Shedding and extensive and prolonged environmental contamination of goat farms of Q fever patients by *Coxiella burnetii*

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Abstract
**Background:** A majority (>70%) of Q fever patients in South Korea do not have a history of animal contact. Therefore, unconscious environmental exposure is suspected. The aim of this study was to investigate exposure of Q fever patients to environmental contamination and animal shedding.

**Methods:** Two goat farmers were enrolled. One was diagnosed with Q fever 3 years ago (Farm 1). Among 20 goats on Farm 1, five were tested randomly and found to be Q fever PCR-positive. Three of the five were Q fever ELISA-positive. Two of five environmental samples taken in 2015 were PCR-positive. In 2018, 17 of 18 environmental samples were PCR-positive. On Farm 2, 54 of the 77 goats were PCR-positive, and 63 were ELISA-positive. Twelve of 14 environmental samples were PCR-positive. Repeat administration of oxytetracycline to goats led to a gradual reduction in PCR-positive tests over a 5-month period. However, PCR-positivity of the farm environment persisted for 5 months.

**Conclusion:** The environment on farms owned by Q fever patients was contaminated extensively and persistently, even after antibiotic treatment of goats and environmental decontamination. Undetected environmental contamination can be a major source of sporadic Q fever infection in South Korea.

**KEYWORDS**
*Coxiella burnetii*, environment, goat farm, Q fever, shedding
1  INTRODUCTION

Coxiella burnetii is an obligate intracellular Gram-negative bacterium that causes Q fever, a widespread zoonotic disease, in humans and animals (Angelakis & Raoult, 2010). It can infect a wide range of animals or livestock, and the main reservoirs are domestic ruminants such as sheep, goats, and cattle (Enright et al., 1971). In animals, Q fever is usually asymptomatic and sometimes causes reproductive problems (Rodolakis, 2009). Q fever may have various clinical manifestations in humans, including asymptomatic infection, febrile episodes with atypical pneumonia, hepatitis, myocarditis or pericarditis and meningoencephalitis (Angelakis & Raoult, 2010). After acute infection, a small portion of human patients progress to chronic Q fever (Parker et al., 2006). Currently, the dichotomy between ‘acute’ and ‘chronic’ Q fever is breaking down since various clinical manifestations that do not match the definition of conventional ‘acute’ or ‘chronic’ Q fever have been reported (Kampschreur et al., 2015; Million & Raoult, 2015). C. burnetii is transmitted to humans mainly via inhalation of contaminated aerosols from animal reservoirs that shed C. burnetii in birth products, faeces and milk (Maurin & Raoult, 1999).

In South Korea, the seroprevalence of C. burnetii in cattle is 0.4–10.5% (Lyoo et al., 2017; Na et al., 2016; Kim et al., 2014a; Seo et al., 2017; Hwang et al., 2020). Also, anti-C. burnetii antibodies have been detected in 13.0–41.4% of goats (Seong et al., 2020; Gang et al., 2016; Jung et al., 2014). In 1994, one study that examined C. burnetii antibody titres in 70 human samples from the general population reported titres of 1:20 against the C. burnetii Phase I antigen (Park et al., 1994). In 2002, a routine health screening reported IgG antibody titres against the Phase I antigen of ≥1:32 in 1.5% of samples (Kim et al., 2006). Q fever seroprevalence was 11.0% (Park et al., 2018a) among Korean dairy cattle farmers and 9.1% among Korean cattle slaughterhouse workers (Park et al., 2018b), both of which are high-risk groups for Q fever (Parker et al., 2006).

Epidemiologic studies of human Q fever in South Korea show that 14–23% of confirmed patients have high-risk occupations (agricultural farmers, livestock raisers, veterinarians and slaughterhouse workers); however, the majority of patients (77–86%) do not have high-risk occupations or contact with animals (Kim et al., 2021; Heo et al., 2019). These findings suggest that many reservoirs of C. burnetii are undetected in South Korea. Thus, unconscious exposure to airborne contaminants, or shedding by animals, can be major transmission routes in South Korea. This study was designed to investigate the duration and extent of environmental contamination by C. burnetii around animal farms with the aim of determining whether the environment is a potential source of Q fever in individuals with no recent animal contacts. Therefore, shedding and environmental contamination were investigated on two farms whose owners were diagnosed with acute Q fever.

