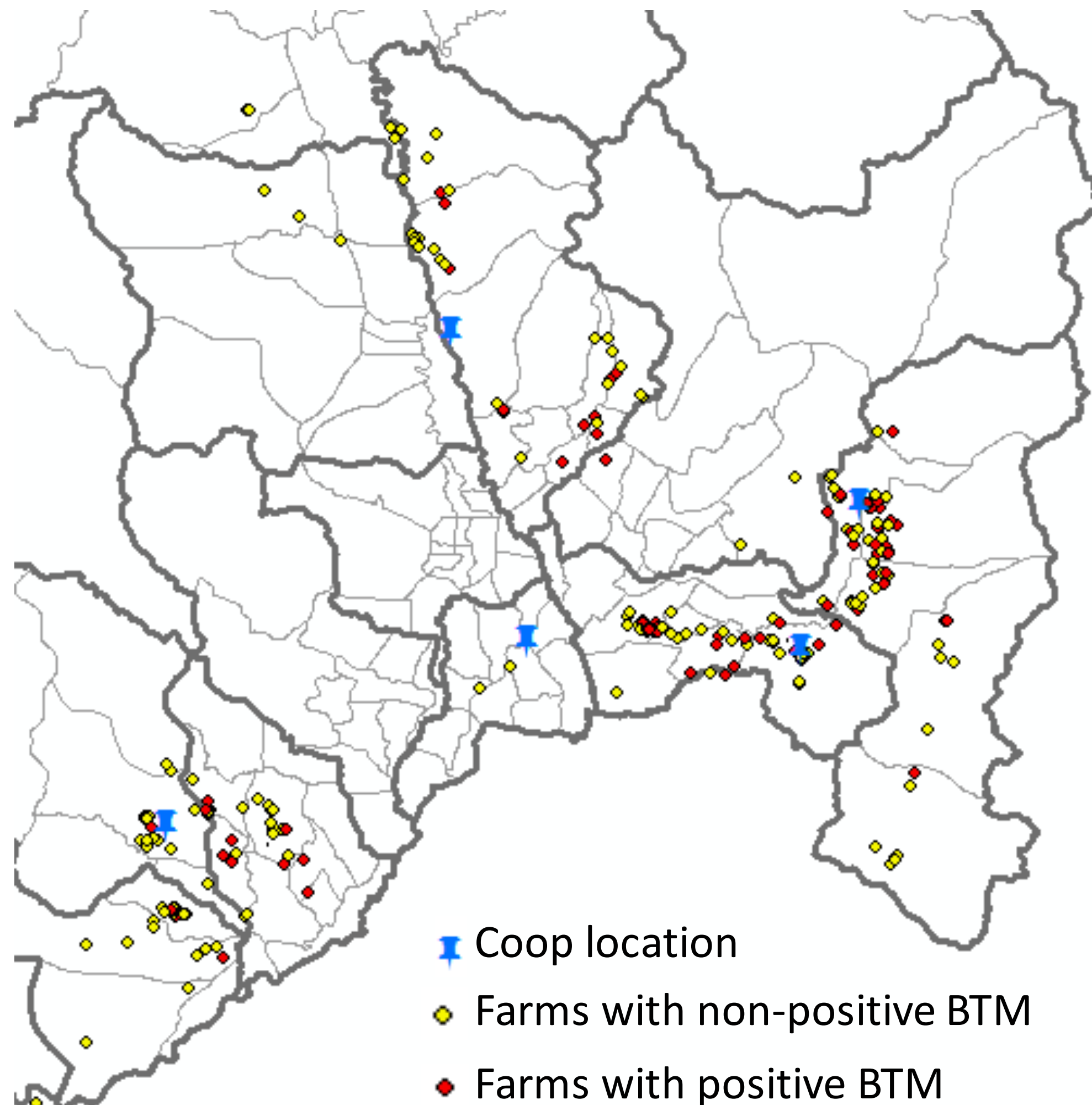
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# Distribution of farms by bulk tank milk (BTM) results

June 2015



Morans I statistic  
(z-score was 2.35  
and p-value 0.02)

# Discussion and conclusions

- Prevalence of *C. burnetii* infection among farmers was similar to the survey in Chiang Mai in 2012 (16% in farmers)
- The importance of biosecurity should be emphasized – cleaning especially after calving
- BTM screening could be a good indicator of *C. burnetii* infected herd – prevention and control could be implemented
- Public health significance:
  - Increase awareness of Q fever among farmers, veterinarians and health care professionals
  - Provide policy makers regarding Q fever prevention and controls



