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# Serologic Survey and Risk Factors for *Coxiella burnetii* Infection among Dairy Cattle Farmers in Korea

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## ABSTRACT

**Background:** The zoonotic disease Q fever is caused by *Coxiella burnetii* and usually affects high-risk human populations. We conducted a serological survey of dairy cattle farmers in Korea to determine seroreactivity and identify risk factors for *C. burnetii* infection.

**Methods:** This cross-sectional study included 1,824 of 7,219 dairy cattle farms (25.3%) in the study region. The selected dairy cattle farmers visited the nearest public health centers or branches with completed questionnaires. Serum samples from the farmers were tested using an indirect immunofluorescence assay to detect phase II *C. burnetii* immunoglobulin (Ig) G or M antibodies.

**Results:** A total of 1,222 dairy cattle farmers from 784 dairy cattle farms (43.0%) participated in this study, and 11.0% (134/1,222) exhibited seroreactivity, defined as a phase II antigen IgG or IgM titer  $\geq 1:16$ . In the multivariate analysis, male sex, residence in Gyeonggi Province, a larger herd size, and ocular/oral contact with birth products during calf delivery were significantly associated with a higher risk of *C. burnetii* infection. Furthermore, the risk was significantly lower among farmers who always wore protective gloves while cleaning cattle excretion, compared to those who sometimes or rarely wore protective gloves.

**Conclusion:** Dairy cattle farmers should exercise caution by avoiding ocular/oral contact with birth products during calf delivery and by using protective equipment (including gloves).

**Keywords:** *Coxiella burnetii*; Dairy Cattle; Farmers; Risk Factors; Serologic Tests; Korea

## INTRODUCTION

Q fever is a widespread zoonotic disease caused by *Coxiella burnetii*, a bacterial pathogen that frequently infects both domestic (primarily cattle, sheep, and goats) and wild ruminants.<sup>1</sup> In animals, Q fever is usually asymptomatic or leads to reproductive disorders (e.g., abortion and stillbirth).<sup>2</sup> In humans, acute Q fever is similarly asymptomatic in most cases, or may involve non-specific, mild influenza-like symptoms. However, a small minority of patients may develop chronic Q fever infection.<sup>3</sup>

**Disclosure**

The authors have no potential conflicts of interest to disclose.

**Author Contributions**

Conceptualization: Lim HS, Chu H. Data curation: Park JH, Yoo SJ, Chu H, Hwang KJ. Formal analysis: Park JH. Investigation: Yoo SJ, Park JH, Lim HS. Writing - original draft: Park JH.

*C. burnetii* is transmitted to humans mainly via the inhalation of contaminated aerosols from infected animals that have shed *C. burnetii* in birth products, milk, and feces,<sup>4</sup> although the pathogen may also be transmitted via other routes, such as direct contact with contaminated materials and ingestion of unpasteurized milk.<sup>5</sup> *C. burnetii* infections mostly occur among high-risk populations that have contact with animals, such as livestock farmers, slaughterhouse workers, and veterinarians.<sup>6</sup> However, a large Q fever outbreak (> 4,000 cases) was reported in the Netherlands during 2007–2010.<sup>7</sup>

In Korea, Q fever was designated as a National Notifiable Infectious Disease in 2006, and approximately 10 cases of sporadic Q fever have been reported annually during 2006–2011.<sup>8</sup> *C. burnetii* infection involves two antigenic states, phases I and II. The levels of anti-phase II antibodies are high in acute disease, whereas elevated anti-phase I antibody levels are observed in chronic disease.<sup>6</sup> A serologic study of slaughterhouse workers in Korea identified reactive phase II antibodies among 10.2% of participants.<sup>9</sup> However, information regarding the serologic results and risk factors of *C. burnetii* infection in other high-risk groups, such as dairy farmers and veterinarians, remains limited.

In the current study, dairy cattle farmers, the largest group of dairy farmers in Korea, were asked to participate in a serologic survey to evaluate the seroreactivity to *C. burnetii* and risk factors for infection.