2  MATERIALS AND METHODS

2.1  Patient enrolment

The medical records of 51 confirmed or probable Q fever patients diagnosed at a university hospital from January 2015 to February 2018 were examined retrospectively. Four Q fever patients with high-risk occupations and recent contact with animals were identified. Acute Q fever was defined as follows: seroconversion to the Phase II antigen; Phase II immunoglobulin G titres that differed between paired sera by more than fourfold (Biggs et al., 2016); or positive PCR or culture results with appropriate clinical findings. Cases with probable acute Q fever were defined as those with a Phase II IgG titre ≥1:128 in a single sample (Korea Disease Control and Prevention Agency, 2021a; Maurin & Raoult, 1999). Chronic Q fever was defined as follows: a Phase I IgG titre ≥1:800 (with the Phase I IgG titre being higher than the Phase II IgG titre); or a positive PCR or culture of C. burnetii from blood or clinical specimens. The C. burnetii antibody titre was determined in an immunofluorescence assay (IFA) using a commercial kit (Focus Diagnostics, Cypress, CA, USA). PCR was performed as described previously (Fournier & Raoult, 2003).

The four patients (two raised goats, one raised cattle and one raised deer) were contacted by telephone and asked to participate in the study. Only one goat farm owner agreed to participate (Farm 1). However, another goat farm was identified with the help of the Chungcheongbuk-do Institute of Veterinary Service and Research; on this farm, the owners (a couple) had been diagnosed with acute Q fever and some of their goats had suffered abortion (Farm 2).

2.2  Sampling and testing of the animals and environment for C. burnetii

Each farm was visited and samples were taken from the environment and from the animals. Farm 1 was located in peri-urban area 3 km from the city in Chungbuk Province of South Korea. It was an indoor farm with an area of 165 m². The farm had 20 goats in 2015 and 55 goats in 2018. Farm 2 was also a peri-urban indoor farm (661 m²) in Chungbuk Province and was located 10 km outside the city. It had 77 goats. Farms 1 and 2 were both breeding animals for meat production. For environmental sampling, dacron swabs premoistened with transport medium were used to collect samples from ground soil, feeding trays, straw, faeces and manure. Soil was also collected from fields near the farms (if the local farmers agreed to this). One swab was used per sampling spot, and the surface of the sampling spot was scrubbed by the swab 10–15 times. The amount of collected sample differed according to the nature of the collection spot; however, all were less than 1000 mg since small collecting swabs were used rather than a bulk collection system. Commercially available transport medium for Rickettsia (Asan pharmaceutical company, Korea) was used. Sampling spots of Farm 1 in 2015 were the floors of the four maternal-baby cages, and ground soil. Sampling spots in 2018 included all areas sampled in 2015 plus the feeding trays, water trays, soil, manure and soil from the field adjacent to the farm. Sampling spots on Farm 2 were soil, the floor of the barn, the floor of the maternal-baby cages, the floor or the male barns, the water trays, the feeding trays, the water in the water tray, and the floor of the farm owner’s house. Subsequent environmental samples were collected from the same spots at the next sampling visit with 5–6 week interval (Tables 1 and 2).
TABLE 1  PCR results from animal and environmental samples, and ELISA results from animal samples (Farm 1)

| Date       | Test Type                  | Female Positive/Total (Percentage) | Male Positive/Total (Percentage) | Number of positive results/number of tested environmental samples (positive percentage) |
|------------|----------------------------|------------------------------------|----------------------------------|-------------------------------------------------------------------------------------|
| Sep. 2015  | PCR (blood)                | 0/4 (0.0)                          | 0/1 (0.0)                        |                                                                                      |
|            | PCR (vaginal swab)        | 2/4 (50.0)                         | -                                |                                                                                      |
|            | ELISA (blood)             | 3/4 (75.0)                         | 0/1 (0.0)                        |                                                                                      |
|            | PCR (soil)\*              |                                    |                                  | 2\*/5\* (40.0)                                                                     |
| Aug. 2018  | PCR (blood)               | NA                                 | NA                               |                                                                                      |
|            | PCR (vaginal swab)        | NA                                 | -                                |                                                                                      |
|            | ELISA (blood)             | NA                                 | NA                               |                                                                                      |
|            | PCR (soil, manure)\*      |                                    |                                  | 17\*/18\* (94.4)                                                                   |

\*In 2015, environmental samples were collected from the floor of each maternal-baby cage and from the floor of the goat farm. The five maternal-baby cages had been empty for 3 years prior to testing.

\*Two samples from the floor of Q fever-affected maternal-baby cages were positive by PCR.

\*In 2018, environmental samples were collected from the floor of each maternal-baby cage, from the floors of different areas of the farm, from feeding trays, from water trays and from manure and soil in the field (the goats had no direct contact with the field).

\*Most of the collected samples were PCR-positive, except for one sample collected from the water tray.

