

## Seroprevalence of *Coxiella burnetii* Antibodies among Ruminants and Occupationally Exposed People in Thailand, 2012–2013

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**Abstract.** Little is known about the burden of Q fever in Thailand. We conducted a serological study to describe the prevalence of anti-*Coxiella burnetii* antibodies among ruminants and occupationally exposed persons in response to the report of the first two Q fever endocarditis patients in Thailand in 2012. We randomly selected ruminant sera from brucellosis surveillance and examined sera of 661 occupationally exposed subjects from two provinces of Thailand: Chiangmai and Nakornratchasima. Animal and human sera were tested using commercial enzyme-linked immunosorbent assay (ELISA). Environmental samples, vaginal swab, and milk from cows in Chiangmai farms with detectable anti-*C. burnetii* serum antibodies were tested using polymerase chain reaction (PCR). Among the 1,632 animal sera tested, 64 (3.9%) were seropositive. The prevalence was highest in dairy cattle (4.6%, 45/988), followed by goats (3.5%, 18/516) and sheep (2.1%, 1/48). The prevalence of anti-*C. burnetii* antibodies in each species varied significantly by province: the prevalence in cattle was higher in Chiangmai (5.5% versus 0%), however, the prevalence in sheep and goats was higher in Nakornratchasima (5.9% versus 1.0%). Four out of 60 milk samples were positive by PCR (6.7%). No environmental samples were positive. Among 661 human samples, 83 (12.6%) were ELISA positive. Seroprevalence was statistically higher in Chiangmai compare with Nakornratchasima (42.8% versus 3.0%). *Coxiella burnetii* infection exists in Thailand, but the prevalence varies by geographic distribution and animal reservoirs. Further studies focusing on the burden and risk factors of *C. burnetii* infection among high-risk groups should be conducted.

### INTRODUCTION

Q fever is a zoonotic disease, caused by *Coxiella burnetii*, a gram-negative intracellular bacteria.<sup>1</sup> Ruminants, including sheep, goats, and cattle, are the most common reservoirs of the disease.<sup>1</sup> Q fever can be transmitted through inhalation of dust contaminated with infected animal birth products or secretions, such as the placenta.<sup>1</sup> A study in the United States showed that *C. burnetii* can persist in the environment for over 1 year after an outbreak in animals.<sup>2</sup> Other routes of transmission, such as food borne and human-to-human transmission, have been reported, but they are rare.<sup>1</sup> *Coxiella burnetii* infection in humans can cause flu-like symptoms, with some patients developing more severe symptoms, such as pneumonia and hepatitis.<sup>3</sup> Additionally, it can cause serious chronic infections, including endocarditis, which can be fatal if not diagnosed and treated promptly.<sup>3</sup>

*Coxiella burnetii* can infect people of all ages; previous studies showed that seroprevalence is highest among people aged 30–60 years.<sup>4,5</sup> The prevalence of anti-*C. burnetii* antibodies in humans increases among those who work closely with ruminants, underlining the significance of animal–human transmission.<sup>3,6,7</sup> Q fever outbreaks have occurred in both humans and animals, with a negative impact on the economy.<sup>8</sup> The largest reported outbreak of Q fever was in the Netherlands during 2007–2010, with approximately 4,000

human cases. More than 50,000 dairy goats were euthanized for outbreak control purposes.<sup>8</sup>

In Thailand, Q fever was first reported in 1966,<sup>9</sup> but few studies have been conducted to understand the burden of Q fever.<sup>10,11</sup> Laboratory capacity to diagnose Q fever was not established before 2012. In 2012, a collaborative research between Khon Kaen University in Khon Kaen Province, Thailand, the Emerging Tropical Infections Research Unit in Marseille, France, and the U.S. Centers for Disease Control and Prevention (CDC) Division of Vector-Borne Diseases in Fort Collins, CO, identified the first two clinical and laboratory-confirmed Q fever endocarditis cases in Thailand.<sup>12</sup> This raised concerns of the Thailand Ministry of Public Health (MoPH), which held a consultative meeting in June 2012 with the National Institutes of Health (NIH), National Institute of Animal Health (NIAH), World Health Organization, and CDC to identify key strategic objectives for Q fever collaboration and research.

