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Detection of *Coxiella burnetii* in Cattle

Yo-Han Kim and Doo Kim[1]

Laboratory of Veterinary Internal Medicine, College of Veterinary Medicine, Kangwon National University, Chuncheon 200-701, Korea

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Abstract : *Coxiella burnetii* is an obligate intracellular rickettsial organism and the causative agent of Query fever, a zoonosis that occurs worldwide. In Korea, *C. burnetii* infection had occurred in humans and animals. However, the studies were only conducted in geographically limited area for detection of *C. burnetii*. The objective of this study was to detect *C. burnetii* in Korean native cattle and dairy cattle nationwide by real-time PCR. The total of 807 blood samples from 622 Korean native cattle and 185 dairy cows, 170 individual milk samples of dairy cows, and 348 bulk tank milk samples of dairy herds were collected nationwide. From blood samples, *C. burnetii* was detected in 17 (2.7%) out of 622 Korean native cattle and 2 (1.1%) of 185 dairy cows. From milk samples, *C. burnetii* was detected in 27 (15.9%) out of 170 individual milk samples of dairy cows. And *C. burnetii* was detected in 84 (24.1%) of 348 bulk tank milk samples. In conclusion, this study revealed that the detection rates are considerably high in cattle and the infection of *C. burnetii* has been continuously occurring in cattle of Korea. In order to prevent the hazards of a zoonosis Q-fever that occur both humans and domestic animals, further studies are needed to clarify the epidemiology of Q-fever of domestic animals and humans in Korea.

Key words **:** *Coxiella burnetii*, real-time PCR, Korean native cattle, dairy cattle.

Introduction

Coxiella burnetii is an obligate intracellular rickettsial organism and the causative agent of Query fever (Q-fever), a zoonosis that occurs worldwide (22). In human, the disease may appear in 2 forms, acute and chronic. Acute Q-fever usually presents a flu-like, self-limiting disease accompanied by atypical pneumonia and hepatitis. In chronic Q-fever, endocarditis, hepatitis, osteomyelitis or infected aortic aneurysms may occur (19,24). In animals, *C. burnetii* infections are generally asymptomatic, except for manifestations as reproductive disorder in female (2).

C. burnetii infections in humans are thought to occur primarily via aerosols generated from urine, feces, milk, and birth products of infected animals (18). The bacterium is present in high numbers in the amniotic fluid, placenta and fetal membranes of parturient ewes, goats and cattle (1). Over 10^9 bacteria per gram of placenta are released from infected animals at the time of delivery (3), and the animals may continue to shed infectious particles long after abortion (5). Shedding of *C. burnetii* in milk by infected dairy cattle is also well documented. *C. burnetii* is shed for extended periods in the milk of dairy cattle (12) and *C. burnetii* shedding in milk was associated with chronic subclinical mastitis in dairy cows (4).

The reported prevalence of Q-fever is considerably high and is continuously increasing in domestic animals (14,27). *C. burnetii* was detected in 8.7% of individual dairy cow milk samples and 66.7% of bulk tank milk samples by *IS1111* element based real-time PCR in Hungary (13).

In Korea, there were several studies of Q-fever in humans and animals (8,16,21). These previous reports suggested that *C. burnetii* infection had occurred in humans and animals in Korea. However, these studies were only conducted in geographically limited area for detection of *C. burnetii* and were also insufficient in identifying characterization of *C. burnetii* detected in Korea. The objective of this study was to detect *C. burnetii* in Korean native cattle and dairy cattle nationwide by real-time PCR.

Materials and Methods

Standard DNA of *C. burnetii*

The chromosomal DNA extracted from phenol-killed, purified, and lysophilized cells of the *C. burnetii* Nine Mile strain, Phase II was procured for standard DNA (152.7 ng/μl) from National Institute of Health, Korea Centers for Disease Control and Prevention, Korea.

Samples

The total of 807 blood samples consisted of 622 Korean native cattle and 185 dairy cows, 170 individual milk samples of dairy cows, and 348 bulk tank milk samples of dairy herds were used in this study and those were collected from August 2010 to October 2011. The 200 μl of buffy coat was collected after centrifuging 1 ml of blood at 13,000 rpm for 1 min from each blood sample and stored at −20°C until being processed. The milk samples were also stored at −20°C.

Real-time PCR assay

DNA was extracted from each buffy coat and milk sample by Accu Prep Genomic DNA Extraction Kit (Bioneer, Korea)

[1]Corresponding author.
E-mail : kimdoo@kangwon.ac.kr

(9). For detection of *C. burnetii* in samples, trans-3 (forward primer, 5'-GTA ACG ATG CGC AGG CGA T-3') and trans-4 (reverse primer, 5'-CCA CCG CTT CGC TCG CTA-3') were synthesized by the Bioneer (Korea). Both trans-3 and trans-4 primers were designed to amplify a 243-bp fragment of the *IS1111* transposon-like repetitive gene in the *C. burnetii* strains (26).

