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RESEARCH ARTICLE

Epidemiology of Q fever in ruminants in the north-east and the north region of Thailand, 2012 to 2013

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Abstract

Objective: To investigate the Q fever status in ruminants especially in the North-East and the North regions of Thailand.

Material and Methods: From 2012 to 2013, 1044 placentas were collected from cows, buffaloes, goats, sheep, and wildlife ruminants from both regions by convenience sampling. Macroscopic examination was initially done, and followed by the extraction of DNA from the placentas. DNA of the placenta samples were identified for *Coxiella burnetii* by the real-time PCR technique targeting the IS1111 gene.

Results: All samples had no gross lesions. In total samples 76.63% (800/1044) were PCR positive with in the North-East 76.19% (685/899), and the North 79.31% (115/145).

Conclusion: This study indicated that the placentas, collected from ruminants in the North-East and the North, had positive results of *C. burnetii* by real-time PCR, with none of abortions. The high percentage of positive results might because of contamination from the bedding, manure, and environment. Further studies, the immunohistochemistry technique should be performed to definitively identify infected placentas.

Keywords: Q fever, Ruminants, North-east region, North region, Thailand

Introduction

The zoonotic disease Q fever is caused by the gram negative bacteria, *Coxiella burnetii*. The main transmission route for humans is inhalation, while other routes include direct contact with hay, bedding, wool, surfaces or other items contaminated by feces, urine, milk and especially placenta and related fluids from infected ruminants (OIE, 2010). The organism has a low infectious dose - a single bacterium (Ormsbee et al., 1978). Cattle, sheep and goats are the main reservoirs, but other domestic and wild animals can also be infected. The clinical signs in animals include abortion, stillborn or weak offspring and infertility problems. The most significant clinical sign for ruminants is late abortion, and abortion storms have been documented, especially after a new introduction to a previously immunologically naïve herd or flock (OIE, 2010).

Q fever in humans has two forms: acute and chronic. The acute form is described as a "flu-like" syndrome. The chronic form may be associated with valvulitis/endocarditis, and may be associated with abortion in pregnant woman. Both forms can be cured with appropriate antibiotic therapy, primarily including doxycycline, but with a shorter course for acute disease and a longer course for chronic. Immuno-compromised people and patients with an underlying heart valve defect are at greater risk of a serious complication. With all of its properties, *C. burnetii* is one of the category B bioterrorist agents. (Angelakis and Raoult, 2010; OIE, 2016; Porter et al., 2011; Rodolakis, 2006; Woldehiwet, 2004) People at higher risk of exposure include veterinarians, farmers, workers in farms, slaughter houses, wool plants, and laboratories. (Georgiev et al., 2013; Nielsen et al., 2013; OIE, 2016; Porter et al., 2011; Whitney et al., 2009; Whitney et al., 2013; Woldehiwet, 2004)

In Thailand, one study showed some seropositive result by microimmunofluorescent antibody test from human samples (Suputtamongkol et al., 2003), and human Q fever-endocarditis cases were found in the north-east recently (2010 to 2012) (Pachirat et al., 2012; Watt et al., 2014). However, there is a lack of data regarding disease situation in animal. Therefore, this information leads us to investigate *C. burnetii* in ruminants in the North-East and the North region and to identify risk factors related to Q fever infection in ruminants that might raise public health awareness and concern about the pathogen as well as prevent human cases in the future.

Materials and Methods

Study area

The cross-sectional study in the North-East (17 provinces) and the North region of Thailand (7 provinces) was performed.

Questionnaire

We did a pilot study to test our questionnaires, and edited some questions for our study. The general data of the owners and animals were collected by the provincial veterinary officers in a one-page, check-the-box style form included with the sample. The data consisted of name and career of the owner, animal species, age, number of parturitions, and appearance of placenta (normal, retained).

Sample collection

From July 2012 to September 2013, placentas were collected. Sample sizes from the population of beef cow (DLD, 2011a), dairy cow (DLD, 2011c), buffalo (DLD, 2011b), goat and sheep (DLD, 2011d) in the North-East region (n = 3,282,448) and the North (n = 594,001) of Thailand in 2011 were calculated. Calculations using the WinEpi program (www.winepi.net) with 95% confidence interval, 2% expected minimum prevalence, indicated that 149 samples from each region were needed. The provincial and regional veterinary officers collected at least 5 cotyledons/placenta by convenience sampling from any ruminants giving birth on each farm. All farmers in this study were willing to participate and send their ruminant placentas to detect the *C. burnetii*. Other sources of placentas were on the shelf in the fresh markets, a zoo, and vendors who did not have any store. Each placenta sample was kept in sterile plastic bag. Frozen placentas and questionnaires were sent to immunology section, National Institute of Animal Health (NIAH) in Bangkok.

