

## Seroprevalence of *Coxiella burnetii* Infection in Dairy Cattle and Non-symptomatic People for Routine Health Screening in Korea

We report results on the seroprevalence of antibodies to *Coxiella burnetii* in cattle and healthy people in Korea. Upon agreement with dairy owners, serum samples from 414 dairy cattle were collected between March and June 2001 and samples from 205 people for health screening were collected between April and December 2002. The sera were analyzed for the presence of anti-*C. burnetii* phase II antibodies using an indirect microimmunofluorescence test; strong fluorescence at a 1:32 dilution was regarded as positive. The overall seroprevalence of *C. burnetii* in cattle in Korea was 25.6%, with regional variation from 8.9 to 59.3%. Of the positive serum samples, 75.5% had antibody titers  $\geq 1:256$ . By contrast, only 1.5% of people in a rural area were seropositive, and most of the positive samples had low antibody titers. In conclusion, this study showed that relatively high seropositivity of *C. burnetii* in dairy cattle, accordingly, the studies on the high-risk groups are needed to evaluate the seroprevalence for this organism in Korea.

Key Words : *Coxiella burnetii*; Seroepidemiological Studies; Seroprevalence; Korea; Q Fever

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## INTRODUCTION

Q fever is a zoonotic disease that is caused by *Coxiella burnetii*, a microorganism that frequently infects domestic ungulates, as well as wild mammals in many genera (1). In animals, Q fever is rarely symptomatic, except for manifestations as reproductive disorders in females. The disease is transmitted to humans incidentally by inhalation of aerosols from infected cattle and sheep (1, 2). In humans, *C. burnetii* infection may be asymptomatic, acute, or chronic. Acute Q fever may manifest as pneumonia, hepatitis, or both. Chronic Q fever is rare, with endocarditis presenting as the most common complication (1, 3).

Q fever has been reported in almost every country, except New Zealand (4). In Canada, 6 to 20% of cats have anti-*C. burnetii* antibodies (5). In Japan, 60 to 84% of cattle with reproductive disorders are seropositive (6). A study conducted in southern France showed that 5 to 8% of endocarditis

cases in humans were due to *C. burnetii*, and the prevalence of acute Q fever was 50 cases per 100,000 inhabitants (7). Researchers have suggested that the incidence of Q fever is chronically underestimated because clinical manifestations of the disease are often nonspecific or even absent. Therefore, concerns with the disease focus on the importance of detection (1, 3, 8).

In Korea, there is a little information concerning the epidemiology of *C. burnetii* infection in either animals or humans. A few cases of acute Q fever in humans have been reported (9, 10). One study showed that the seroprevalence of anti-*C. burnetii* antibodies was less than 1% in healthy people in Korea (11).

We examined the prevalence of antibodies to *C. burnetii* in dairy cattle nationwide and in people for health screening in a rural area of Korea, and used the data to evaluate the impact of Q fever in both animals and humans in Korea. The analyses were done retrospectively.

## MATERIALS AND METHODS

### Subjects

Upon agreement with dairy owners, serum samples from 414 dairy cattle were collected on 31 farms from March to June, 2001. No clinical history of reproductive problems in the herds was obtained. All of the sampled cattle were more than 24 months old and female.

Serum was collected from people who visited Kangwon National University Hospital for health examinations between April and December 2002. The subjects were interviewed to confirm the absence of symptoms of respiratory tract infection during the preceding two weeks. All the sera were stored at -70°C until tested.

Informed consent was obtained from all people for health screening and the animals are treated by the ethical guidelines of Kangwon National University. This study was approved by the ethics committee of Kangwon National University Hospital.

### Indirect microimmunofluorescence antibody (IFA) assay

*Coxiella burnetii* phase II antigen (Nine Mile whole-cell antigen) was prepared as previously described at the National Institute of Infectious Diseases (NIID) in Tokyo, Japan (12), and dotted onto Teflon-printed glass slides. Each serum sample was diluted 1:16 with phosphate-buffered saline (PBS), overlaid on the antigen dots, and incubated for 45 min at 37°C in a moist chamber. The slides were subsequently washed twice for 5 min in PBS plus 0.05% Tween-20 and then incubated with a 1:1,400 dilution of fluorescein isothiocyanate (FITC)-conjugated rabbit anti-bovine IgG (Sigma-Aldrich, St. Louis, MO, U.S.A.) or FITC-conjugated rabbit anti-human IgG (DakoCytomation, Glostrup, Denmark) for 45 min at 37°C in a moist chamber. The slides were again washed twice using the same method and examined using fluorescence microscopy (Axioskop 2, Zeiss, Germany) at 200× magnification. We considered a sample positive if the 1:32 serum dilution

resulted in strong fluorescence. All sera that produced positive or equivocal reactions at 1:32 were further analyzed using 2-fold serial dilutions up to 1:4,096. The end point was the highest dilution showing complete fluorescence. Approximately 10% of the sera were divided and tested concurrently at Kangwon National University and at the NIID for quality control of test reproducibility; there was greater than 95% concordance between the results from the two laboratories.

## RESULTS

Table 1 shows the regional prevalence of antibodies against *C. burnetii* in dairy cattle. Each province had a relatively high prevalence, ranging from 8.9 to 59.3%; the overall national prevalence was 25.6%. Of the positive sera (n=106), 80 (75.5%) had high antibody titers ( $\geq 1:256$ ).