Real-time PCR assay was performed with a model Rotor-gene 3000 (Corbett Research, Australia) under following conditions. For detection of *C. burnetii*, the PCR mixture (10 μl) included 5 μl of 2 X SYBR Green Premix Ex Taq (TAKARA BIO INC, Japan), 0.25 μl of 2.5 pM of each trans primer, 2 μl of template DNA, and distilled water to make up the reaction mixture volume. The cycling conditions for PCR included an initial denaturation at 95ºC for 30 s, followed by 40 cycles at 95ºC for 5 s, 60ºC for 20 s, and 72ºC for 10 s. Cycle threshold (*Ct*) values were determined by using fluorescence $10^{1.5}$ as the baseline. To determine the DNA concentration of each sample, a standard curve was generated from the *Ct* values of the amplification plots based on the standard *C. burnetii* DNA concentration with Rotor-Gene Real-Time Analysis Software 6.0 (Corbett Research, Australia). Assay specificity was confirmed by subjecting the PCR products to 1.5% agarose gel electrophoresis, DNA sequencing with the PCR product and melting curve analysis with a ramp from 72ºC to 95ºC at increments of 1ºC each step.

Results

The standardized real-time PCR assay was used for the detection of *C. burnetii* DNA in blood and milk samples from cattle. According to the calculated *Ct* values of standard DNA, the detection limit of *C. burnetii* was 0.2 bacteria/μl of blood and milk samples.

From blood samples of Korean native cattle, *C. burnetii* was detected in 17 out of 622 samples (2.7%) and the detection rates of Gangwon-do, Chungcheongnam-do and Jeju-do were 1.7%, 6.0% and 12.5%, respectively (Table 1). From blood samples of dairy cows, *C. burnetii* was detected in 2 out of 185 samples (1.1%). Detection rate of Gyeonggi-do was 1.8% and *C. burnetii* was not detected in Chungcheongnam-do (Table 2). The numbers of *C. burnetii* detected in blood samples ranged between 0.2 and 156.0 bacteria/μl.

From milk samples, *C. burnetii* was detected in 27 out of 170 individual milk samples (15.9%) of dairy cows and detection rates of Gyeonggi-do and Chungcheongnam-do were 4.9% and 32.8%, respectively (Table 3). And *C. burnetii* was detected in 84 out of 348 bulk tank milk samples (24.1%) of dairy herds in 9 provinces (Table 4). Among the 9 provinces, Chungcheongnam-do showed the highest detection rate (38.0%) of *C. burnetii* infection and Jollanam-do and Chungcheongbuk-do showed the lowest detection rates (10.0%) of *C. burnetii* infection. The numbers of *C. burnetii* detected in milk samples ranged between 0.2 and 747.2 bacteria/μl.

Table 1. Detection of *C. burnetii* from blood samples of Korean native cattle

Province	Samples	Positive	Detection rate (%)
Gangwon-do	532	9	1.7
Chungcheongnam-do	50	3	6.0
Jeju-do	40	5	12.5
Total	622	17	2.7

Table 2. Detection of *C. burnetii* from blood samples of dairy cows

Province	Samples	Positive	Detection rate (%)
Gyunggi-do	110	2	1.8
Chungcheongnam-do	75	0	0
Total	185	2	1.1

Table 3. Detection of *C. burnetii* from individual milk samples of dairy cows

Province	Samples	Positive	Detection rate (%)
Gyunggi-do	103	5	4.9
Chungcheongnam-do	67	22	32.8
Total	170	27	15.9

Table 4. Detection of *C. burnetii* from bulk tank milk samples of dairy herds

Province	Samples	Positive	Detection rate (%)
Gangwon-do	30	8	26.7
Gyeonggi-do	50	10	20.0
Gyeongsangnam-do	50	18	36.0
Gyeongsangbuk-do	39	10	25.6
Jeollanam-do	30	3	10.0
Jeollabuk-do	31	4	12.9
Jeju-do	38	9	23.7
Chungcheongnam-do	50	19	38.0
Chungcheongbuk-do	30	3	10.0
Total	348	84	24.1

Discussion

Since the first report in 1937, Q-fever has been reported almost worldwide (2). A study in the Spanish Basque region reported a Q-fever pneumonia prevalence of 18.8% in patients with community acquired pneumonia (24). In Greece, the prevalence of acute Q-fever was 4.7% in a cohort of 3,686 patients with atypical pneumonia (25). Until 2006, the number of notifications had ranged between 1 and 32 cases annually, with an average of 17 cases per year in Netherlands. However, between 2007 and 2011, the Netherlands experienced the largest documented Q-fever outbreak to date with a total of 4,108 notified acute Q-fever patients (27). And the outbreaks in humans were mainly associated with intensive dairy goat farming in Netherlands (9).

