SEROEPIDEMIOLOGIC SURVEY IN THAILAND OF *COXIELLA BURNETII* INFECTION IN CATTLE AND CHICKENS AND PRESENCE IN TICKS ATTACHED TO DAIRY CATTLE

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Abstract. A seroepidemiologic survey of *Coxiella burnetii* in cattle and chickens in Thailand was carried out using indirect fluorescent antibody test. Nine of the 130 serum samples from cattle were positive for antibodies against *C. burnetii*, with antibody titers ranging from 32 to 64. Only one of 113 serum samples from chickens was seropositive, with antibody titer of 16. No *C. burnetii*-specific DNA was detected using restriction fragment length polymorphism-nested PCR in spleens of cattle and chickens. However, coxiella DNA was detected in two of 102 engorged *Rhipicephalus microplus* ticks attached to dairy cattle. These results indicated that infestation of *C. burnetii* among cattle and chickens is considerably low in Thailand.

Keywords: *Coxiella burnetii*, cattle, chicken, tick, Thailand

INTRODUCTION

Q fever is a worldwide zoonotic disease caused by an obligate intracellular bacterium, *Coxiella burnetii* (Svraka *et al*, 2006). An indirect fluorescent antibody test (IFAT) is the most suitable for serosurveillance of Q fever in humans and coxiellosis in animals because of its simplicity to perform and high sensitivity (Hirai and To, 1998). Since the 1990s, detection of coxiella DNA by a PCR method has been widely utilized to diagnose human Q fever and animal coxiellosis (Stein and Raoult, 1992; Hirai and To, 1998).

C. burneti exists in bimodal cycles, which are usually independent but occasionally overlaps (Hirai and To, 1998). The basic cycle involves many species of wild animals and their ectoparasites, and the second cycle involves livestock. Among the ectoparasites, ticks are considered to be the natural primary reservoirs for the spread of coxiella infection in wild animals and for coxiella transmission from

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wild animals to livestock, such as cattle (Hirai and To, 1998). Cattles are the main reservoir of *C. burnetii* and are responsible for coxiella infection in animals and humans. The coxiella microorganisms then are dispersed into the environment through animal excretions, such as saliva, urine and feces. Infected cows also shed *C. burnetii* through their placenta, birth fluid, colostrum and milk into the environment. Thus, the most probable route of infection with *C. burnetii* in animals and humans is the respiratory system via inhalation of infectious aerosol or airborne dust (Derrick *et al*, 1942; Tigertt *et al*, 1961). In addition, because *C. burnetii* is excreted into cow's milk over a long period of time, the microorganism is also regarded as a causative agent of food-borne diseases.

Clinical cases of Q fever in humans caused by ingestion of milk or milk products have been reported (Huebner *et al*, 1949; Babudieri, 1953; Enright *et al*, 1956). Also there have been many reports during the past few decades on seropositivity of antibodies against *C. burnetii* in cattle (Biberstein *et al*, 1974; Martin *et al*, 1982; Marrie *et al*, 1985; Muramatsu *et al*, 1997). Seropositivity in cattle with reproductive disorders are higher than that in healthy cattle (To *et al*, 1995, 1998a).

On the other hand, a zoonotic correlation between coxiella and chickens is unclear. In 1959, transovular transmission of *C. burnetii* in chickens was reported (Sobeslavsky and Syrucek, 1959), but in 1978 it was reported that vertical transmission and ovarian infection of coxiella was not found in experimentally infected chickens (Sethi *et al*, 1978). Recently, chicken eggs and egg products have been suspected to be an infection source of Q fever in humans in Japan (Kishimoto, 2006). Using nested-PCR targeting coxiella outer membrane protein gene (*com1*), coxiella DNA was detected in commercial chicken eggs and mayonnaise (Tatsumi *et al*, 2006). However, no coxiella DNA has been detected from chicken eggs and egg products in other studies (Komiya *et al*, 2004; Hirai *et al*, 2005; Kishimoto, 2006). Epidemiologic studies on *C. burnetii* in chickens have reported prevalences of antibodies against *C. burnetii* in chicken serum samples collected in 1995 and 1996 ranging from 1% to 3% (To *et al*, 1998b). Seropositivity of antibodies against *C. burnetii* in serum samples of aged non-laying chickens is 7% (Muramatsu *et al*, 2006).

