



Clinical and Genetic Features of *Coxiella burnetii* in a Patient with an Acute Febrile Illness in Korea

Seung Hun Lee,^{1*} Jung Yeon Heo,^{2*}
Hae Kyung Lee,¹ Yeong Seon Lee,¹
Hye Won Jeong,² and Seon Do Hwang¹

¹Division of Zoonoses, Center for Immunology & Pathology, Korea National Institute of Health, Korea Centers for Disease Control & Prevention, Cheongju, Korea; ²Division of Infectious Diseases, Department of Internal Medicine, Chungbuk National University College of Medicine, Cheongju, Korea

*Seung Hun Lee and Jung Yeon Heo contributed equally to this work.

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Address for Correspondence:

Seon Do Hwang, DVM, PhD
Division of Zoonoses, Center for Immunology & Pathology, Korea National Institute of Health, Korea Centers for Disease Control & Prevention, 187 Osongsaengmyeong 2-ro, Osong-eup, Heungdeok-gu, Cheongju 28159, Republic of Korea
E-mail: hwangsd@korea.kr

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Q fever, a zoonosis caused by *Coxiella burnetii* (an obligate intracellular bacterium), occurs worldwide. Not only cattle, sheep, and goats, but also other species (birds, arthropods, and ticks) may also act as reservoirs for *C. burnetii*. *C. burnetii* can survive for months in the environment; thus, transmission to humans can occur through direct (ingestion or skin inoculation) and indirect (aerosol inhalation) contact of contaminated environs (1). This caused several large outbreaks of Q fever in Europe (2). Q fever in humans is characterized by a nonspecific febrile illness that might be accompanied by various degrees of pneumonia or hepatitis. Since Q fever was first described in 1937 by Derrick, it has been considered an under recognized infectious disease because the nonspecific symptoms pose a diagnostic challenge (3). Previous serologic and bacteriologic studies have suggested that *C. burnetii* might be extensively distributed among host animals in Korea (4,5). Recently, reports of Q fever in Korea have increased, based on serologic test results (6). However, the microbiologic characteristics of *C. burnetii* have not been reported in Korean patients with Q fever; this is necessary to understand the route of infection and epidemiologic risk. To determine the detailed relationship between *C. burnetii* from different geographic origins and hosts, we present the first mo-

lecular analysis of *C. burnetii* from a patient with Q fever in Korea. Although Q fever is an important zoonotic infection with a worldwide distribution, no human isolates of *Coxiella burnetii* have been identified in Korea. For the first time, we identified the nucleotide sequence of *C. burnetii* from a 32-year-old man with an acute febrile illness in Korea. Diagnosis of acute Q fever was confirmed by seroconversion using indirect immunofluorescence antibody assays. Phylogenetic analysis demonstrated high sequence similarity (99.6%–100%) with *C. burnetii* 16S rRNA sequences identified from the reservoir. These results are the first genetic analysis of *C. burnetii* in a human case of Q fever in Korea.

Keywords: *Coxiella burnetii*; Q Fever; Korea; 16s rRNA

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A previously healthy 32-year-old man, an office worker living on the outskirts of Cheongju, Korea was hospitalized for an acute febrile illness in March 2016. He presented with a 5-day history of fever and headache. Physical examination of the chest, abdomen, and skin was initially unremarkable. On admission, his vital signs were: body temperature, 39.6°C; blood pressure, 140/80 mmHg; heart rate, 88 beats/min; and respiratory rate, 20 breaths/min. Complete blood count revealed a normal platelet ($217 \times 10^3/\mu\text{L}$) and white blood cell ($5,720/\mu\text{L}$) count, with 77% neutrophils and 17% lymphocytes. Chemistry test results showed elevated levels of C-reactive protein (8.27 mg/dL), aspartate aminotransferase (71 IU/L), and alanine transaminase (76 IU/L). Computed tomography revealed multiple, sub-centimeter lymph nodes in the porta hepatis, inter-aortocaval, and para-aortic areas. Other laboratory and imaging findings were within normal limits. Intravenous ceftriaxone 2 g per day was administered as empiric antibiotic treatment of the febrile illness. There was no bacterial or fungal growth from blood samples obtained for culture prior to antibiotic administration. Because of prolonged fever (> 7 days) despite antibiotic therapy, a serum sample was collected from the patient for specific *C. burnetii* anti-

body and nucleic acid detection on hospital day 4. However, he had no history of animal contact. The patient was discharged in an afebrile state after 9 days in hospital as his laboratory findings had normalized.