As a result of this collaboration, laboratory capacities for Q fever diagnosis have been strengthened to include the capacity for immunofluorescence assay and polymerase chain reaction (PCR). This capacity has allowed further investigation and understanding of the burden of Q fever in Thailand and identification of appropriate prevention measures. In this study, we analyzed blood samples from ruminants and humans occupationally exposed to ruminants to determine the level of exposure to *C. burnetii* and explored the presence of *C. burnetii* in the dairy farm environment.

### MATERIALS AND METHODS

**Study area.** We selected two provinces from two regions of Thailand based on the number of ruminants: Chiangmai

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and Nakornratchasima are among the top five provinces with ruminants in the north and northeast, respectively.<sup>13</sup> The majority of people in these provinces work in agriculture<sup>14</sup> (Figure 1).

**Study samples.** Samples came from various sources. Ruminant sera from both Chiangmai and Nakornratchasima came from samples collected for annual brucellosis surveillance. The ruminant serum samples were collected by the authorities from the Bureau of Disease Control and Veterinary Services from January 2012 to September 2013. Eight animal samples from all 204 cattle farms in the two provinces were selected through this routine surveillance to test for antibodies to *C. burnetii*. For Chiangmai, human sera were collected from dairy farmers, livestock officers, and wildlife caretakers in response to the results of seroprevalence in animals. In addition, six farms in the area with seropositive results in Chiangmai were selected for the environmental study conducted in September 2013. Five samples of environmental swabs from each farm were taken from the birthing areas and stalls of dairy cattle, sheep, and goats. Moreover, serum samples, vaginal swabs, and milk from all the ruminants in these six farms were collected. Human serum samples from Nakornratchasima Province came from a brucellosis investigation.

**Laboratory analysis.** Animal serum samples from the annual brucellosis surveillance in animals were tested using commercial enzyme-linked immunosorbent assay (ELISA) (IDEXX®, Westbrook, ME) for the presence of IgG antibody to

Q fever. The assays were performed by the Serology Section, NIAH, Department of Livestock Development, following the manufacturer's instructions. Sample per positive percentages (S/P%) was calculated using optical density (OD) values as  $100 \times (OD_{\text{sample}} - OD_{\text{negative control}}) / (OD_{\text{positive control}} - OD_{\text{negative control}})$ . S/P%  $\geq 40\%$  was defined as positive, and S/P%  $< 40\%$  was defined as nonpositive.

For animal samples from the investigation in six farms in Chiangmai, milk, vaginal swab samples, and swabs from the environment were tested for the presence of *C. burnetii* DNA using real-time PCR to identify the insertion element IS1111. The forward primer used was 5'-ccgatcattgggcgct-3' and the reverse primer is 5'-cggcgggtgttaggc-3'. Hybridization probe method was used in real-time PCR, and the probe sequence was 5'-ttaacacgccaagaacgtatcgctgtg-3'.<sup>15</sup> All laboratory samples from animals and environment were tested at the Veterinary Research and Development Center in Lampang Province. Human serum samples were tested by commercial ELISA (Verion-Serion) for the presence of Q fever antibody by staff from Thailand NIH. Serology results were divided into positive, equivocal, and negative according to the manufacturer's criteria. The outcome of interest was seropositive versus nonpositive test results.

