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# Livestock Prevalence Of Brucellosis And Q Fever In Thailand

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## **Livestock Prevalence of Brucellosis and Q fever in Thailand**

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## **ABSTRACT**

### **Background**

Brucellosis and Q fever may impart high morbidity in humans and economic losses among livestock. Yet, a systematic investigation has not been performed in Thailand, where a significant proportion of the rural population may be vulnerable to these zoonotic diseases.

### **Objectives**

We surveyed the seroprevalence of brucellosis and Q fever in livestock from Thai communities at the border with Cambodia, evaluated risk factors for seropositivity, and performed a risk assessment for potential transmission to farmers.

### **Methods**

We selected herds of beef and dairy cattle and small ruminants (sheep and goats) for Sa Kaeo province in 2015 using a two-stage random sampling design. Rose Bengal, ELISA and complement fixation assays were performed to evaluate brucellosis seroprevalence, while ELISA was performed to evaluate Q fever seroprevalence. We interviewed farmers to evaluate potential risk factors for transmission among herds and to the community.

### **Results**

We surveyed a total of 520 individuals from 143 farms (15 small ruminant flocks, 117 beef cattle herds and 11 dairy cattle herds). Brucellosis seroprevalence in beef cattle and small ruminants was respectively 2.6% (0.7-7.9) and 13.3% (2.3-41.6). Q fever seroprevalence in beef cattle, dairy cattle and small ruminants was respectively 4.3% (1.6-10.2), 27.3% (7.3-60.7) and 33.33% (13.0-61.3). We found no significant association between known risk factors for herd-transmission and seropositivity of the farms. Lack of disinfectant use (64.3%-90.9%) and consumption of placenta by farmers (40%-80.8%) were frequent among farms.

## **Discussion**

This study identified a significant burden associated with brucellosis and Q fever among livestock and a potential risk for spillover transmission to farmers via consumption of placenta and lack of disinfectant use. Efforts should therefore be made to implement routine surveillance and prevention of brucellosis and Q fever in livestock and evaluate the potential burden of these zoonotic diseases among subsistence farming populations in the region.

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## INTRODUCTION

The Food and Agriculture Organization reported a dairy farms' production growth rate of 15.40 from 1983 to 2001 [1]. Livestock production has been rapidly growing in Thailand, generating increased risks to animal and human health [2]. Under the "One Health" concept, collaboration between public health officers, medical doctors and veterinarians is paramount to set up interacting surveillance systems for animal and human health. Brucellosis and Q fever are major veterinary public health zoonoses caused by *Brucella spp.* and *Coxiella burnetii*, respectively. Sheep, goats and cattle are the main reservoirs. In herds and flocks both diseases can lead to series of abortion and infertility issues and result in decrease in both milk and offspring production. In animals, both vertical and horizontal transmission can take place and the main entry point are mucous membranes [3-7]. Organisms are excreted in milk, urine and feces. Amniotic fluids and placenta of infected animals are also major sources of bacteria spread ( $10^9$  *Coxiella*/g placenta and  $10^{13}$  *Brucella* CFU/g of cotyledons' tissue) [8-10]. Transmission to humans is related to consumption of raw meat or dairy products, inhalation of contaminated materials or direct contact through breaks in the skin [5,7-9]. These bacteria are extremely resistant in the environment (up to 240 days for *Brucella spp.* and 300 days for *Coxiella burnetii* [9,11]). There is potential contact and transmission between the domestic and wildlife species through sharing of pasture, seasonal movements of herds and trade [12-13]

Because of their low apparent impact, brucellosis and Q fever have not been a public health priority in Thailand. However ninety percent of Thai farms are still subsistence ones [14], with poor biosecurity measures and high risk of occupational exposure to zoonotic diseases. In these



communities, both zoonoses have the potential to impart high morbidity in humans and economic losses among livestock.