We used an indirect immunofluorescence antibody (IFA) assay from a commercial kit (IF0200G, IF0200M; Focus Diagnostics, Cypress, CA, USA) to examine specific antibodies to *C. burnetii*. The test was performed according to the manufacturer's instructions. First, the serum sample was screened at a 1:16 dilution against *C. burnetii* phase I and phase II antigens. In sequence, positive samples were diluted twofold from 1:16 to 1:2,048. Diagnosis of acute Q fever is serologically defined as a fourfold rise in the titer of phase II immunoglobulin G (IgG) from serum in the acute phase to the convalescent phase. We confirmed seroconversion between paired serum samples: the initial serum sample (taken on hospital day 4) demonstrated negative results in phase II IgM and IgG; whereas in the serum sample obtained 9 weeks later, the phase II IgG and IgM titers had increased to $\geq 1:2,048$ and 1:16, respectively.

Initially, no *C. burnetii* specific genes (16S rRNA, *IS1111*, and *outer membrane protein [OMP]*) were detected in the whole blood samples. After bacterial amplification of *C. burnetii* through animal experiments, nucleic acid of *C. burnetii* specific genes *IS1111*,

OMP, and 16S rRNA were detected using polymerase chain reaction (PCR); the genes were confirmed as *C. burnetii* specific strains using sequencing results. Four-week-old Balb/c mice were purchased from Orientbio (Seoul, Korea). All animals were maintained under Animal Biosafety Level 3 (ABL-3) conditions. All appropriate guidelines for the use and handling of infected animals were followed for the animals infected with *C. burnetii*. Buffy coats of *C. burnetii*-infected specimens were inoculated into immune-depressed Balb/c mice via the intraperitoneal route. On post-inoculation day 49, the spleens, livers, and lungs of the mice were harvested. Genomic DNA was extracted from inoculated mouse spleens using G-spin Total DNA Extraction Mini kit (Intronbio, Seongnam, Korea), according to the manufacturer's protocol. The extracted DNA specimens were stored at -20°C until analyzed. Nested PCR (nPCR) was used to amplify 16S rRNA of *C. burnetii*. First-round PCR was performed with the primers Cox16SF1 (50-CGTAGGAATCTACCTTRTAGWGG-30) and Cox16SR2 (50-GCCTACCCGC TTCTGGTACAATT-30), which produced amplicons with 1,321–1,429 bp. Then, nPCR was performed using the primers Cox16SF2 (50-TGAGAACTAGCTGTTGGRR-AGT-30) and Cox16SR2, which produced amplicons with 624–627 bp (7). Amplicons obtained from nPCR using the *Coxiella* specific primers Cox16F2 and Cox16R2 were analyzed using