**Data analysis.** Data were entered in Microsoft excel, and analyzed using Stata, version 11 (STATA Corp, College Station, TX) statistical software. Proportions were calculated to describe the distribution of Q fever in animals and humans.  $\chi^2$  and Fisher's exact tests were used to determine

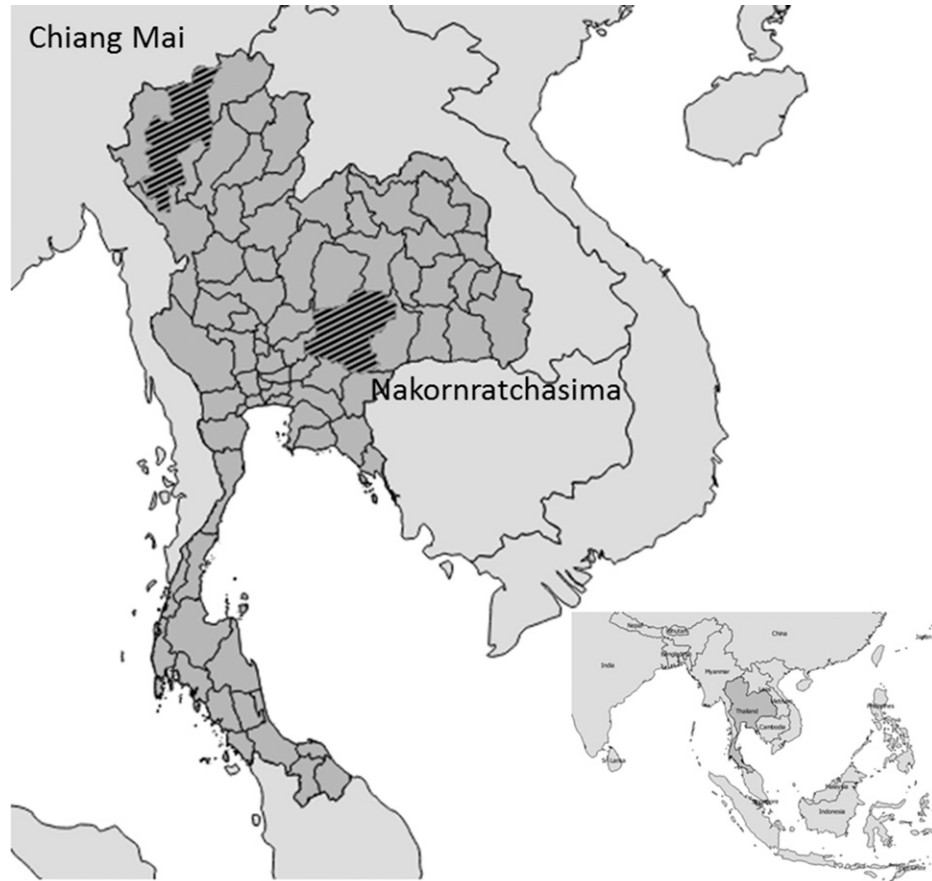


FIGURE 1. Study areas where samples for Q fever were collected, Thailand 2012–2013.

the significant difference between proportions. Univariate analysis was applied to estimate prevalence ratios and 95% confidence intervals to assess the association between the covariates and seropositivity.

### ETHICAL CONSIDERATIONS

This study was conducted as part of the outbreak investigation by the bureau of epidemiology team. Thus, it is exempted from the ethical review by the Ethical Committee of Thailand MoPH.

### RESULTS

Animal testing of cows, sheep, and goats in Chiangmai Province was performed on serum samples, vaginal swabs, and milk. Serologic testing was performed on 780 cattle serum samples and on 112 sheep and goat serum samples. For cattle, 5.5% ( $N = 43$ ) of serum samples were positive by Q fever ELISA, and serum samples from both sheep and goats were all ELISA negative. Vaginal swabs were tested from 74 dairy cattle, 19 sheep, and two goats and all were found to be negative by PCR. Sixty cow's milk samples were tested and four (6.7%) were positive by PCR. None of the environmental samples from the six ruminant farms in Chiangmai were positive.

Animal testing in Nakornratchasima was only done on serum samples. Sera from 208 cows were tested by ELISA, with 1% ( $N = 2$ ) positive. There were also 272 sheep and goats tested in Nakornratchasima with 5.9% ( $N = 16$ ) positive. The seroprevalence among cattle in Chiangmai (5.5%) was significantly different than seroprevalence among cattle in Nakornratchasima (1%) (Table 1).