TABLE 2  PCR results of animal/environmental samples, and ELISA results of animal samples (Farm 2)

| Date       | Test Type                  | Female Positive/Total (Percentage) | Male Positive/Total (Percentage) | Number of positive results/number of tested environmental samples (positive percentage) |
|------------|----------------------------|------------------------------------|----------------------------------|-------------------------------------------------------------------------------------|
| 13. Feb. 2018 | PCR (blood, vaginal swab) | 43/55 (78.2)                      | 11/22 (50.0)                    |                                                                                      |
|            | ELISA (blood)             | 42/55 (76.4)                      | 21/22 (95.5)                    |                                                                                      |
|            | PCR (soil, dust)\*        |                                    |                                  | 12\*/14\* (85.7)                                                                   |
| 23. Mar. 2018 | PCR (blood, vaginal swab) | 23/55 (41.8)                      | 4/22 (18.2)                     | 14/14 (100.0)                                                                       |
| 30. Apr. 2018 | PCR (blood, vaginal swab) | 19/23 (82.6)                      | 0/4 (0.0)                       | 14/14 (100.0)                                                                       |
|            | PCR (soil, dust)\*        |                                    |                                  |                                                                                      |
| 15. Jun. 2018 | PCR (blood, vaginal swab) | 1/19 (5.3)                        | -                               | 14/14 (100.0)                                                                       |
|            | PCR (soil, dust)\*        |                                    |                                  |                                                                                      |
| 16. Jul. 2018 | PCR (blood, vaginal swab) | 1/1 (100.0)                       |                                  | 14/14 (100.0)                                                                       |

\*Environmental samples were collected from different areas of the goat farm floor, from the feeding trays, and from the water trays.

\*All environmental samples were PCR-positive (the exceptions were one sample from the farm floor and one from the water tray [collected on the first sampling day]). All samples were persistently PCR-positive during follow-up environmental samplings.

Samples were also collected from four female and one male goat on Farm 1 in 2015. In 2018, the owner of Farm 1 refused animal sampling, so it was not performed. On Farm 2, all 55 female and 22 male goats were sampled at the first visit. At the first visit, 10 ml of blood was collected from all goats by jugular vein puncture and collected into EDTA vacutainers with the help of the Chungcheongbuk-do Institute of Veterinary Service and Research. Vaginal swabs were collected from female goats by veterinarians using sterile polyester swabs. Current levels of Q fever infection were determined by PCR of blood or vaginal swab samples. PCR-positive goats were sampled repeatedly at 5- to 6-week intervals. Seropositivity was examined by ELISA (IDEXX Laboratories, USA).

For PCR, DNA was extracted from environmental swabs and vaginal swabs after vortexing the swab-containing media for 25 min in 3500 rpm. Blood samples were treated with lysing beads (IDEXX, USA) and homogenised for 2 min at 2500 rpm using a homogeniser (Bertin, France). DNA extraction was performed using a DNA extraction kit (Nextractor, Korea) and PCR was carried out to detect a...
2.3 Follow-up tests for C. burnetii after treatment and environmental decontamination

On Farm 2, animals positive for C. burnetii by PCR received intramuscular (IM) injections of oxytetracycline (20 mg/kg; Berri et al., 2002; Angelakis & Raoult, 2010). One cycle of oxytetracycline treatment comprised two consecutive doses given at a 4-day interval. This one cycle treatment was repeated at 5- to 6-week intervals until the PCR results became negative. The farm environment was decontaminated with 5000 ppm hypochlorite solution. After each cycle of oxytetracycline treatment and environmental cleaning, animal and environmental sampling was repeated.

On Farm 1, the owner was diagnosed with Q fever in 2015, and samples from animals and the environment were collected; therefore, environmental samples were collected again in 2018. The owner was also retested for C. burnetii antibodies by IFA. He refused to allow animal sampling in 2018 since the animals showed no symptoms.

2.4 Ethics statements

The study was conducted in line with the study protocol approved by the Institutional Review Board of Chungbuk National University Hospital (IRB No. 2018-05-029). All participants provided informed consent.

3 RESULTS

3.1 Farm 1

In 2015, the patient (a 56-year-old male) was diagnosed with acute Q fever; symptoms included fever, myalgia and headache. IFA revealed more than fourfold rise in the IgG titre against C. burnetii Phase II antigen (from 1:256 to >1:2048). The Phase I IgG titre was <1:16. The patient was raising 20 goats. Although none were symptomatic, we performed Q fever tests on five randomly selected goats. Two samples were PCR-positive (two vaginal swabs) and three were ELISA-positive (three blood samples from female goats). Also, we collected five environmental samples (from the floor of the five maternal-baby cages); two were PCR-positive (Table 1).