Human brucellosis was first reported in Thailand in 1963 [12,15]. It was reported for the second time in 2003 [16] and has since been considered a re-emerging zoonosis in Thailand. According to human passive surveillance data, from 2004 to 2013, there were 153 reported cases to the World Organization for Animal Health (OIE) . The majority of the cases were due to *B.melitensis* and *B.abortus* (respectively 88.2% and 6.5%)[17]. Q fever was first reported in Thailand in 1966 [18]. According to prevalence surveys, Q fever represented 1.3% of the hospitalizations for fever in Northern Thailand in 2003 and accounted for 1.0% of unexplained acute febrile illness in 2001-2002. The prevalence of asymptomatic persons is estimated around 0.4-2.6% [19].

Since the creation of the ASEAN (Association of South-East Asia Nations), there has been an increasing trade between Thailand and Cambodia [20], not only in terms of livestock but also of workforce. In addition, both countries have agreed to increase bilateral trade by 3-fold by 2020 [21]. Sa Kaeo province, Thailand's point of entry to and from Cambodia, will thus require a strengthened and wider surveillance system. In 2010, the Department of Livestock Development (DLD) and the National Institute of Animal Health (NIAH) started a national 'brucellosis free' campaign for dairy cows. This program consists in annual testing of dairy farms and culling of infected animals and has been a success in Sa Kaeo province so far (no positive dairy herds in Sa Kaeo province over the past 3 years – unpublished data). This program is not widely applied to beef cattle and small ruminants and the seroprevalence of Brucellosis is thus unknown in these

production types. No systematic data has ever been collected for Q fever in beef and dairy cattle and small ruminants in Sa Kaeo province. In addition, no systematic study of the association between brucellosis and Q fever seroprevalence and potential risk factors has previously been conducted in Thailand.

The general aim of the study was to determine the seroprevalence of brucellosis and Q fever at the herd and flock level among cattle and small ruminant farms from a rural-based farming community at the Thai-Cambodian border. Our specific objectives were to (i) determine the seroprevalence with its 95% confidence interval at the herd and flock level (ii) identify risk factors associated with *Brucella spp.* and *Coxiella burnetii* seropositivity at the herd/flock level, (iii) evaluate the presence of occupational health exposures that might represent a risk for spillover infections to humans. Successful completion of these aims would provide the DLD and the Ministry of Public Health (MoPH) tools to stratify the risks factors for herd or flock positivity and target interventions designed to reduce the disease burden in these populations.

## **METHODS**

### **Study design and study population**

A cross-sectional study was conducted in Khlong Hat and Aranyaprathet districts, the two largest districts bordering Cambodia in Sa Kaeo province, Thailand in June 2015. The study unit was the herd or flock, defined as animals from one unique production group (beef cattle, dairy cattle, sheep or goats) owned by the same household and kept in the same location. The target populations for brucellosis were beef cattle herds and small ruminant flocks. The target populations for Q fever were beef cattle and dairy cattle herds and small ruminant flocks in the

two districts mentioned above. Herds and flocks were homogeneously distributed across the two districts. The study was granted IACUC ethical approval.

### **Sampling strategy and sample size**

Data collection was based on a 2-stage random sampling. A list of herds and flocks present in each district was provided by the DLD. The total number of cattle herds and small ruminant flocks to be sampled in order to generate herd/flock-level prevalence estimates was calculated with StatCalc (Epi-Info) based on Q fever expected prevalence, for a total number of beef herds/flocks of 1756, an estimated expected herd/flock prevalence of 10% for all species [17,22] and a confidence level of 95%. The number of herds/flocks to be sampled was distributed across the 2 districts in proportion to their weight in the total population. Since the expected prevalence was the same for all species within each district, the number of herds or flocks to sample was calculated using the proportion of each species in each district. After sample size calculation, the herds and flocks were randomly selected from this list. Herd/flock criteria for eligibility to participate in investigation were being within the district administrative borders and being owned by a Thai farmer. Herd/flock criteria for exclusion were not meeting the inclusion criteria, declining to participate in the study and being unable to answer more than 50% of the questionnaire.