Human seroprevalence was determined for samples from Chiangmai and Nakornratchasima provinces. Seroprevalence of the subjects from two provinces combined was 12.6% (83/661). Most of the people in the study had an occupation that would put them in contact with livestock and other animals. In Chiangmai, 42.8% ( $N = 68$ ) of 159 subjects were seropositive by ELISA. The majority of those tested were men (79.2%), but proportions of seropositivity were similar between men (42.1%) and women (45.5%) (Table 1). For 155/159 subjects, an occupation was identi-

fied. The occupations were farmer, zoo worker, and livestock officer. The prevalence ratio showed a significantly higher seroprevalence for farmers compared with zoo workers. Seropositivity for the three livestock officers tested was 33.3% and this did not significantly differ from zoo workers.

Testing of samples from Nakornratchasima showed a lower seroprevalence. Of the 502 human sera, 15 samples (3.0%) were positive by ELISA. An additional 23 samples were scored as equivocal by the ELISA test. Of the 502 samples, 468 were from farmers, and all the 15 positive samples were farmers. Occupation data were missing for the other 34 persons, all with a negative ELISA result. The difference in seroprevalence between Chiangmai (42.8%) and Nakornratchasima (3.0%) provinces was statistically significant ( $P < 0.0001$ ).

### DISCUSSION

We analyzed blood samples from ruminants and humans occupationally exposed to ruminants to determine the magnitude of *C. burnetii* exposure and explored the presence of *C. burnetii* in the dairy farm environment. In ruminants, the overall seroprevalence was 4.5%, with dairy cattle (4.6%) having a slightly higher seroprevalence than sheep and goats (4.2%). The prevalence of anti-*C. burnetii* serum antibodies among people occupationally exposed to ruminants was 12.6%. Significant differences were observed between the two provinces analyzed. Chiangmai had significantly higher seroprevalence in dairy cattle and humans, whereas Nakornratchasima had higher seroprevalence among sheep and goats but much lower for humans. A limitation of the study is that different sampling strategies in the two provinces (routine surveillance for abortion versus an outbreak setting) could influence the results. Further studies are needed in Thailand to explore how environmental factors, farm practices, and the density of animal reservoirs in each area impact exposure to *C. burnetii*.

The prevalence of anti-*C. burnetii* serum antibodies among ruminants in our study (4.5%) was lower compared with a study performed in Thailand in 1967 where the seroprevalence among cattle, sheep, and goats combined was 5.4%.<sup>9</sup> Furthermore, the seroprevalence among cattle in our study (4.6%) was lower compared with another study in Thailand published in 2014 where the prevalence of Q fever

TABLE 1  
Human seroprevalence to *Coxiella burnetii* in Chiangmai and Nakornratchasima provinces, Thailand 2012–2013

Province	Characteristics	N	Seropositive N (%)	Prevalence ratio (95% CI)	P value
Chiangmai	Total	159	68 (42.80)	–	–
	Gender				
	Men	126	53 (42.06)	0.93 (0.60–1.42)	0.7215
	Women	33	15 (45.50)	Reference	–
	Occupation				
	Farmer	48	33 (68.75)	2.23 (1.58–3.16)	< 0.0001
	Livestock officer	3	1 (33.33)	1.08 (0.21–5.50)	0.9231
	Unknown	4	2 (50.00)	1.63 (0.59–4.50)	0.3516
Zoo worker	104	32 (30.77)	Reference	–	
Nakornratchasima	Total	502	15 (2.99)	–	–
Chiangmai	Total	159	68 (42.80)	14.31 (8.43–24.3)	< 0.0001
Nakornratchasima	Total	502	15 (2.99)	Reference	–

CI = confidence interval.