Three years later (in 2018), the patient agreed to a follow-up IFA test and allowed re-sampling of the environment. The patient had no symptoms, and his C. burnetii IgG titre against Phase II antigen was >1:2048; the Phase I IgG titre was <1:16. The number of goats on the farm had increased to 55, and none had symptoms. In 2018, the patient refused animal sampling. Therefore, we just collected 18 environmental samples (from the floor of each farm sector, from the manure and from the field onto which the farmer spread the manure (the goats did not have direct contact with this field). Of the 18 environmental samples, 17 (94.4%) were PCR-positive (Table 1).

3.2 Farm 2

Both the farmer (a 66-year-old male) and his wife (a 58-year-old female) had acute Q fever in 2018; symptoms were fever, myalgia, headache and arthralgia. The patient’s IgG titre against C. burnetii Phase II antigen was 1:512, and that against Phase I antigen was 1:1024. His wife’s antibody titre against Phase II C. burnetii antigen was ≥1:2048, and that against Phase I antigen was 1:128.

Six female goats on Farm 2 recently suffered abortions or premature delivery within 40 days of onset of the patients’ symptoms. The Chungcheongbuk-do Institute of Veterinary Service and Research conducted a diagnostic investigation of the goats, including detection of bacterial, viral, and protozoal pathogens: Q fever was diagnosed. PCR revealed that 54 of 77 goats (70.1%) were positive for C. burnetii. In addition, 63 of 77 goats (81.8%) were positive for anti-C. burnetii antibodies.

PCR-positive goats were treated with sequential cycles of oxytetracycline. After each cycle, the farm environment was decontaminated using hypochlorite solution. After treatment and environmental decontamination, the animals and environment were subjected to repeat PCR testing. Even after treatment with oxytetracycline, a large portion of goats remained PCR-positive after each cycle (Table 1). Consecutive oxytetracycline treatments led to the number of PCR-positive animals decreasing slowly over 5 months (Table 2). Vaginal swabs from one female goat remained PCR-positive after four cycles of oxytetracycline treatment; ultimately, the animal was culled.

Most environmental samples remained PCR-positive for C. burnetii IS1111 after decontamination with hypochlorite solution (Table 2).

4 DISCUSSION

We found that the animals of Q fever patients harbored high levels of C. burnetii and that the environment was heavily contaminated. Shedding of C. burnetii by infected animals and subsequent contamination of the environment persisted even after repeated animal treatment and environmental decontamination. C. burnetii DNA was still detected in the environment of the farm in 2018, affected by Q fever in 2015, even though the farm animals had no symptoms thereafter and the farmer received antibiotics at the time of diagnosis.
South Korea started national surveillance for Q fever after designating it as a national notifiable infectious disease in 2006 (Korea Disease Control and Prevention Agency, 2021b). Human Q fever cases in South Korea have increased rapidly since 2015, reaching more than 160 cases per year in 2018 and 2019 (0.32 cases/100,000 persons per year; Korea Disease Control and Prevention Agency, 2021b). The reason for the rapid increase of Q fever in South Korea is not clear. Animal reservoirs, especially goats, are a major suspect as the number of goat farms has increased continuously (Korean Ministry of Agriculture, Food and Rural Affairs, 2021). In South Korea, about 8.6–19.1% of samples collected from goats between 2009 and 2019 were Q fever-seropositive by ELISA (Kim et al., 2014b; Seong et al., 2020; Jung et al., 2014). In some areas of South Korea, seropositivity of goats is as high as 36.8% (Kim et al., 2014b). Here, we found that goats raised by human Q fever patients showed high rates of Q fever infection: 40–70% were PCR-positive and 60–82% were seropositive. People who come from urban to rural areas tend to select goat farming because it is relatively easy to breed goats; however, they lack knowledge about zoonotic diseases, including Q fever, and how to prevent them (Choi et al., 2010; Park et al., 2018a). The findings presented herein suggest that the increased incidence of Q fever among goats in South Korea may be one of the main causes of the rapid increase of Q fever in humans.