## METHODS

### Study population

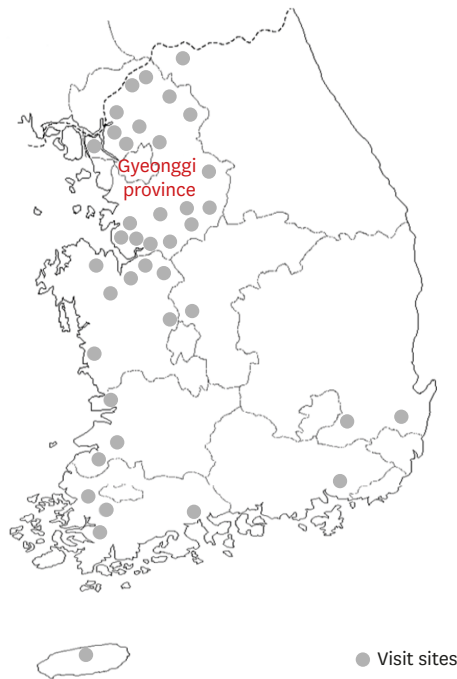
According to the Korean Statistical Information Service,<sup>10</sup> 2,889 (40.0%) and 4,330 (60.0%) dairy cattle farms were located in Gyeonggi Province and other Korean provinces, respectively, during the second quarter of 2008. In Gyeonggi Province, we selected densely populated areas with  $\geq 50$  dairy cattle farms per city/county/district or town/township (15 and 5 sites, respectively). In other Korean provinces, densely populated areas with  $\geq 20$  dairy cattle farms per town/township were selected (20 sites, **Fig. 1**). Of the 7,219 available dairy cattle farms, 1,824 (25.3%) were included in the study (912 farms in Gyeonggi Province and 912 farms in other Korean provinces). We requested the participation of up to 2 dairy cattle farmers per farm.

### Data collection

The selected dairy cattle farms were informed about the study and received questionnaires (developed in-house) by registered mail. The questionnaires comprised inquiries about the demographic characteristics, farm-related factors, and work hygiene-related factors, with the intent to collect information about potential risk factors. The dairy cattle farmers were then requested to visit the nearest of 15 public health centers or 25 public health center branches, where researchers identified or administered the participants' questionnaires by interview during their appointments. Blood samples were also obtained from the participants for *C. burnetii* antibody testing. This survey was conducted during November–December 2008 in Gyeonggi Province and during June–July 2010 in other Korean provinces.

### Serologic analysis

Serum samples obtained by centrifuging the collected blood were sent in a sealed icebox to the Korea Centers for Disease Control and Prevention (KCDC). An indirect immunofluorescence assay (IFA), the reference method of serologic diagnosis for Q fever,<sup>6</sup>



**Fig. 1.** Sites of serologic survey visits to evaluate the prevalence of *Coxiella burnetii* infection among dairy cattle farmers in Korea.

was used to detect phase II *C. burnetii* antibody immunoglobulin G (IgG) and immunoglobulin M (IgM) (Focus Diagnostics, Cyprus, CA, USA) according to the manufacturer's instruction. A positive phase II IgG or IgM antibody reaction at a dilution of  $\geq 1:16$  was considered seroreactive.

### Statistical analysis

A univariate logistic regression was used to calculate the odds ratios (ORs) with 95% confidence intervals (95% CIs). Subsequently, variables identified as significant ( $P < 0.05$ ) in the univariate analysis were included in a multivariate logistic regression. A backward stepwise logistic regression was applied, and all variables that met the 10.0% significance level in the likelihood ratio test were maintained in the final model. The indicator of statistical significance was set at  $< 0.05$ . SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for all analyses.

### Ethics statement

This study was reviewed and approved by the Institutional Review Board of Dongguk University Gyeongju Hospital (No. 2008-08-15 and 2010-10-11). Informed consent was obtained from all participants.

## RESULTS

A total of 1,222 dairy cattle farmers from 784 dairy cattle farms (43.0%) participated in this study (Fig. 2), including 704 (57.6%) farmers in Gyeonggi Province and 518 (42.4%) in other provinces. The participants comprised 773 (63.3%) men and 449 (36.7%) women, with a mean age of  $51.4 \pm 8.9$  years. The average duration of work was  $20.6 \pm 8.6$  years, and the average herd size was  $81.6 \pm 69.6$  dairy cattle.