The mean age of the subjects was 43.7 yr (standard deviation 15.9, range 19-82), and the male-to-female ratio was 1:0.86 (n=110:95). Three (1.5%) of the 205 healthy people tested were seropositive. Of these, one had a serum sample with a high antibody titer (1:512), and two had samples with low antibody titers (1:64) (Table 2).

## DISCUSSION

This study examined the seroprevalence of *C. burnetii* infection. Table 2. Presence of anti-*C. burnetii* antibodies in people for health screening (n=205)

Titer of IgG antibodies to <i>C. burnetii</i>	No. of cases
$\leq 1:16$	203
1:32	0
1:64	2
1:128	0
1:256	0
1:512	1
Total	205

Table 1. Regional seroprevalence of antibodies against *C. burnetii* in dairy cattle

Province	No. tested	Number of sera with this titer								Seropositivity ( $\geq 1:32$ )
		$\leq 1:16$	1:32	1:64	1:128	1:256	1:512	1:1024	$\geq 1:2048$	
Kangwon	46	37	1	1	2	1	2	0	2	9 (19.6%)
Jeju	51	46	0	1	0	1	0	0	3	5 (9.8%)
Kyeonggi	27	11	0	1	2	5	3	1	4	16 (59.3%)
Chungnam	45	41	1	0	0	1	1	0	1	4 (8.9%)
Chungbuk	44	33	0	1	0	3	2	2	3	11 (25.0%)
Kyeongnam	51	37	2	1	0	1	3	0	7	14 (27.5%)
Kyeongbuk	50	31	4	2	2	0	2	2	7	19 (38.0%)
Jeonnam	50	40	1	1	1	2	1	2	2	10 (20.0%)
Jeonbuk	50	32	0	1	1	5	5	3	3	18 (36.0%)
Total (%)	414	308 (74.4)	9 (2.2)	9 (2.2)	8 (1.9)	19 (4.6)	19 (4.6)	10 (2.4)	32 (7.7)	106 (25.6)

tion in cattle and people for health screening in Korea. The sera of cattle showed a high prevalence (25.6%) of anti-*C. burnetii* antibodies, with 75.5% of the positive sera having high titers ( $\geq 1:256$ ). By contrast, the sera of healthy people showed a relatively low prevalence (1.5%) of anti-*C. burnetii* antibodies.

The sera of dairy cattle were collected regardless of the disease status of each animal, which is a limitation of this study. Since we could not find an association between a history of reproductive failure and seropositivity of the cows because of the absence of the data on the disease status, no prediction concerning seropositive status and reproductive problems can be made. However, *C. burnetii* infection is prevalent in all areas of Korea; every region had a seroprevalence above 8%, and the national prevalence was 25.6%. Moreover, the high prevalence of high-titer sera provides evidence that the disease might be very active in this country. In neighboring Japan, the seroprevalence of *C. burnetii* in healthy cattle ranges from 2 to 46%; in cattle with reproductive disorders, the range was 60 to 84% (6). Bildfell et al. reported that bovine placentitis was highly associated with the presence of *C. burnetii* (13). In addition, 9% of abortions in goats are reported to be caused by this microorganism (14). This is the first report to provide data that suggest that *C. burnetii* infection might be one of the important causes of reproductive problems in cattle in Korea. Further studies based on the isolation of *C. burnetii* are needed to elucidate the etiologic role of this microorganism in the reproductive problems of cattle in this country. Furthermore, the high seroprevalence of *C. burnetii* among cattle suggests the possibility of contamination of the environment around farms. Since *C. burnetii* is widely distributed in wild animals and ticks and causes Q fever to humans (1, 3), it is necessary to evaluate the environmental hazards associated with *C. burnetii* infection that may threaten public health in the near future. In addition, the survey on the high-risk group such as farmers and butchers is strongly needed.

The finding that people in a rural area demonstrated a relatively low seroprevalence of *C. burnetii*, as well as lower titers, suggests that infection with this microorganism seems to be relatively low in the rural area of Korea. In a previously published report (11), less than 1% of both healthy people and patients with fever of unknown origin tested positive for anti-*C. burnetii* antibodies using an IFA assay. Another report showed none of the sera from 70 healthy people was reactive for *C. burnetii* antigen (15). Recent data showed that 11 out of 448 healthy people were reactive (16). With sera from acute febrile episode, 11.5% were reactive (17). On the other hand, our group previously reported that only one of 88 patients with community-acquired pneumonia was diagnosed with Q fever with an IFA assay using both phase I and II antigens (18).

Despite the low seroprevalence of *C. burnetii* in humans, we cannot exclude the risk of Q fever to individuals with high-risk occupations, such as farmers, veterinarians, and meat-

processing workers, considering the high rate of infection observed in cattle in this study. Previous Korean report showed two of 46 stock breeders had an antibody titer of 1:20 (15), and 5 sera from 202 abattoir workers reacted with phase II antigen (16).

In conclusion, *C. burnetii* appears to be a highly prevalent pathogen in cattle in Korea and, accordingly, the studies on the high-risk groups are needed to evaluate the seroprevalence for this organism in Korea.

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