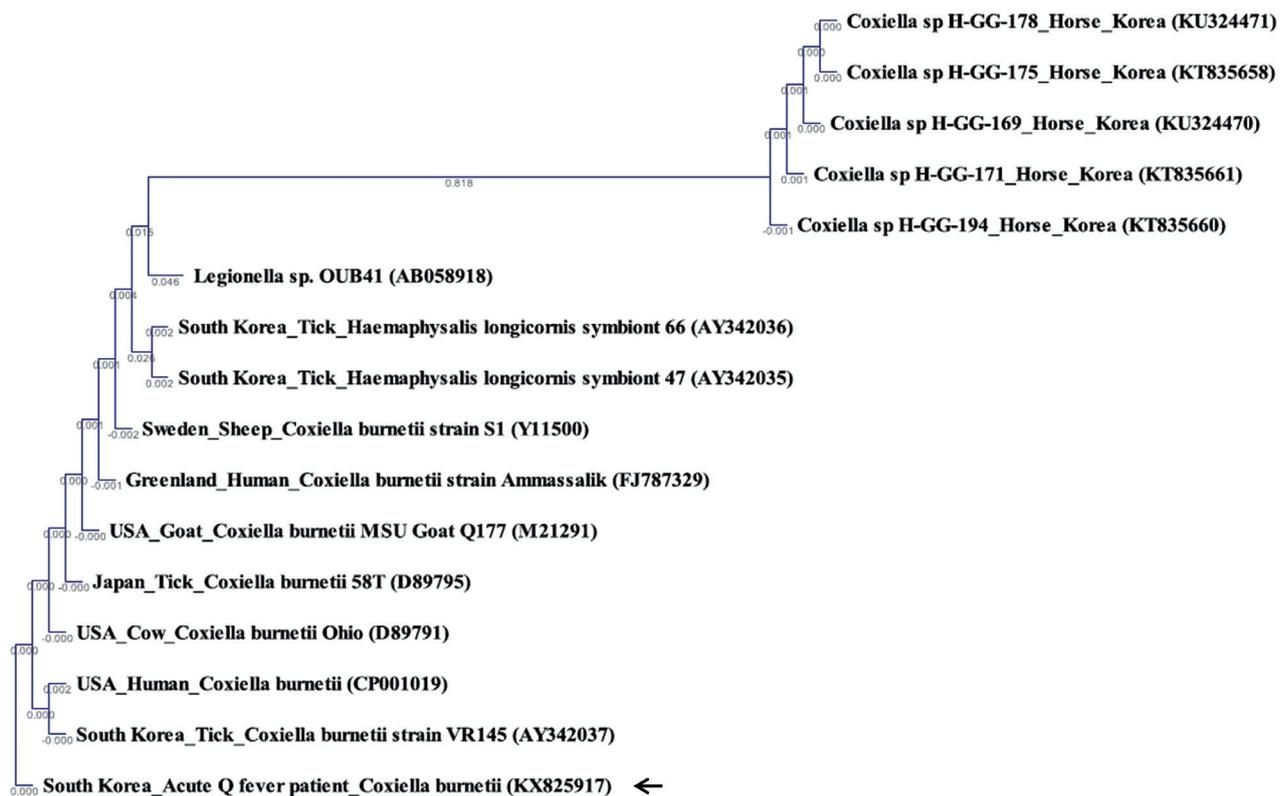


Fig. 1. Phylogenetic tree constructed by Jukes-Cantor/Neighbor joining methods based on 16S rRNA sequences of *Coxiella burnetii*. The sequence of *C. burnetii* obtained in this study is marked using arrows. The accession numbers of sequences obtained from GenBank are shown with the sequence names and countries of origin. Numbers on the branches indicate bootstrap support (1,000 replicates). Scale bars indicate phylogenetic distances of 0.35 nucleotide substitutions per position. The sequences of human *C. burnetii* (KX825917) were analyzed by Chungbuk National University Hospital and the Korea National Institute of Health.

CLC Main Workbench 7.6.4 software (CLC Bio, Aarhus, Denmark) by Jukes-Cantor/Neighbor Joining algorithms. Sequences of *Coxiella* 16S rRNA were aligned to determine homology. The stability of the proposed branching order was assessed by bootstrapping (1,000 replicates). The sequence of *C. burnetii* 16S rRNA obtained from the human patient's blood and replicated in Balb/c mice was deposited in GenBank (KX825917). The *C. burnetii* 16S rRNA sequence was compared to published sequences from GenBank. Phylogenetic analysis (Jukes-Cantor/Neighbor Joining) showed high similarity (99.6%–100%) with other strains of *C. burnetii* 16S rRNA sequences (Fig. 1). The sequence results were identical to those obtained from ticks in Korea (AY342037). Compared with *C. burnetii* 16S rRNA nucleotides (Y11500, D89791, D89795, FJ787329, M21291, and CP001019) and in view of regional variation, 16S rRNA sequences of this human case in Korea were different from the following *C. burnetii* strains: USA, Japan, Greenland, and Sweden (Y11500, D89791, D89795, FJ787329, M21291, and CP001019) (Fig. 2).