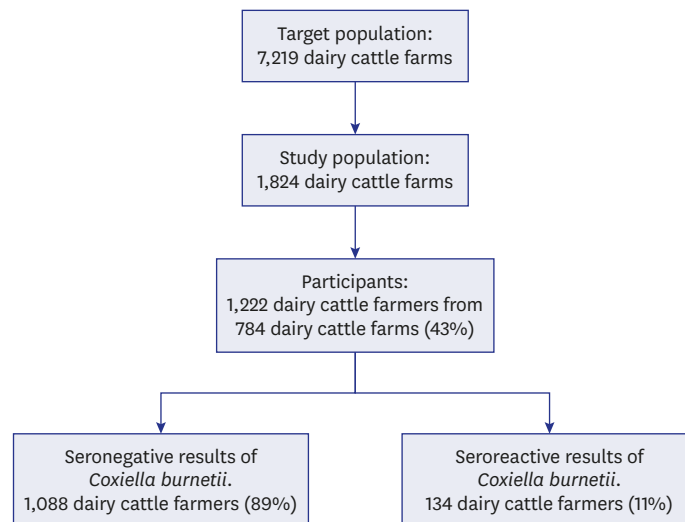


Fig. 2. Flowchart of serologic study participation.

### Seroreactivity of *C. burnetii* and serologic profiles

Overall, 134 of 1,222 participants (11.0%) exhibited seroreactivity for *C. burnetii*. The phase II IgG antibody titer cutoffs ranged from < 1:16 to 1:1,024, and 133 participants (10.9%) had IgG titers of  $\geq$  1:16. The phase II IgM antibody titer cutoffs ranged from < 1:16 to 1:16; however, only 1 participant (0.1%) had an IgM titer of  $\geq$  1:16 (Table 1).

### Univariate analysis of *C. burnetii* seroreactivity and associated risk factors

Generally, sex, region, and herd size were associated with a higher risk of *C. burnetii* infection. Men were significantly more frequently seroreactive than women ( $P = 0.034$ ), and the Gyeonggi Province region had a significantly higher prevalence of seroreactive dairy cattle farmers ( $P = 0.001$ ), compared to the other provinces. A large herd size ( $\geq$  100 dairy cattle) was significantly more likely to associate with *C. burnetii* seroreactivity, compared to a small herd size (< 50 dairy cattle) (OR, 1.99; 95% CI, 1.20–3.32) (Table 2). Additionally, the farming of beef or Korean native cattle was significantly associated with a lower *C. burnetii* infection ( $P = 0.002$ ). However, the status as a farm owner or family member ( $P = 0.191$ ), work type (e.g., feeding, milking, cleaning cattle excretion, compost production, calf delivery, and artificial insemination), and the consumption of raw milk were not significantly associated with *C. burnetii* infection ( $P = 0.562$ ) (Table 3), although the latter factor was rare (4.3% of participants).

Table 1. Distribution of titers against *Coxiella burnetii* phase II antigen among dairy cattle farmers in Korea

Titer	IgG		IgM	
	No.	(%)	No.	(%)
< 1:16	1,089	(89.1)	1,221	(99.9)
1:16	31	(2.5)	1	(0.1)
1:32	43	(3.5)	0	(0.0)
1:64	30	(2.5)	0	(0.0)
1:128	21	(1.7)	0	(0.0)
$\geq$ 1:256	8	(0.7)	0	(0.0)
Total	1,222	(100.0)	1,222	(100.0)

IgG = immunoglobulin G, IgM = immunoglobulin M.

**Table 2.** Associations of general characteristics with *Coxiella burnetii* seroreactivity among dairy cattle farmers in Korea

Variables	Total	Seroreactivity, No. (%)	OR (95% CI)	P value <sup>a</sup>
<b>Sex</b>				
Male	773	96 (12.4)	1.53 (1.03–2.28)	0.034
Female	449	38 (8.5)	Reference	
<b>Age, yr</b>				
< 45	219	23 (10.5)	Reference	0.987
45–59	797	84 (10.5)	1.00 (0.62–1.63)	
≥ 60	206	27 (13.1)	1.29 (0.71–2.32)	
<b>Duration of work, yr</b>				
< 15	282	29 (10.3)	Reference	0.627
15–29	740	84 (11.4)	1.12 (0.71–1.75)	
≥ 30	169	20 (11.8)	1.17 (0.64–2.14)	
<b>Region</b>				
Gyeonggi Province	704	96 (13.6)	1.99 (1.34–2.96)	0.001
Others	518	38 (7.3)	Reference	
<b>Herd size (dairy cattle)</b>				
< 50	387	33 (8.5)	Reference	0.209
50–99	610	67 (11.0)	1.32 (0.85–2.05)	
≥ 100	217	34 (15.7)	1.99 (1.20–3.32)	
<b>Education</b>				
Middle school or less	655	70 (10.7)	0.94 (0.65–1.34)	0.721
High school or more	565	64 (11.3)	Reference	