# Acknowledgements

- Five dairy cooperatives and farmers
- Drs. Soawapak Hinjoy, Teerasak Chuxnam, Ms.Punnarai Smithsuwan, IEIP-Q team
- Chiang Mai One Health Team (Animal health, and public health officers)
- U.S. CDC and Thai-US.CDC Collaboration
- Maharaj Chiang Mai Hospital, Thai – NIH, Thai-NIAH, Veterinary Research Laboratory – Lampang, Bureau of Epidemiology
- Department of Epidemiology and Biomedical consulting laboratory – UNC-CH



Thank you for your attention



# Preliminary analysis: Multivariate analysis of factors associated with seropositivity among farmers, Chiang Mai, Thailand 2015

## GEE with outcome 1:64 (Outcome = 90, N = 532)

Variable	OR	95%LCI	95%UCI	p-value	Type3 p-value
Cooperative					0.0028
- Coop1 vs Coop5	2.3724	0.5187	10.8501	0.2654	
- Coop2 vs Coop5	4.6556	1.0721	20.2165	0.0401	
- Coop3 vs Coop5	7.1733	1.7265	29.8038	0.0067	
- Coop4 vs Coop5	3.2345	0.7145	14.6421	0.1276	
Farm size					0.7463
- 1 - 20 cows				ref	
- 21 - 40 cos	0.8	0.3044	2.1022	0.6507	
- >40 cows	0.7105	0.3007	1.6788	0.436	
Age (10 year)	1.2509	1.0139	1.5433	0.0368	0.0339
Ever been in contact with birth products during calving	1.9404	0.9769	3.8539	0.0583	0.0371
Ever used glove in the farm	0.6194	0.3655	1.0497	0.0751	0.0708
Working in farm with positive BTM	2.7918	1.5084	5.1672	0.0011	0.0013

## Study among farmers

## Dairy farms

## Screening of Bulk Tank Milk

Farmers

Cows

Bulk Tank Milk (BTM)

Interview & collect samples 3 times every 6 months\*  
- Questionnaire – farm practice, and individual farmers

Screening using ELISA every 6 months

ELISA (P)\*

ELISA (S)\*\*

ELISA (N)\*\*\*

1<sup>st</sup>

IFA phase I  
persistently >1:800?

Yes

No

Refer to specialist

ELISA (P/P,P/S,S/P)

ELISA (PN)

ELISA SS, SN

2<sup>nd</sup>

Retested with ELISA

## Field investigation

Cows – 8 cows/farm  
- Vaginal swab for PCR  
- Blood for ELISA

-Other animals  
-Environment samples e.g., soil

PCR

Active case finding

\* P: Positive  
\*\* S: Suspect  
\*\*\* N: Negative

Prospective study among farmers F/U every 6 months

Milk screening every 6 months and placenta screening

Field investigation in the farms with milk or placenta positive



# Methods: laboratory study

- Farms:

- Milk and Cow sera: commercial ELISA kit (IDEXX CHECKIT™ Q-fever Test Kit)
- Vaginal swab samples, ticks, other animals whole blood, and environmental samples: Real time PCR, target gene - IS1111 at the Veterinary Research Center (following the US.CDC protocol)

- Farmers:

- Indirect Immunofluorescence Assay (IFA) – antibody to *C.burnetii* phase I and phase II Antigen at the Thai – NIH, following the US.CDC protocol

# Discussion

- Number of cows especially older cows, proximity to the BTM positive farms were positively associated with the odds of positive BTM
- Cleaning right after birth and quarantining newly purchased animals were the protective factors
- Role of stall base needs further study
  - Rubber mats could be a sustainable source of bacteria if cleaning is not properly performed
- Newly purchased animal – less exposure to *C.burnetii*