Seroprevalence of antibodies against *C. burnetii* conducted using CFT in both animals and humans in Thailand reported a value in cattle of 6.1% (Sangkasuwan and Pongpradit, 1967), and that in febrile and non-febrile patients ranging from 0.4% to 2.6% (Puranavej *et al*, 1968). More recent epidemiologic surveys have reported prevalence of anti-*C. burnetii* antibodies in febrile disease patients in Thailand of 1.3% (Suputtamongkol *et al*, 2003) and 0.7% (Suttinont *et al*, 2006). All patients diagnosed with Q fever in Thailand are rice farmers and their farm animals are cattle and chickens (Suputtamongkol *et al*, 2003), suggesting that cattle and/or chickens are important zoonotic reservoirs of *C. burnetii* in Thailand.

In order to support this notion, we surveyed seroprevalence of anti-*C. burnetii* antibodies in serum samples from retail meat cattle and chickens in Thailand. We also used nested PCR to detect coxiella DNA in tissue samples of retail meat cattle, chickens and in ticks attached to dairy cattle.

MATERIALS AND METHODS

Samples

A total of the 130 serum samples from

cattle and 141 from chickens were collected in Bangkok, Thailand in December 2005, and were stored at -20°C until tested. In addition, 113 cattle spleen samples from three markets and 120 chicken spleen samples from two other markets in Bangkok, during October 2005 to January 2006, a total of 128 engorged ticks [102 *Rhipicephalus microplus* (formerly *Boophilus microplus*) and 26 *Haemaphysalis longicornis*] were collected from dairy cattle reared at 6 farms in two provinces, Phrae and Lumphun, Thailand. Each aliquot of the spleen sample was minced well. The tick was disrupted with a 180 µl of ATL buffer (Qiagen, Tokyo, Japan) in a 1.5 ml-sized Eppendorf type tube by using a plastic pestle. Genomic DNA was extracted from 10 mg each of the minced spleen aliquot or the disrupted tick using a commercial kit (QIAamp® DNA Mini Kit; Qiagen) (Muramatsu *et al*, 2008). The DNA samples were also stored at -20°C until used.

IFAT assay

IFAT was performed as previously described (Muramatsu *et al*, 1997, 2006). In brief, serum samples were assayed starting at 16-fold dilution together with secondary fluorescein-5'-isothiocyanate (FITC)-conjugated rabbit anti-bovine (H+L) IgG or FITC-conjugated rabbit anti-chicken (H+L) IgG antibodies (ICN Biomedicals, Carlsbad, CA). Cattle sera having an antibody titer higher than 32 and chicken sera higher than 16 are considered positive. The chi-square test was used to estimate association in bivariate analysis of seropositivity between cattle and chicken samples.

Restriction fragment length polymorphism (RFLP)-nested PCR assay

Nested PCR to detect *C. burnetii com1* was performed as previously described (Zhang *et al*, 1998a). Briefly, the first amplification for the nested PCR was performed with the primer pair OMP1 (5'-AGT AGA AGC ATC CCA AGC ATT G-3') and OMP2 (5'-TGC CTG CTA GCT GTA ACG ATT G-3') for 36 cycles of 1 minute each at 94°C, 54°C and 72°C. Second PCR with the other primer pair OMP3 (5'-GAA GCG CAA CAA GAA GAA CAC-3') and OMP4 (5'-TTG GAA GTT ATC ACG CAG TTG-3') were performed for 36 cycles of 30 seconds at 94°C, 20 seconds at 54°C and 1 minute at 72°C. PCR amplicon was purified by using a commercial kit (QIAquick gel extraction kit; Qiagen, Tokyo, Japan). Subsequently, the amplicon was subjected to restriction fragment length polymorphism (RFLP) analysis using *Sal*I and *Ssp*I restriction endonucleases as previously described (Zhang *et al*, 1998b), Nested PCR amplicons and enzyme digested products were analyzed by 1.5% and 2% agarose gel-electrophoresis, respectively. Visualization and identification of DNA fragments were performed with ethidium bromide staining and UV illumination.

RESULTS

The prevalence of IgG antibodies against *C. burnetii* in cattle and chicken sera was 9/130 (7%) and 1/141 (1%), respectively (Table 1). Seropositivity in cattle is significantly higher than that in chickens (chi-square value = 5.704 with Yates' correction).

We detected coxiella DNA from 2/126 (2%) *R. microplus* samples by means of RFLP-nested PCR of *C. burnetii com1* (samples no. 25 and 27, Table 1; Fig 1). No coxiella DNA was detected from *H. longicornis* samples. All DNA samples extracted from 113 cattle and 120 chicken spleens were negative for coxiella DNA (Table 1).