Laboratory investigation

Macroscopic examination of placenta was observed before DNA extraction. DNA was extracted from the cotyledon using Qiagen DNAeasy blood and tissue kit 250 (Qiagen S.A., France) according to the instructions of the manufacturer. *C. burnetii* DNA was detected by real time PCR targeting the IS1111 gene, using Taqman probe IS1111-p822S (TgTCggCgTTTATTgggTTggTCCC, final concentrate 0.05 µM), primer IS1111-F801 (AATTTCATC-

gTTCCCggCAg, final concentrate was 0.5 µM), and IS1111-R901 (gCCgCgTTTACTAATCCCCA, final concentrate was 0.5 µM). The real-time PCR condition was processed as previously described (Christensen et al., 2006), in a Roche LightCyclerÒ 2.0 PCR machine. DNA/RNA-free water was used as negative control. The positive control was *C. burnetii* Nine Mile strain from cell culture, provided by AFRIMS. Samples were deemed positive if the cycle threshold was less than 39.

Statistical analysis

Percent positive real-time PCR results were calculated and crude odds ratios (OR) via the R program version 1.0.143 was evaluated. Some calculations included several species together as categories of ruminants. "Dairy ruminant" means dairy cow and dairy goat. "Beef ruminant" means beef cow, meat goat, buffalo, and sheep.

Results

Totally we evaluated 1,044 placenta samples from farms, middle merchants, beef shops, a zoo and other places. We summarized the placentas from middle merchants and beef shops together in "Fresh market" in table 1. We received the placentas from 17 provinces from the North-East, and 7 provinces from the North (figure 1). We put data on the map by using ArcGIS version 10.5.1 software. In the North-East 76.19% (685/899), the North regions 79.31% (115/145), and the total 76.63% (800/1044) of the samples were PCR positive. The wildlife ruminant placentas were a deer (0/1), a wild sheep (0/1), and a nyala (1/1). We did not receive any placenta from dairy goat. The age of animal were 1 to 16 years range (mode = 4 years). The parturition times were 1 to 12 range (mode = 1). The cycle threshold (Ct) values from real-time PCR were 9.24 to 37.79 (median = 32.18, mean = 30.88, standard deviation = 4.32). The distribution of positive samples' Ct was shown in figure 2.

The odds ratio analysis was shown in table 2. We could not find any risk factor associated with *C. burnetii* infection in this study, because no factor was significantly different from any other based on the OR.

Discussion

The strength of this study was that the sample used was placenta, the target organ of *C. burnetii*. This was the first *C. burnetii* placenta study in ruminants in Thailand. Additionally, three quarters of our placenta samples were positive for *C. burnetii* infection, but the offspring appeared healthy. None of the animals involved in this study had aborted, and most of the samples were normal placentas. This indicated that both areas of the investigation had the reservoir animals of *C. burnetii*. This information is contradicted to other previous studies that the pathogen can cause the problems in reproductive system (Rai et al., 2011; Reichel et al., 2012). This contradiction necessarily needs another investigation to explain. Importantly due to high positive percentage results, public health awareness from the pathogen should be concerned. The high positive percentage was might because the *C. burnetii* exposed animal can shed the pathogen in a huge number during the parturition period as in previous published research (van der Hoek et al., 2010).

Due to the high sensitivity of PCR, its' positive result was absolutely higher than serology tests. Another etiology was the contamination from the environment (Kersh et al., 2010) during collection of the placenta might increase the positive results. Figure 2 displayed that more than 50% of our positive results had Ct greater than 28. While this would still indicate that *C. burnetii* was present on the premises, it is important to know whether the organism was in the placental tissue itself. Immunohistochemistry (IHC) technique will be required to precisely localize the pathogen within the cells of the positive placenta in the further study. Besides abortions, normal deliveries in *C. burnetii*-infected goats should be considered as a major zoonotic risk for Q fever in human (Roest et al., 2012). Unfortunately, we did not know the status of our animals before this study. So in Thailand, the placenta positive animals can have normal deliveries (possibly *C. burnetii* subtypes that do not cause disease in animals) but we cannot ignore the fact that Q fever is a zoonotic disease.