The number of animals to be tested within a herd or flock in order to reach a certain confidence of detecting at least one positive animal was calculated with the following function (adapted from Musallam et al. [23]):

$$K(j) = \left[ 1 - (1 - p)^{\frac{1}{d_j}} \right] * \left[ j - \frac{d_j}{2} \right] + 1$$

where  $k$  is the number of animals to be sampled from each herd or flock;  $p$  is the probability of detecting at least one positive animal (set as 95%) ;  $d$  is the expected individual level prevalence of the disease (10% here) and  $j$  is the herd or flock size.

In each selected herd/flock, individuals, males and females, were randomly selected. Individual animal criterion for eligibility to participate in investigation was being older than 6 months of age. Individual animal criteria for exclusion were not meeting the inclusion criteria, being pregnant and impossibility of drawing blood.

### **Data collection**

For each animal sampled, the age, sex and body score of the animal were recorded. In parallel, a survey of the farm was conducted to collect epidemiological information on the farmer's socio-economic status, the herd/flock health history, the farm characteristics, the management practices, the workers' health and migrations and trade domestically and with Cambodia.

Interviews were conducted in Thai, by a public health officer from the MoPH. We interviewed the owner when possible otherwise a member of the owner's household was interviewed. In addition, Global Positioning System (GPS) coordinates were taken as close to the herd as possible with Google Maps App or a GPS tool depending on the availability of service.

### **Serological analyses**

Blood samples were obtained from the jugular or the coccygeal vein and identified with individual tag numbers. Sera were tested for *Brucella spp.* and *Coxiella burnetii* at the National Institute of Animal Health, Bangkok, Thailand following the OIE guidelines [3-4,7]. Brucellosis was tested by Rose Bengal Test (RBT) and indirect Enzyme Linked ImmunoSorbent Assay

(iELISA). For the RBT, any observed agglutination by naked eye was considered positive. For the iELISA, the cut-off Optic Density (OD) was set at 80%. A test sample with an OD equal to or above 80% was considered positive. Animals presenting negative results in both RBT and iELISA were considered non-infected. Animals presenting positive results in both tests were considered infected. When the tests were discordant, the sample was further tested with Complement Fixation Test (CFT). For the CFT, sera giving a titer equivalent to 20 ICFTU/ml or more were considered to be positive. If the CFT was negative, the animal was considered non-infected. If the CFT was positive, the animal was 'suspect' and was resampled for new testing. While waiting for confirmation of the 'suspect' cases, those will be considered as 'positive'. The sensitivity and specificity for the brucellosis parallel CFT/iELISA combined to RBT in series are 99.70% and 100% for cattle and 99.16% and 100% for small ruminants respectively [24-27]. Q fever testing consisted in iELISA. If the titer was lower than 40 then the test was considered negative and the animal was considered non-infected. If the titer was higher than 40, then the test was considered positive and the animal was considered infected. The sensitivity and specificity of the iELISA is 95% and 98% for all species [28-29]. A herd/flock was considered positive for brucellosis or Q fever if at least one animal in the herd/flock tested positive for brucellosis or Q fever.

### **Data management and statistical analyses**

The prevalence for brucellosis and Q fever was calculated at the herd/flock level, across all species and per species with their 95% confidence interval. It was calculated as the number of positive herds/flocks over the total number of herds/flocks visited. The 95% confidence interval was calculated using continuity correction.

To conduct the risk factors' analysis and the risk assessment for farmers, variables of interest were chosen based on biological plausibility and frequencies among the herds/flocks sampled. We calculated prevalence ratio and their 95% confidence interval for each selected variables when comparing seropositive herds/flocks and seronegative herds/flocks in order to test for associations between exposure and positivity of the herd. For variables representing practices known to be risk factors for disease spillover to humans, presence was attested by calculating proportions within our sample. The selected variables are listed in Tables 2 and 3. The analysis was performed in both Excel and R. The herds/flocks GPS coordinates were entered and mapped in ArcGIS 10 (ESRI 2010).