The genome sequence of *C. burnetii* strain 9Mi/I was determined and 20 unique copy of the *IS1111* transposase

gene were identified (23). The average number of *IS1111* genes per *C. burnetii* genome varied from 7 to 110 genes. However, the majority isolates have between 10 and 30 *IS1111* genes (17). High number of the *IS* gene in the *C. burnetii* genome and the unique regions has proved to be a useful tool in differentiating isolates of *C. burnetii* into groups (10). Real-time PCR performed with primers based on a repetitive, transposon like element (Trans-PCR) was proved to be highly specific and sensitive for the detection and the quantitative information of *C. burnetii* infection in clinical samples (29).

When infection occurs via the respiratory route, *C. burnetii* is internalized within eukaryotic cells in phagosome (19). Infected animals were bacteremic for 7-14 days, beginning 6 days after infection (28). After the short bateremia, *C. burnetii* was found in many organs in chronic infection. For this reason, detection rates of *C. burnetii* in blood samples were generally lower than those of milk samples. In this study, *C. burnetii* was detected only in 2.7% and 1.1% of blood samples of Korean native cattle and dairy cows, respectively.

In Netherlands, prevalence of *C. burnetii* infection of individual milk samples of lactating cows and bulk tank milk samples of dairy herds tested by PCR were 8.7% and 56.6%, respectively (20). The prevalence of *C. burnetii* infection in bulk tank milk samples of U.S. dairy herds tested by trans-PCR was 94.3%. Q-fever infection rates of individual cows in a bulk tank positive dairy herd based on *C. burnetii* shedding in their milk over 3 year (2002 to 2004) by Trans-PCR were from 52.8 to 23.5% (15). In Hungary, *C. burnetii* was detected in 8.7% of individual milk samples of dairy cows and 66.7% of bulk tank milk samples of dairy herds by *IS1111* element based real-time PCR (13). In this study, *C. burnetii* was detected in 15.9% of individual milk samples of dairy cows and in 24.1% of bulk tank milk samples of dairy herds in 9 provinces. The detection rate of *C. burnetii* of bulk tank milk samples was lower than those of other countries. However, this study revealed that chronic intramammary infection with *C. burnetii* was widespread in dairy cows of Korea.

Ingestion of *C. burnetii*-contaminated milk or milk products may result in serological conversion potentially indicating infection but not necessarily clinical disease (11). In addition, pasteurization of milk is defined by the Codex alimentarius Committee for Food Hygiene as 'pasteurization conditions are designed to effectively destroy the organisms *Mycobacterium tuberculosis* and *Coxiella burnetii*' (6). Thus the international definition points to the need for the destruction of *C. burnetii* to protect the health of milk consumers (7).

In conclusion, this study revealed that the detection rates of *C. burnetii* are considerably high in cattle and the infection of *C. burnetii* has been continuously occurring in cattle of Korea. In order to prevent the hazards of a zoonosis Q-fever that occur both humans and domestic animals, further studies are needed to clarify the epidemiology of Q-fever of domestic animals and humans in Korea.

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소에서 *Coxiella burnetii*의 검출

김요한 · 김두[1]

강원대학교 수의과대학 수의내과학교실

요 약 : *Coxiella burnetii*는 세포 내 기생하는 리케치아의 한 종류로 전세계에서 발병하는 인수공통 전염병인 큐열의 원인체이다. 국내에서 *C. burnetii*의 감염이 사람과 동물에서 보고되고 있지만 제한적인 지역에서만 실시되었다. 본 연구의 목적은 real-time PCR을 이용하여 소의 혈액과 유즙 시료에서 *C. burnetii*의 전국적인 검출률을 조사하는 것이다. 총 1,325 개 시료(한우 혈액 622 개, 젖소 혈액 185 개, 젖소 개체 유즙 170 개 및 젖소 집합유 348 개)를 전국적으로 채취하였다. 채취한 시료에서 Genomic DNA를 추출하였으며 *C. burnetii* 검출을 위한 real-time PCR을 실시하였다. 한우 혈액 622 개 중 17(2.7%) 개에서 *C. burnetii*가 검출되었고 젖소 혈액 185 개 중 2 (1.1%) 개에서 *C. burnetii*가 검출되었다. 젖소의 개체별 유즙 170 개 중 27(15.9%) 개에서 *C. burnetii*가 검출되었으며 집합유 348 개 중 84(24.1%) 개에서 *C. burnetii*가 검출되었다. 본 연구는 국내의 소가 *C. burnetii*에 높게 감염되어 있는 것을 확인하였으며, 인수공통질병인 큐열에 의한 가축과 사람의 피해를 예방하기 위하여, 가축과 사람의 큐열에 대한 추가적인 역학적 연구가 필요하다고 생각된다.

주요어 : *Coxiella burnetii*, Real-time PCR, 한우, 젖소