Considering the mode of transmission of C. burnetii (inhalation of the aerosolised pathogen, as well as direct contact with infected animals), it could be inferred that many environments in South Korea are contaminated with C. burnetii. Currently, no studies have been published regarding detection of C. burnetii in environmental samples from South Korea. The large differences in the incidence of human Q fever in many regions of South Korea (from 0.01 cases per 100,000 persons per year in Incheon city to 0.53 cases per 100,000 persons per year in Chungbuk province) (Kim et al., 2021) suggest that the distribution of infected animal reservoirs may vary substantially nationwide. During a large Q fever outbreak in the Netherlands, control measures focusing on areas around Q fever-infected dairy farms led to the successful control of the outbreak (Roest et al., 2011). Further geological and epidemiological investigations, including of the regional incidence of human Q fever, animal Q fever, and environmental contamination, will provide more accurate information about Q fever-risk areas.

In this study, we found that eradicating C. burnetii from infected goat herds was challenging. A previous study showed that treatment of Q fever-infected sheep with oxytetracycline was not very effective (Astobiza et al., 2010), nor was it effective in Q fever-infected goats or cows (Álvarez-Alonso et al., 2018). Currently, oxytetracycline treatment of Q fever-infected animals lacks scientific evidence (Plummer et al., 2018). However, there are some reports of success after using oxytetracycline as a treatment for Q fever (Berri et al., 2002; Angelakis & Raoult, 2010). Here, we repeated the treatment cycle four times over a period of 5 months (two shots of oxytetracycline with a 4-day interval in-between). One goat remained positive after four cycles of treatment, so it was culled to prevent further spread of Q fever on the farm.

A study evaluating the shedding of C. burnetii in goat herds after an outbreak of Q fever-induced abortion revealed that vaginal mucus, faeces, and milk samples were PCR-positive in 32% of animals after 30 days from the abortion outbreak (Rousset et al., 2009). This long duration of shedding may be a reason for the decreased effectiveness of the treatment, since re-infection of treated goats by non-responders is possible. The environmental investigation revealed that the farms where owners and goats had been infected and treated in 2015 remained positive by PCR in 2018. Manure and soil samples from an adjacent field, which was enriched with manure, were also PCR-positive, suggesting that excreted C. burnetii persisted in manure and was then transferred to the field soil. C. burnetii in the field soil can then contaminate plants and other agricultural equipment.

A limitation of the study is that we did not perform cultures to find out whether the PCR-positive samples contained viable C. burnetii. However, we did confirm that the animals and environments around the Q fever patients harbor C. burnetii. A thorough epidemiological study, including animal tests and environmental surveys, will be helpful to identify hidden reservoirs of C. burnetii.

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AUTHOR CONTRIBUTIONS
Hyeon Seop Byeon: conceptualisation; investigation; resources; writing – original draft. Nattan Stalin: visualisation; writing – original draft. Jun Hyoung Kim: resources; validation. Seong Tae Han: investigation; resources. Mun Hui Chae: methodology; validation. Mi Na Han: methodology; resources. Byeongwoo Ahn: conceptualisation; data curation. Yong-Dae Kim: formal analysis; validation. Hee-Sung Kim: resources; validation. Hyewon Jeong: conceptualisation; data curation; formal analysis; writing – original draft; writing – review & editing.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

ETHICS STATEMENT
This study was approved by the institutional review board of Chungbuk National University Hospital (IRB no. 2018-05-029). All participants provide informed consent. Animals were handled and sampled in line with recommended guidelines. All animal sampling and treatment were conducted in strict accordance and adherence to relevant policies regarding animal handling as mandated under the act on the prevention of contagious animal diseases of Korea.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.
The peer review history for this article is available at https://publons.com/publon/10.1002/vms.3780.
The Netherlands: History, onset, response and reflection. Epidemiology & Infection, 139(1), 1–12.

Rousset, E., Berri, M., Durand, B., Dufour, P., Prigent, M., Delcroix, T., Touratier, A. & Rodolakis, A. (2009). Coxiella burnetii shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. Applied and Environmental Microbiology, 75, 428–433.

Seo, M.-G., Ouh, I.-O., Lee, S.-H., Kim, J. W., Rhee, M. H., Kwon, O.-D., Kim, T.-H. & Kwak, D. (2017). Prevalence of Coxiella burnetii in cattle at South Korean national breeding stock farms. PLoS One, 12, e0177478.

Seong, M.-H., Bak, J.-S., Youn, D.-K., Kim, H.-S., Ko, B.-H., Ham, J.-M. & Jeong, M.-H. (2020). Seroprevalence of Coxiella burnetii in Korean native goats in Gyeongnam province. Korean Journal of Veterinary Service, 43, 211–216.

Vaidya, V., Malik, S., Kaur, S., Kumar, S. & Barbuddhe, S. (2008). Comparison of PCR, immunofluorescence assay, and pathogen isolation for diagnosis of Q fever in humans with spontaneous abortions. Journal of Clinical Microbiology, 46, 2038–2044.

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