## **RESULTS**

### **Estimation of the herd/flock level seroprevalence**

A total of 143 herds/flocks, 117 beef herds, 11 dairy herds, 12 goat flocks and 3 sheep flocks, were visited and 520 samples were tested. For the analysis, sheep and goats were regrouped under the “small ruminants” denomination, to mirror the similarity in management practices. The location of the farms is shown in Figure 1. To assess the clustering of positive animals, we compared number of animals and farms being positive for each disease. We found that 3 beef animals, 3 beef herds, 5 small ruminants and 2 flocks were positive for brucellosis. 6 beef animals, 5 beef herds, 4 dairy animals, 3 dairy herds, 14 small ruminants and 5 flocks were positive for Q fever. Brucellosis herd-prevalence was 2.6% (95%CI 0.7-7.9) in beef cattle and 13.3% (95%CI 2.3-41.6) in small ruminants. Q fever herd-prevalence was 4.3% (95%CI 1.6-10.2) in beef cattle, 27.3% (95%CI 7.3-60.7) in dairy cattle and 33.3% (95%CI 13.0-61.3) in small ruminants. 2 flocks and 1 beef cattle herd were infected with both brucellosis and Q fever.

The prevalence calculations at the herd-level and their 95% confidence intervals are presented in Table 1.

### **Analyses of prevalence ratio**

Table 2 presents the univariate analyses' results for risk factor variables, for each production group and for each disease. Variables with the strongest apparent association for disease among beef cattle were throwing the placenta in the field (PR=3.9; 95%CI 0.4-43.0) for brucellosis, giving placenta to pets for both brucellosis and Q fever (PR=1.4; 95%CI 0.9-2.2 for both diseases). In dairy cows, a strong association between water source and Q fever seropositivity was found (PR=2.2; 95%CI 0.9-5.9 for ground water source and PR=0.1; 95%CI 0.0-1.8 for tap or pond water source). In small ruminants, the strongest association was for sharing of pasture (PR=3.6; 95%CI 0.3-39.9) for brucellosis, use of tap or pond water source for both diseases (PR=0.7; 95%CI 0.4-1.2 for brucellosis and PR=0.6; 95%CI 0.3-1.5 for Q fever), passing of wildlife (PR=1.5; 95%CI 0.8-2.8) and pets roaming on the farm (PR=1.6; 95%CI 0.7-3.7) for Q fever. No variable was significantly associated with seropositivity of the herd as shown by the 95% confidence intervals.

### **Risk assessment**

Table 3 presents farmers and households' practices that are typically considered occupational hazards for brucellosis and Q fever. Consumption of placenta by farmers was performed in 80.8% of the beef cattle farms, 40.0% of the dairy farms and 53.9% of the small ruminant farms. 63.0% of the beef cattle farms, 72.7% of the dairy farms and 93.3% of the small ruminant farms mentioned wearing protective equipment such as boots, masks, gloves but 50.9% of the beef

cattle farms, 90.9% of the dairy farms and 46.7% of the small ruminant farms admitted protective equipment was never worn. Disinfectant to clean stalls was used in 34.6% of beef farms, 9.1% of dairy farms and 35.7% of small ruminant farms.

## **DISCUSSION**

This study was the first systematic study conducted in Thailand to look at brucellosis and Q fever seroprevalence in different production type. As the Thai brucellosis-free campaign has proven to be successful in dairy cattle, this study aimed at investigating which diseases and production types to target next. Brucellosis prevalence was the highest among small ruminants flocks and the lowest among beef cattle herds. Brucellosis in sheep and goats could represent a public health threat in provinces of Thailand where flocks are a large part of livestock production, such as Southern Thailand. Beef cattle do not, in our study, represent a large occupational public health threat since few contacts happen between farm workers and cattle.