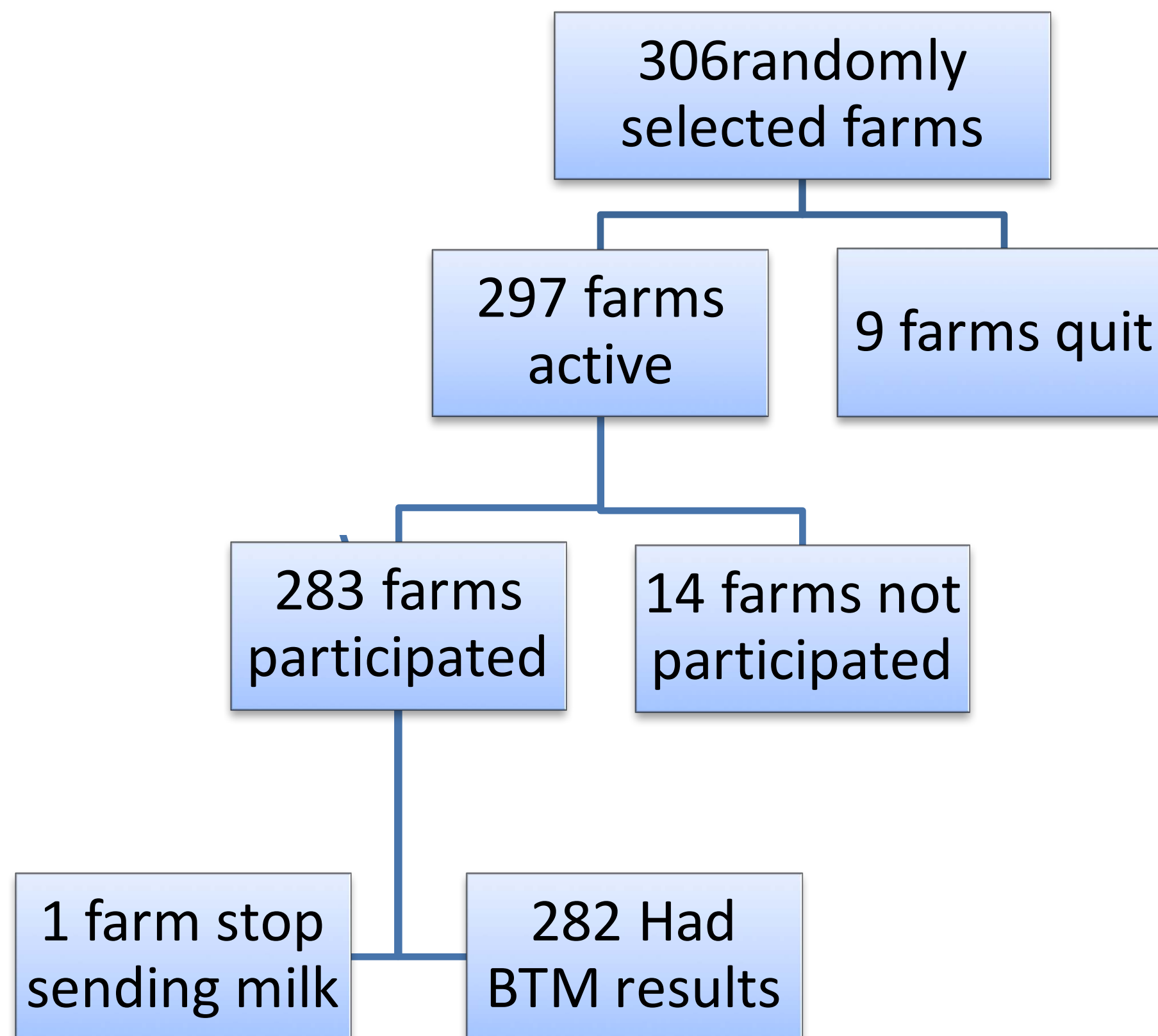


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# Aim1: Farm study

## Farms enrollment



## Farm characteristics

- 282/306 (92%) randomly selected farms participated
- Farm size: Participation:N (%)
  - 1 – 20 cows: 40 (69.0%)
  - 21 – 40 cows: 96 (98.0%)
  - > 40 cows (40-125): 146 (97.3 %)
- Coops: farm(%response)
  - Coop1 78 (88.6%)
  - Coop2 73 (93.6%)
  - Coop3 52 (100.0%)
  - Coop4 40 (95.2%)
  - Coop5 39 (92.7%)



# Exposure and outcome assessment at the farm level

- Data collection: face to face interview
- Exposure:
  - Farm level (farm characteristics, farm management)
  - Farmer level (demographic information, job description)
- Outcome:

Outcomes - BTM	Definitions
<u>Negative</u>	Negative ELISA
<u>Positive</u>	First positive, and second positive or suspect, or First suspect and second positive
<u>Suspect</u>	First suspect, and second negative or suspect, or First positive, and second negative
<u>Non-positive</u>	combination of negative and suspect BTM

# Aim 1: BTM screening

- ELISA Results of all 282 randomly selected farms:
  - 115 farms (40.8%) had positive results
  - 134 farms (47.5%) had negative results
  - 33 farms (11.7%) had suspect results

Characteristics	N	Prevalence	95%LCI	95%UCI
<b>Overall</b>	282	38.72%	32.66%	44.79%
Dairy cooperatives				
- Coop1	78	55.16%	42.99%	67.34%
- Coop2	73	37.36%	25.53%	49.18%
- Coop3	52	36.51%	21.94%	51.08%
- Coop4	40	25.69%	7.91%	43.47%
- Coop5	39	22.48%	7.64%	37.32%
Farm size				
- 1 - 20 cows	40	31.62%	16.28%	46.96%
- 21 - 40 cows	95	36.67%	26.53%	46.82%
> 40 cows	147	47.18%	38.96%	55.40%