This is the first report to identify the nucleotide sequence of 16S rRNA from a patient with acute Q fever in Korea. Since first described in 1992, there have been reports describing the clinical characteristics of Q fever in Korea (8–10). *C. burnetii* has been isolated from the raw milk of dairy cows and has been detected in ticks (11–13). However, the diagnosis of Q fever in humans has mostly been achieved only via serologic testing (such as IFA assays), except in cases of chronic endocarditis, where the diagnosis has been confirmed by nucleic acid detection (14). Isolation of *C. burnetii* is not routinely recommended because it is a difficult and dangerous process. Furthermore, the PCR test performed on clinical specimens has low sensitivity (5%–24%) in acute Q fever and specimens must be obtained within the first 2 weeks of symptom onset and before antibiotic administration

(15). Hence, the microbiologic characteristics of *C. burnetii* in Korean patients has previously not been studied. However, it is essential to understand the microbiologic characteristics of *C. burnetii*—obtained by genetic analysis—to gain insight into the route of infection or epidemiologic risk. Although epidemiologic investigation showed that dairy goat and dairy sheep farms might lead to large outbreaks of human Q fever in the Netherlands, genetic analysis of *C. burnetii* played a key role in elucidating the sources of Q fever outbreaks (16,17).

In Korea, phylogenetic analysis of 16S rRNA sequences for *C. burnetii* has not previously been reported. In our study, we investigated the origin of Q fever in our patient by comparing the 16S rRNA sequence isolated with those isolated from other countries and hosts. The results of our phylogenetic analysis suggest that the source of Q fever in this case from Korea may not have been from a foreign country.

In our study, phylogenetic analysis of the 16S rRNA nucleotide sequence demonstrated that it was not related to the nucleotide sequences of *Coxiella*-like bacteria (CLB) in horses and ticks. A few cases of CLB identified from horses and ticks have been reported in Korea (8,13). A previous study showed that the sequences of CLB 16S rRNA were closely related to *C. burnetii*, indicating diversity within the genus *Coxiella* (18). Despite genetic similarities, CLB is genetically and etiologically distinct from *C. burnetii*. Although a few cases of Q fever in humans in Korea were linked to livestock such as goat and cattle, the primary source of *C. burnetii* was uncertain in most cases (19). Ticks can transmit both *C. burnetii* and CLB; hence, they might be a source leading to the emergence of Q fever (20). Therefore, it is necessary to confirm differences between our human isolate and CLB in Korea.