The questionnaire also solicited information about the use of personal protective equipment (e.g., glasses, masks, gloves, aprons, and boots) during work (e.g., milking, cleaning cattle excretion, artificial insemination, and calf delivery); ocular or oral contact with milk, feces, and birth products during work; and the habit of showering after work. In the univariate analysis, the ORs of *C. burnetii* infection were significantly lower among those who always wore protective gloves during milking (0.57; 95% CI, 0.39–0.84) and when cleaning cattle excretion (0.62; 95% CI, 0.40–0.96), compared to those who sometimes or rarely wore protective gloves. Additionally, ocular or oral contact with birth products during calf delivery had a significantly higher OR (1.50; 95% CI, 1.00–2.24), compared to no such contact with these products (Table 4). All results from the univariate analysis of the association of *C. burnetii* seroreactivity and work hygiene-related factors are available in the supplemental materials (Supplementary Tables 1–4).

### Multivariate analysis between *C. burnetii* seroreactivity and risk factors

The following factors identified as significant ( $P < 0.05$ ) in the univariate analysis were included in the multivariate model: sex, region, herd size, farm beef or Korean native cattle, wearing protective gloves during milking and while cleaning cattle excretion, and ocular/oral contact with birth products during calf delivery. After a backward stepwise regression, six variables remained in the model. Of these variables, male sex (OR, 1.79; 95% CI, 1.14–2.82), residence in Gyeonggi Province (OR, 2.10; 95% CI, 1.30–3.40), a large herd size ( $\geq 100$  dairy cattle; OR, 2.02; 95% CI, 1.18–3.45), and ocular/oral contact with birth products during calf delivery (OR, 1.54; 95% CI, 1.02–2.33) were significantly associated with a higher risk of *C. burnetii* infection. Furthermore, the risk of infection was significantly lower among farmers who always wore protective gloves while cleaning cattle excretion (OR, 0.63; 95% CI, 0.39–0.99), compared to those who sometimes or rarely wore protective gloves (Table 5).

**Table 3.** Associations of farm-related factors with *Coxiella burnetii* seroreactivity among dairy cattle farmers in Korea

Variables	Total	Seroreactivity, No. (%)	OR (95% CI)	P value <sup>a</sup>
Farm owners or their families				
Yes	1,180	128 (10.8)	0.55 (0.22–1.35)	0.191
No	33	6 (18.2)	Reference	
Separation between stalls and milking parlors				
Yes	1,037	118 (11.4)	Reference	
No	158	16 (10.1)	0.88 (0.51–1.52)	0.642
Purchase of dairy cattle (within 1 year)				
Yes	232	24 (10.3)	0.90 (0.57–1.44)	0.672
No	963	109 (11.3)	Reference	
Farm beef or Korean native cattle				
Yes	471	35 (7.4)	0.52 (0.35–0.78)	0.002
No	744	99 (13.3)	Reference	
Farm dogs				
Yes	574	70 (12.2)	1.25 (0.87–1.79)	0.220
No	641	64 (10.0)	Reference	
Consumption of raw milk				
Yes	52	7 (13.5)	1.27 (0.56–2.88)	0.562
No	1,167	127 (10.9)	Reference	
Feeding				
Yes	1,053	114 (10.8)	0.94 (0.56–1.57)	0.812
No	166	19 (11.4)	Reference	
Milking				
Yes	1,090	119 (10.9)	0.94 (0.53–1.66)	0.831
No	130	15 (11.5)	Reference	
Cleaning cattle excretion				
Yes	1,022	107 (10.5)	0.74 (0.47–1.16)	0.193
No	198	27 (13.6)	Reference	
Compost production				
Yes	796	90 (11.3)	1.13 (0.77–1.65)	0.543
No	423	43 (10.2)	Reference	
Calf delivery				
Yes	1,054	114 (10.8)	0.89 (0.53–1.47)	0.637
No	166	20 (12.0)	Reference	
Artificial insemination				
Yes	338	38 (11.2)	1.04 (0.70–1.54)	0.858
No	882	96 (10.9)	Reference	