Q fever prevalence was the highest among flocks and dairy cattle herds and was also lowest among beef cattle herds. Q fever seems to be mostly of a concern in dairy farms for Sa Kaeo province, especially if we consider the close contact that farmers have with dairy cows, consumption of milk, as well as the importance of breeding and thus exposure to amniotic fluids and placenta. It would be useful to get more dairy farms tested to get a more precise confidence interval for the prevalence. Here again, beef cattle do not represent a significant public health threat.



Our study did not allow us to identify any significant risk factors for livestock transmission of brucellosis and Q fever either due to lack of power of our study or due to widespread contamination. Water source for livestock and placenta management could be risk factors that need to be further investigated. Studies on the transmission of brucellosis and Q fever via water consumption are scarce. If the OIE and the Center for Disease Control [3-4,6-7] report potential contamination via water ingestion for both animal and human populations, the link to water source is unclear. Tap water has been shown to be protective against ovine brucellosis and ground water and pond water seem to be associated with higher risk of contamination with Q fever for livestock and for exposed professions such as farmers or veterinarians [30-33]. Placenta management on the other hand is a well-known risk factor for both diseases for herd contamination and human infection [34-35]. Feeding of placenta to the pets or throwing placenta in the field and contamination of the pasture has been shown to be a risk factor for farm endemicity for brucellosis and Q fever.

We did identify potential risk factors for transmission to farmers. If 63.0% to 93.3% of the farms mention specific clothing or footwear, 46.7 to 90.9% of the interviewees report that those are never worn, which shows a gap between knowledge and practice. The lack of use of personal protective equipment has been mentioned before in livestock rearing in South-East Asia [36] but studies specific to Thailand have been focusing on poultry and swine farming so far [37]. In addition, with a large proportion of farmers (40%-80.8%) cooking and eating the placenta, brucellosis and Q fever have the potential to be public health threats for Sa Kaeo livestock farmers.

There are several limitations to our study. Non-piloting of the questionnaire, methods variations between interviewers and presence of the local veterinarians during the interviews might have introduced bias. In addition, since the sample size calculations were based on a Q fever expected prevalence of 10%, our results might not reflect the true prevalence in the population for brucellosis and Q fever in beef cattle. The absence of statistically significant results in our risk factor analysis shows a potential lack of power due to difficulty to meet the within-herd sample sizes and due to sample size calculations that did not include risk factor analysis (for logistics purposes). However the sample size was robust enough to calculate prevalence and their 95% confidence interval for dairy cows and small ruminants. Our results also add to the pre-existing studies by testing for Q fever with iELISA on sera, which is more sensitive and specific than the usual Polymerase Chain Reaction testing on placenta [38]. Most importantly the two districts sampled are highly agricultural area and, since herds and animals were randomly sampled, the results are likely generalizable to Sa Kaeo province.

The seroprevalence found for all production types and both diseases is of concern not only in terms of public health but also in terms of economic costs: non-brucellosis and non-Q fever free statuses imply testing of each animal before trade [39]. Achieving brucellosis and Q fever-free regional and national-wide statuses via systematic slaughtering as per OIE guidelines is thus recommended for Thailand. Dairy cattle and small ruminants should be prioritized in future control programs. In addition, larger studies are needed to be able to conclude which farms' characteristics are important for Sa Kaeo province and Thailand to control and raise awareness for, to avoid reinfection of the herds. Finally we recommend that the MoPH invest in human community surveillance to assess spillover to human populations and target at-risk populations.

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## TABLES

**Table 1:** Estimated herd/flock seroprevalence of brucellosis and Q fever and their 95% confidence intervals by production group.