Table 1
Seropositivity and prevalence of IgG antibody against *C. burnetii* in cattle and chickens tested by IFA, and detection of coxiella DNA from cattle, chickens and ticks by nested PCR and identification of the PCR products by RFLP analysis.

Prevalence of IgG antibody (*n*)			Detection of *C. burnetii* DNA		
Serum	Positive ratio	Antibody titers	Genomic DNA[a]	Positive ratio	Identification by RFLP
Cattle (130)	7% (9)	32 (7), 64 (2)	Cattle (113), Chicken (120)	Not detected	Not tested
Chicken (141)	1% (1)	16 (1)	Ticks (128)	1.6% (2[b])	*C. burnetii* (2)

[a]Each genomic DNA was extracted from cattle spleen, chicken spleen and whole bodies of engorged ticks attached to dairy cattle.
[b]Both ticks positive for nested PCR were *R. microplus* (sample nos. 25 and 27 in Fig 1).

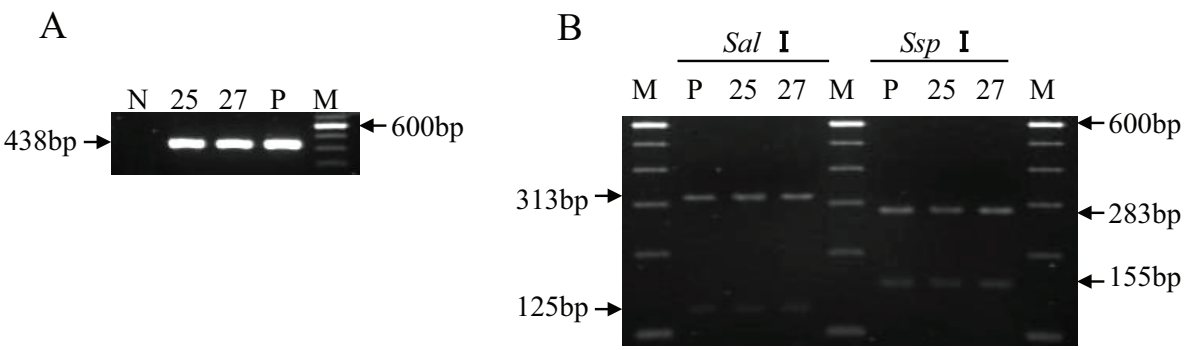

Fig 1- RFLP nested PCR of *C. burnetii com1* from *R. microplus* ticks. A. Nested PCR amplicons separated by 1.5% agarose gel-electrophoresis. B. RFLP analysis of amplicons digested with *Sal*I and *Ssp*I and separated by 2% agarose gel-electrophoresis. Lane M, 100 bp DNA size markers (Invitrogen, CA); lane P, positive control (*C. burnetii* Nine Mile II, ATCC VR-616), lanes 25 and 27, *R. microplus*; lane N, negative control.

DISCUSSION

Several decades have passed since a seroepidemiologic survey has been conducted for *C. burnetii* infection in cattle in Thailand, which reported (using complementary fixation test) an average seropositive ratio of 6.1% in cattle, from 18 areas, with the highest seropositive ratio of 9.2% in cattle from Bangkok. It is difficult to compare the seropositive ratio between the results obtained in the current and the previous studies, because the serological method used in each survey was different (IFAT being used in our study). However, the results of the both surveys suggest that the seropositive ratio in cattle has been maintained at a low level in Thailand.

Epidemiologic information on *C. burnetii* infection in chickens worldwide is scarce in comparison with that for cattle.

In this study, both seropositive rate and the distribution of IFAT antibody titer against *C. burnetii* in chickens were very low, and no coxiella DNA was detected. Our results suggest that chicken is not important for the lifecycle of *C. burnetii* in Thailand.

This is the first report in Thailand on the detection of *C. burnetii* in engorged *R. microplus* ticks attached to dairy cattle, which provides evidence that *R. microplus* has ability for transmitting *C. burnetii* to cattle. The failure to detect coxiella DNA in *H. longicornis* ticks, which are known to be vector of *Coxiella*-like microorganisms (Lee *et al*, 2004) may reside in the small number of samples. No coxiella DNA was detected as well in cattle and chicken spleens, reflecting the low rate of detection of *C. burnetii* (coxiella DNA) in the ticks.

It is essential in the prevention of coxiella infection that epidemiologic information on microorganisms be obtained in more details, not only in Thailand but in neighboring countries where such epidemiologic information are equally scarce.

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