**Table 4.** Associations of significant ( $P < 0.05$ ) work hygiene-related factors with *Coxiella burnetii* seroreactivity among dairy cattle farmers in Korea

Variables	Total	Seroreactivity, No. (%)	OR (95% CI)	P value <sup>a</sup>
During milking				
Wearing protective gloves				
Always	684	60 (8.8)	0.57 (0.39–0.84)	0.004
Sometimes or rarely	403	58 (14.4)	Reference	
No milking	130	15 (11.5)	0.78 (0.42–1.42)	0.412
While cleaning cattle excretion				
Wearing protective gloves				
Always	794	75 (9.4)	0.62 (0.40–0.96)	0.032
Sometimes or rarely	221	32 (14.5)	Reference	
No cleaning	198	27 (13.6)	0.93 (0.54–1.62)	0.804
During calf delivery				
Contact with birth products via eyes or mouth				
Yes	331	45 (13.6)	1.50 (1.00–2.24)	0.048
No	716	68 (9.5)	Reference	
No calf delivery	166	20 (12.0)	1.31 (0.77–2.22)	0.324

**Table 5.** Multivariate logistic regression analysis of variables associated with *Coxiella burnetii* seroreactivity among dairy cattle farmers in Korea

Variables	OR (95% CI)	P value
Sex		
Male	1.79 (1.14–2.82)	0.012
Female	Reference	
Region		
Gyeonggi Province	1.88 (1.24–2.86)	0.003
Others	Reference	
Herd size (dairy cattle)		
< 50	Reference	
50–99	1.31 (0.83–2.06)	0.249
≥ 100	2.02 (1.18–3.45)	0.010
Farm beef or Korean native cattle		
Yes	0.66 (0.43–1.01)	0.054
No	Reference	
Wearing protective gloves during cleaning cattle excretion		
Always	0.63 (0.39–0.99)	0.046
Sometimes or rarely	Reference	
No cleaning	1.13 (0.59–2.14)	0.715
Contact with birth products via eyes or mouth during calf delivery		
Yes	1.54 (1.02–2.33)	0.040
No	Reference	
No calf delivery	1.56 (0.85–2.88)	0.154

OR = odds ratio, CI = confidence interval.

## DISCUSSION

In this study, we observed an overall seroreactivity rate of 11.0% against *C. burnetii* and seroprevalence rate (phase II IgG or IgM titer  $\geq 1:32$ ) of 8.3% (102/1,222). These patterns applied to dairy cattle farmers nationwide. The *C. burnetii* seroprevalence was considerably higher among these farmers than among non-symptomatic people who attended routine health screenings in Korea (seroprevalence rate = 1.5%).<sup>11</sup> Another Korean study of slaughterhouse workers, a high-risk group for Q fever, reported a seroreactivity rate of 10.2%, similar to that observed among dairy cattle farmers in our study.<sup>9</sup> However, among European studies, a higher seroprevalence was reported among dairy cattle farmers in the Netherlands, compared to their Korean counterparts,<sup>12</sup> and a study of dairy cattle farmers in Denmark reported a seropositivity rate (defined as an IgG or IgM titer  $\geq 1:256$ ) of 3.1%; again, this exceeded our findings (0.7%; 8/1,222).<sup>13</sup> Regarding other Asian studies, a seroprevalence of 35.6% was reported among cattle farmers and farm residents in China.<sup>14</sup> In summary, we observed reduced seroprevalence and seroreactivity among dairy cattle farmers in Korea than among their counterparts in other countries.