Most of the samples were cow placentas, because farmers can sell them with expensive price for consumption. As a result, we could not compare the positive percentage by species, because the number of samples/species was distinctive from each species. The awareness of *C. burnetii* infection was during the parturition period in every species of ruminants. Dairy farms might need more biosafety attentions, because the close contact between human to the animal such as bathing, milking time, and artificial insemination were more often than beef farms. Furthermore, the

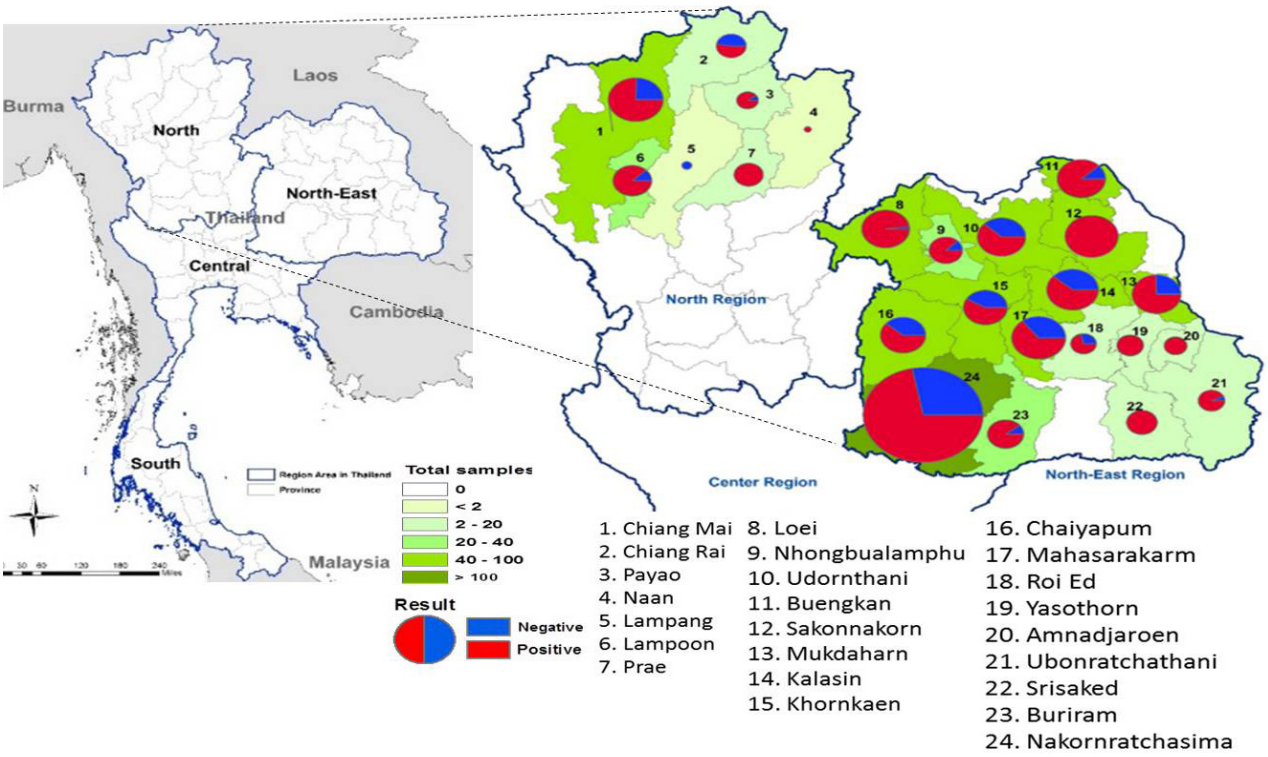

Figure 1. The number of placenta samples and location of sample collection from 2012 to 2013 in the north-east and the north region of Thailand

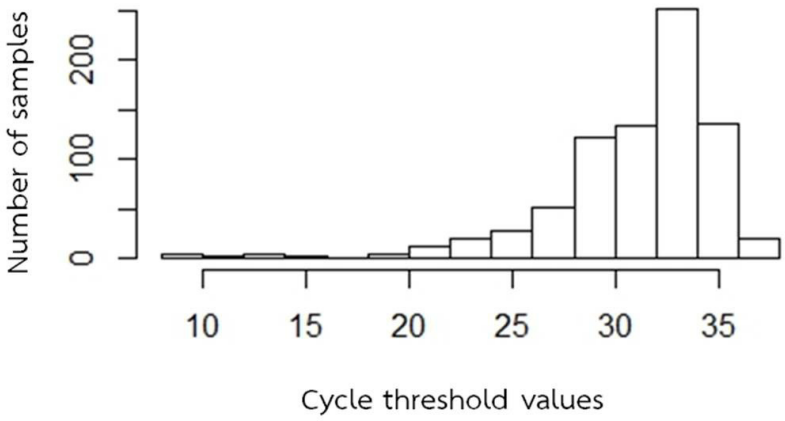

Figure 2. The distribution of positive placentas'cycle threshold (n = 800) by Real-Time PCR (min Ct = 9.24, max Ct = 37.79)

animal life in dairy farms was longer than in beef farms. This may serve as baseline information for future surveillance efforts, as well as any follow up to possible future human cases.