C. burnetii may be more widespread in South Korea than pre-

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
South Korea_Acute Q fever patient_Coxiella burnetii (KX825917)	1	100.00	100.00	99.81	99.61	94.22	92.29	93.64	99.61	96.53	96.53	37.46	37.46	37.46	37.46	37.46
South Korea_Tick_Coxiella burnetii strain VR145 (AY342037)	2	0	100.00	99.81	99.61	94.22	92.29	93.64	99.61	96.53	96.53	37.46	37.46	37.46	37.46	37.46
USA_Goat_Coxiella burnetii MSU Goat Q177 (M21291)	3	0	0	99.81	99.61	94.22	92.29	93.64	99.61	96.53	96.53	37.61	37.61	37.61	37.61	37.61
USA_Human_Coxiella burnetii (CP001019)	4	1	1	1	99.42	94.03	92.10	93.45	99.42	96.34	96.34	37.46	37.46	37.46	37.46	37.46
USA_Cow_Coxiella burnetii Ohio (D89791)	5	2	2	2	3	93.83	91.91	93.26	100.00	96.15	96.15	37.46	37.46	37.46	37.46	37.46
Sweden_Sheep_Coxiella burnetii strain S1 (Y11500)	6	30	30	30	31	32	97.96	88.05	93.83	90.94	90.94	35.89	35.89	35.89	35.89	35.89
Greenland_Human_Coxiella burnetii strain Ammassalik (FJ787329)	7	40	40	40	41	42	10	86.13	91.91	89.02	89.02	35.21	35.21	35.21	35.21	35.21
Legionella sp. OUB41 (AB058918)	8	33	33	33	34	35	62	72	93.26	91.71	91.71	37.02	37.02	37.02	37.02	37.02
Japan_Tick_Coxiella burnetii 58T (D89795)	9	2	2	2	3	0	32	42	35	96.15	96.15	37.46	37.46	37.46	37.46	37.46
South Korea_Tick_Haemaphysalis longicornis symbiont 47 (AY342035)	10	18	18	18	19	20	47	57	43	20	99.61	37.02	37.02	37.02	37.02	37.02
South Korea_Tick_Haemaphysalis longicornis symbiont 66 (AY342036)	11	18	18	18	19	20	47	57	43	20	2	36.87	36.87	36.87	36.87	36.87
Coxiella sp H-GG-169_Horse_Korea (KU324470)	12	424	424	423	424	424	434	438	427	424	427	428	99.85	100.00	100.00	99.85
Coxiella sp H-GG-171_Horse_Korea (KT835661)	13	424	424	423	424	424	434	438	427	424	427	428	1	99.85	99.85	99.85
Coxiella sp H-GG-175_Horse_Korea (KT835658)	14	424	424	423	424	424	434	438	427	424	427	428	0	1	100.00	99.85
Coxiella sp H-GG-178_Horse_Korea (KU324471)	15	424	424	423	424	424	434	438	427	424	427	428	0	1	0	99.85
Coxiella sp H-GG-194_Horse_Korea (KT835660)	16	424	424	423	424	424	434	438	427	424	427	428	1	1	1	1

Fig. 2. Comparison of *Coxiella burnetii* 16S rRNA nucleotide sequences. The upper matrix shows percent identity between the partial sequences of the *Coxiella* 16S rRNA gene. The lower matrix presents the number of differences in nucleotide bases.

viously reported. To understand the mode of transmission to humans, further attempts to cultivate *C. burnetii* are needed from animals, ticks, and environmental samples across Korea, and from more patients. Moreover, we believe that additional investigations into the pathogenicity and infectivity of these isolates are required to complete its characterization.

DISCLOSURE

The authors have no potential conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Conceptualization: Hwang SD. Data curation: Lee SH, Heo JY. Investigation: Lee SH, Lee HK, Lee YS, Jeong HW. Writing - original draft: Lee SH, Heo JY.

ORCID

Seung Hun Lee <http://orcid.org/0000-0002-9988-4250>
 Jung Yeon Heo <http://orcid.org/0000-0002-6548-1939>
 Hae Kyung Lee <http://orcid.org/0000-0002-9560-862X>
 Yeong Seon Lee <http://orcid.org/0000-0002-4082-128X>
 Hye Won Jeong <http://orcid.org/0000-0002-1063-8476>
 Seon Do Hwang <http://orcid.org/0000-0002-8679-2250>