In Korea, dairy cattle farms are characterized by family-based management and small-scale farming; in the former, men are more likely to participate in dairy cattle farming. The male-to-female ratio of Q fever prevalence was  $> 2:1$  in both Australia and France, where the extensive occupational exposure of men to Q fever is assumed to influence the ratio.<sup>3</sup> Similarly, a study of dairy cattle farmers in the Netherlands reported a higher seroprevalence among men than among women.<sup>12</sup> We additionally observed regional differences in *C. burnetii* seroreactivity between Gyeonggi Province and other provinces in Korea. The overall seroprevalence among dairy cattle in Korea was 25.6%, and the highest regional rate of



59.3% was observed among dairy cattle in Gyeonggi Province,<sup>11</sup> which corresponded with the high rate of seroreactivity among dairy cattle farmers in this province. Furthermore, a large herd size was associated with *C. burnetii* seroreactivity in our study, consistent with a study from the Netherlands wherein the seroprevalence of *C. burnetii* infection among dairy cattle farmers tended to increase with herd size.<sup>12</sup>

We further identified ocular or oral contact with birth products during calf delivery as a significant correlate of *C. burnetii* seroreactivity. *C. burnetii* are present in large populations in the birth products of infected dairy cattle; accordingly, direct contact with these products facilitates the spread of infection.<sup>15-17</sup> Our analysis revealed that wearing a protective mask during calf delivery was not significantly associated with *C. burnetii* infection. As healthcare personnel have been recommended to use N-95 masks when performing potentially aerosol-generating procedures for patients with suspected or confirmed Q fever,<sup>18</sup> we assume that dairy cattle farmers may be using insufficiently protective masks (e.g., cloth masks and face masks). *C. burnetii* is similarly present in feces, therefore dairy cattle farmers should minimize fecal exposure.<sup>4</sup> We found that among work hygiene-related factors, the consistent use of protective gloves while cleaning cattle excretion yielded a significantly lower OR for infection, compared to the occasional or infrequent use of such gloves. Full compliance with the use of gloves during and around calving was found to protect against *C. burnetii* infection in a cross-sectional study of dairy cattle farmers in the Netherlands.<sup>12</sup> In summary, gloves protect the hands from direct contact with feces and might lower the risk of fecal-oral transmission.

The consumption of raw milk has also been identified as a risk factor in the transmission of *C. burnetii*.<sup>19</sup> We note that dairy cattle farmers in Korea rarely consume raw milk and although this consumption was slightly more frequent among seroreactive dairy cattle farmers, it was not significantly associated with *C. burnetii* infection. Our univariate analysis also revealed a significantly lower number of farm beef or Korean native cattle among seroreactive dairy cattle farmers, although this significance was not maintained in the multivariate analysis. Interestingly, a previous study of the seroprevalence of *C. burnetii* infection among cattle in Korea observed a lower seroprevalence among Korean native cattle (1.7%) than among dairy cattle (10.5%).<sup>20</sup>

To the best of our knowledge, this was the first nationwide cross-sectional study of *C. burnetii* infection among dairy farmers in Korea. However, several factors might affect *C. burnetii* seroreactivity. First, a disproportionately large percentage of the farmers in the study were from Gyeonggi Province. Second, this study was conducted during the winter season in Gyeonggi Province and the summer season in the other provinces. As a Danish study of dairy cattle observed higher seroprevalence rates during the summer and winter,<sup>21</sup> we must consider the potential effects of seasonal differences on seroreactivity among dairy cattle farmers in Korea.

In conclusion, we observed a *C. burnetii* seroreactivity rate of 11.0% among dairy cattle farmers in Korea. Our multiple logistic regression analysis led us to identify several risk factors (male sex, residence in Gyeonggi Province, larger herd size, ocular/oral contact with birth products during calf delivery) and one protective factor (wearing protective gloves while cleaning cattle excretion). To prevent *C. burnetii* infection, dairy cattle farmers must take precautions during calf delivery to avoid ocular or oral contact with birth products and should fully comply with the recommended use of protective equipment, including gloves.



## SUPPLEMENTARY MATERIALS

### Supplementary Table 1

Associations of work hygiene-related factors during milking with *Coxiella burnetii* seroreactivity among dairy cattle farmers in Korea

[Click here to view](#)

### Supplementary Table 2

Associations of work hygiene-related factors while cleaning cattle excretion with *Coxiella burnetii* seroreactivity among dairy cattle farmers in Korea

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### Supplementary Table 3

Associations of work hygiene-related factors during artificial insemination with *Coxiella burnetii* seroreactivity among dairy cattle farmers in Korea

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### Supplementary Table 4

Associations of work hygiene-related factors during calf delivery with *Coxiella burnetii* seroreactivity among dairy cattle farmers in Korea

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