Production group	Total herds	Brucellosis		Q fever	
		N <sup>o</sup> sampled (%)	Seroprevalence (95%CI)	N <sup>o</sup> sampled (%)	Seroprevalence (95%CI)
<b>Beef Cattle</b>	1595	117 (7.3)	2.6% (0.7-7.9)	117 (7.3)	4.3% (1.6-10.2)
<b>Dairy Cattle</b>	141	--	--	11 (7.8)	27.3% (7.3-60.7)
<b>Small ruminants</b>	16	15 (93.8)	13.3% (2.3-41.6)	15 (93.8)	33.3% (13.0-61.3)
Goats	13	12 (92.3)	8.3% (0.4 -40.2)	12 (92.3)	33.3% (11.3-64.6)
Sheep	3	3 (100.0)	33.3% (1.8-87.5)	3 (100.0)	33.3% (1.8-87.5)

**Table 2:** Estimated prevalence ratio of brucellosis and Q fever and their 95% confidence intervals by production group.

<b>Risk factor variables</b>	<b>Brucellosis</b>	<b>Q fever</b>
	Prevalence ratio (95%CI)	Prevalence ratio (95%CI)
<b>Beef cattle</b>		
Mix of livestock species	1.2 (0.7-1.9)	--
Burial of the placenta on the farm	1.2 (0.7-1.9)	1.1 (0.7-1.9)
Placenta fed to pets	1.4 (0.9-2.2)	1.4 (0.9-2.2)
Casting of the placenta in the field	3.9 (0.4-43.0)	1.3 (0.6-2.8)
<b>Dairy cattle</b>		
Use of ground water source for animals	--	2.2 (0.9-5.9)
Use of tap or pond water source	--	0.1 (0.0-1.8)
Implementation of tick control	--	0.7 (0.3-1.6)
<b>Small ruminants</b>		
Share of pasture with other farms	3.6 (0.3-39.9)	--
Use of external manure	1.1 (0.5-2.6)	0.8 (0.4-2.1)
Use of ground water source for animals	1.1 (0.7-1.8)	1.2 (0.5-2.7)
Use of tap or pond water source	0.7 (0.4-1.2)	0.6 (0.3-1.5)
Occupational training by local vets	1.2 (0.8-1.8)	0.9 (0.4-2.0)
Wild animal* seen on the farm	NA	1.5 (0.8-2.8)
Access of pets to the livestock area	NA	1.6 (0.7-3.7)
Spill of secretions from animals in the field	1.1 (0.7-1.6)	1.5 (0.6-3.7)

\* Rodents, rabbits, birds or ruminants

**Table 3:** Assessment of potential risks for farmers and households

Risk assessment variables	Beef cattle		Dairy cattle		Small ruminants	
	Sampled	No (%)	Sampled	No (%)	Sampled	No (%)
<b>Consumption of farm products</b>						
Milk	117	0 (0.0)	11	2 (18.2)	15	0 (0.0)
Meat	117	45 (38.5)	11	0 (0.0)	15	10 (66.7)
Placenta	99	80 (80.8)	10	4 (40.0)	14	7 (53.9)
Sale of placenta on the market	99	24 (24.2)	10	9 (90.0)	14	0 (0.0)
<b>Occupational Health training by local vets</b>	108	40 (37.0)	11	10 (90.9)	15	10 (66.7)
<b>Implementation</b>						
Mention of the use of protective equipment	108	68 (63.0)	11	8 (72.7)	15	14 (93.3)
Absence of actual use of protective equipment	106	54 (50.9)	11	10 (90.9)	15	7 (46.7)
Presence of washing facilities*	107	34 (31.8)	11	1 (9.1)	15	11 (73.3)
Use of disinfectant	107	37 (34.6)	11	1 (9.1)	14	5 (35.7)
Cleaning of the stall after parturition	99	3 (3.0)	10	0 (0.0)	14	1 (7.1)

\*washing facilities represent a sink and/or a towel and/or soap.



## FIGURE LEGEND

**Figure:** Map of the distribution of seropositivity by production type. A) Distribution of beef cattle herds. B) Distribution of dairy cattle herds. C) Distribution of small ruminant flocks.

**FIGURE**