among cattle in Bangkok was 6.9%.<sup>16</sup> All of the three studies used different serological tests, making direct comparisons difficult, but together provide information regarding the existence of Q fever in Thailand. Our findings show that different ruminants in various regions could be the potential source of *C. burnetii* infection in humans. However, reservoir species might differ geographically. Dairy cattle could be a potential reservoir for *C. burnetii* in Chiangmai, while sheep and goats could be the possible major animal reservoir in Nakhonratchasima. Individual characteristics of the ruminants, for example age, as well as the environmental factors such as the presence of other animals in each particular area might also influence the maintenance of *C. burnetii* in ruminants.<sup>17</sup>

The presence of the antibody to *C. burnetii* in each particular herd may not indicate bacterial shedding from the animals. A previous study showed that the animals that shed the bacteria might not have antibody and seroconverted animals might not shed the bacteria.<sup>18</sup> Identification of shedding animals is critical for reducing the source of Q fever infection. Our study found the evidence of *C. burnetii* DNA in milk, but not in the vaginal swabs of the same cow, indicating that milk could be a potential source of infection apart from placenta.<sup>19</sup>

Similar to other studies, our study found that the prevalence of *C. burnetii* serum antibodies among people who were exposed to animals, such as farmers, tended to be higher than that observed in other studies of the general population.<sup>10,20,21</sup> This suggests that farmers might get more exposed to the bacteria as consistent with known routes of *C. burnetii* transmission. The seroprevalence among farmers, livestock, zoo, and wildlife workers with close contact to ruminants in this study (6.4%) was lower compared with other studies. The prevalence of anti-*C. burnetii* serum antibodies among farmers and veterinarians in Malaysia during a Q fever outbreak in 2011 was 43%.<sup>22</sup> In previous studies, the seroprevalence among veterinarians in Japan was 35%,<sup>21</sup> Taiwan 26%,<sup>23</sup> and in the United States 22%.<sup>24</sup> However, comparing the seroprevalence between studies can be challenging due to the discrepancies in serological methods and the cut-off value used, as well as the discrepancies in times when samples were collected. In addition, other environmental factors such as type and density of animal reservoirs might vary geographically.

Although some studies reported that men are more likely to have anti-*C. burnetii* antibodies than women,<sup>23,25–27</sup> our study did not find a statistically significant difference between the seroprevalence of men versus women in Chiangmai Province. The result is similar to that of studies in Spain<sup>28</sup> and in the Netherlands.<sup>29</sup> The role of gender in *C. burnetii* exposure in Thailand might need further investigation if other routes of transmission or other animal reservoirs are found to influence the occurrence in men and women.

Following the identification of the first two confirmed cases of Q fever endocarditis in 2012,<sup>12</sup> the Thailand Bureau of Policy and Strategy, Ministry of Public Health reported approximately 4,000–5,500 people had been diagnosed with endocarditis (6–8/100,000) during 2012 and 2013.<sup>30</sup> Unfortunately, the etiologies of endocarditis were not identified. A multinational multicenter study in America, Europe, and Asia focused on the etiology of infective endocarditis showed that Q fever was positive in approximately 1% of patients

presenting with acute endocarditis.<sup>31</sup> Thus, it is likely that Q fever endocarditis is underreported in Thailand.

The main limitation of our study was missing data as we reanalyzed existing samples and no information related to *C. burnetii* exposures such as age or job description were available. Therefore, it was a challenge in exploring and determining possible risk factors for seropositivity in this study. Human sera from the two provinces came from different sampling strategies; hence, it also limited our ability to compare sampled human populations between regions. Nevertheless, our study has confirmed the presence of Q fever in Thailand.

In conclusion, antibodies to *C. burnetii* were prevalent in ruminants and humans in selected provinces of Thailand. It is likely that people who are exposed to ruminants might be at risk of becoming seropositive. Thus, health education to the high-risk population, and increasing awareness of Q fever among physicians, should be implemented to prevent the occurrence of the disease and to improve Q fever diagnosis. There are gaps in knowledge of both acute and chronic Q fever in Thailand. Therefore, future systematic epidemiological studies are needed to understand the risk factors for Q fever in animals and humans, and to identify the potential reservoirs for Q fever in Thailand.

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