We chose to study in the North-East, and the North region because of human cases (Watt et al., 2014) and human behavior (consuming placenta soup). The number of samples was enough to estimate the positive frequency in these 24 provinces. We did not do random sampling, but instead collected them by convenience sampling. The

Table 1. *Coxiella burnetii* in ruminant placenta samples by Real-Time PCR, in the north-east and the north region of Thailand during 2012 to 2013 (n = 1044)

Data	Real-Time PCR	
	Positive (%) (n=800)	Negative (%) (n=244)
Regions of Thailand		
North-East	685 (85.63)	214 (87.70)
North	115 (14.37)	30 (12.29)
Source of samples		
Farm	753 (94.13)	223 (91.39)
Fresh market*	47 (5.88)	21 (8.61)
Propose-Species of placenta		
Beef cow	418 (52.25)	96 (39.34)
Dairy cow	325 (40.62)	103 (42.21)
Meat goat	31 (3.87)	37 (15.16)
Buffalo	22 (2.75)	3 (1.23)
Sheep	3 (0.37)	3 (1.23)
Wildlife ruminants*	1 (0.12)	2 (0.82)
Appearance of placenta		
Normal placenta	725 (90.63)	218 (89.34)
Retained placenta	20 (2.5)	11 (4.51)
Missing data	55 (6.88)	15 (6.15)

*Fresh market means middle merchant and beef shop
*Wildlife ruminants were a deer, a wild sheep, and a nyala.

samples are not necessarily representative of the whole of both regions. Our sampling method (convenience sampling) did not allow for formal statistical assessment of prevalence. However, a substantial number of samples gave an indication of the extent of *Coxiella* presence in those provinces.

We could not find the risk factor to be infected by *C. burnetii*. From other published studies, the risk of being infected with *C. burnetii* included having at least one seropositive nulliparous female and was increased in herds with seasonal calving and where the fetus and/or the placenta of aborted cows were not systematically removed (Taurel et al., 2011). We could not estimate the increasing number of parity to be a risk factor because we conducted our study in many species not only dairy cows like the research in Denmark (Paul et al., 2012). Our risk factors were not comparable with any other published research.

Biosafety behaviors and sanitary procedures must be used in routine farm management, especially birthing pens. When closed to delivery, animals should be confined in a birthing pen to easily disinfect and remove the debris potentially contaminated with *C. burnetii* organisms. The effective disinfectants including Microchem, 70% alcohol, Chlorox, and formaldehyde solutions require contact time of at least 30 minutes. The birth pen floor should be cement or rubber sheet to easily facilitate and clean up (Anderson et al., 2013; Doung-ngern et al., 2017). People involved in helping animals at delivery should prepare disinfectant solution in a bucket to clean themselves afterward. The consuming placenta soup was safe for eating. It might be

Table 2. Crude odds ratio of Q fever with production use of ruminants, sources, appearance of ruminant from the placentas in Thailand, 2012 to 2013

Variable	Crude odds ratio (95% CI)
Production use of ruminants (n = 1041[a])	
Beef ruminants	Reference
Dairy ruminant[b]	0.93 (0.69-1.24)
Sources of placenta (n = 1044)	
Farm	Reference
Fresh market	0.66 (0.39-1.16)
Appearances of placenta (n = 974[c])	
Normal placenta	Reference
Retained placenta	0.55 (0.26-1.21)

[a]This n was excluded number of wildlife ruminants'placentas
[b]Dairy ruminant means dairy cow.
[c]This n was excluded number of missing data of appearance of placenta

risk for people who cook, clean the raw placenta with no gloves.

This study indicated that the placentas, collected from ruminants in the North-East and the North, had positive results of *C. burnetii* by real-time PCR with none of abortions. The high percentage of positive results were possibly due to the contamination from the bedding, manure, and environment. Further studies should be using the immunohistochemistry technique, to definitively identify infected placentas. The important measure for positive animal was giving education to farmers, veterinarians, and risk people to protect themselves from *C. burnetii* infection such as management of birthing pen, using disinfectant, and wear personal protective equipment (mask, gloves, apron, boots) during animal parturition period.

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