REFERENCES

- Maurin M, Raoult D. Q fever. *Clin Microbiol Rev* 1999; 12: 518-53.
- Tissot-Dupont H, Raoult D. Q fever. *Infect Dis Clin North Am* 2008; 22: 505-14.
- Anderson A, Bijlmer H, Fournier PE, Graves S, Hartzell J, Kersh GJ, Limonard G, Marrie TJ, Massung RF, McQuiston JH, et al. Diagnosis and management of Q fever--United States, 2013: recommendations from CDC and the Q Fever Working Group. *MMWR Recomm Rep* 2013; 62: 1-30.
- Kim JY, Sung SR, Pyun JI, Her M, Kang SI, Lee HK, Jung SC. Seroprevalence of Q-fever in Korean native cattle. *Korean J Vet Res* 2014; 54: 147-50.
- Kim WJ, Hahn TW, Kim DY, Lee MG, Jung KS, Ogawa M, Kishimoto T, Lee ME, Lee SJ. Seroprevalence of *Coxiella burnetii* infection in dairy cattle and non-symptomatic people for routine health screening in Korea. *J Korean Med Sci* 2006; 21: 823-6.
- Korea Centers for Disease Control and Prevention. Disease web statistics system [Internet]. Available at <http://is.cdc.go.kr/dstat/index.jsp> [accessed on 1 October 2016].
- Duron O, Noël V, McCoy KD, Bonazzi M, Sidi-Boumedine K, Morel O, Vavre F, Zenner L, Jourdain E, Durand P, et al. The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, *Coxiella burnetii*. *PLoS Pathog* 2015; 11: e1004892.
- Seo MG, Lee SH, VanBik D, Ouh IO, Yun SH, Choi E, Park YS, Lee SE, Kim JW, Cho GJ, et al. Detection and genotyping of *Coxiella burnetii* and *Coxiella*-like bacteria in horses in South Korea. *PLoS One* 2016; 11: e0156710.
- Lee M, Jang JJ, Kim YS, Lee SO, Choi SH, Kim SH, Yu E. Clinicopathologic features of Q fever patients with acute hepatitis. *Korean J Pathol* 2012; 46: 10-4.
- An SJ, Koo JW, Chung CY, Lee WY. A case of Q fever: person-to-person transmission. *J Korean Pediatr Soc* 1998; 41: 120-4.
- Park HS, Lee EG, Lee SY, Lyu CJ, Son YM, Kim DS, Kim KY, Lee WY. A case of Q fever: associated with pancytopenia, hepatitis, and myocarditis. *Korean J Infect Dis* 1992; 24: 45-54.
- Park JY, Lee WY, Cho SN, Park YS, Park KS, Youn HJ, Kang YB, Koh CM. Isolation and cultivation of a *Coxiella burnetii* strain from raw milk of dairy cows in Korea. *J Korean Soc Microbiol* 1993; 28: 285-93.
- Lee JH, Park HS, Jang WJ, Koh SE, Park TK, Kang SS, Kim BJ, Kook YH, Park KH, Lee SH. Identification of the *Coxiella* sp. detected from *Haemaphysalis longicornis* ticks in Korea. *Microbiol Immunol* 2004; 48: 125-30.
- Moon SY, Choi YS, Park MY, Lee JA, Chung MK, Chung HS, Jung DR, Song JH, Peck KR. Two cases of Q fever endocarditis. *Infect Chemother* 2009; 41: 199-204.
- Fournier PE, Raoult D. Comparison of PCR and serology assays for early diagnosis of acute Q fever. *J Clin Microbiol* 2003; 41: 5094-8.
- Karagiannis I, Schimmer B, Van Lier A, Timen A, Schneeberger P, Van Rotterdam B, De Bruin A, Wijkman C, Rietveld A, Van Duynhoven Y. Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiol Infect* 2009; 137: 1283-94.
- Roest HI, Ruuls RC, Tilburg JJ, Nabuurs-Franssen MH, Klaassen CH, Vellema P, van den Brom R, Dercksen D, Wouda W, Spierenburg MA, et al. Molecular epidemiology of *Coxiella burnetii* from ruminants in Q fever outbreak, the Netherlands. *Emerg Infect Dis* 2011; 17: 668-75.
- Zhong J. *Coxiella*-like endosymbionts. In: Toman R, Heinzen, RA, Samuel JE, Mege JL, editors. *Coxiella Burnetii: Recent Advances and New Perspectives in Research of the Q Fever Bacterium*. New York, NY, Springer, 2012, p365-79.
- Kwak W, Chu H, Hwang S, Park JH, Hwang KJ, Gwack J, Choi YS, Youn SK, Park MY. Epidemiological characteristics of serologically confirmed Q fever cases in South Korea, 2006-2011. *Osong Public Health Res Perspect* 2013; 4: 34-8.
- Duron O, Sidi-Boumedine K, Rousset E, Moutailler S, Jourdain E. The importance of ticks in Q fever transmission: what has (and has not) been demonstrated? *Trends Parasitol* 2015; 31: 536-52.