NOTE *Wildlife Science*

The Prevalence of *Coxiella burnetii* Infection in Wild Korean Water Deer, Korea

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ABSTRACT. The aim of this study was to evaluate the prevalence of *Coxiella burnetti* infection in wild Korean water deer (*Hydropotes inermis argyropus*) in Korea, by using serology and real-time PCR analyses. One hundred ninety-six sera were collected from 4 provinces and tested for anti-*C. burnetii* antibody detection, by means of CHEKIT Q fever ELISA kit; and *C. burnetii* IS1111 insertion sequence detection, by means of real-time PCR. Antibodies were detected in 18 of the 196 (9.18%) serum samples, whereas genomes of *C. burnetii* were detected in 13 of the 196 (6.63%) serum samples. Based on overall high seroprevalence, the public health implications of these findings are important, because they indicate that asymptomatic seropositive or seronegative wild animals may be consistently shedding *C. burnetii*. This is the first study of *C. burnetii* prevalence in Korean water deer in the Republic of Korea that has indicated the presence of infected animals throughout the country.

KEY WORDS: *Coxiella burnetti*, prevalence, Q fever, wild Korean water deer.

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Q fever, caused by *Coxiella burnetii*, is a zoonosis that affects wild and domestic animals worldwide [10]. Farm animals and pets are the main reservoirs of infection, and transmission to humans is mainly accomplished through inhalation of contaminated aerosols [1].

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 [6, 11]. One study showed that the seroprevalence of anti-*C. burnetii* antibodies was less than 1% in healthy people in Korea [12]. Moreover, despite efforts to eradicate coxiellosis from cattle and farm-raised deer, the disease remains a serious risk for human and animal health in Korea [5, 8]. Despite the presence of Q fever in Korea, little is known about its current incidence and geographic distribution in wild animals. Moreover, there are no reports assessing Q fever in wild animals in the Republic of Korea.

There are 2 distinct water deer species in Far East Asia: The Chinese water deer (*Hydropotes inermis inermis*) is native to China along the Yangtze River; and the Korean water deer (*Hydropotes inermis argyropus*) is native to the Korean peninsula [7]. The Korean water deer has been classified as a vulnerable species on the International Union for Conservation of Nature and Natural Resources (IUCN) Red List, due to a serious decline, resulting from poaching and habitat destruction [4]. Recently, however, wild Korean water deer populations are growing in Korea, thus increasing the risk for disease transmission to humans and domestic animals [7].

Therefore, the objective of this was to evaluate by serology and real-time PCR analyses the prevalence of *C. burnetii* infection in wild Korean water deer in Korea.

One hundred ninety-six serum samples were obtained from wild Korean water deer captured in 4 provinces aged over 1 year (Gyeonggi province, 37°30′N and 127°15′E; Chungnam province, 36°21′N and 127°23′E; Jeonbuk province, 35°49′N and 127°09′E; and Jeonnam province, 35°10′N and 126°55′E) in the Republic of Korea, from January 2010 to December 2012. Blood samples were collected from the jugular vein into sterile 10 m*l* anticoagulant-free Vacutainers (BD Biosciences, Franklin Lakes, NJ, U.S.A.). Serum was separated from the samples and stored at −20°C, until ELISA and real-time PCR were performed.

The presence of antibodies against *C. burnetii* was determined using the ELISA CHEKIT Q-fever test (IDEXX Laboratories, Westbrook, ME, U.S.A.), according to the manufacturer's instructions. Briefly, serum samples were prepared at a 1:400 dilution, and specific antibodies consisting of Phase I and II *C. burnetii* were measured, using a peroxidase labeled anti-ruminant immunoglobulin G conjugate. The results are expressed as a percentage of the optical density (%OD) reading of the test sample, which was calculated as follows: $\%OD=100 \times (S - N)/ (P - N)$, where S, N and P are the OD values of the test sample and the negative and positive controls, respectively. On the basis of ELISA, sera were considered to be negative for *C. burnetii,* if the %OD was ≤30; intermediate, if the %OD was between 30 and 40; and positive, if the %OD was >40 [14]. Statistically significant differences ($P<0.05$) of seroprevalence in regions were determined by the χ^2 test, using SPSS 17.0 (SPSS Inc., Chicago, IL, U.S.A.).

The serum samples were prepared by DNA extraction kit for real-time PCR (QIAamp DNA Mini kit; QIAGEN,

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Table 1. Seroprevalence and real-time PCR of *C. burnetii* in 196 serum samples from wild Korean water deer of different regions in the Republic of Korea

Region		Serology			Real-time PCR	
	Tested (n)	Positive	Intermediate	Negative	Positive	Negative
Gyeonggi	35	2 (5.71%)	3 (8.58%)	30 (85.71%)	1 (2.86%)	34 (97.14%)
Chungnam	31	4 (12.90%)	2 (6.45%)	25 (80.65%)	3 (9.68%)	28 (90.32%)
Jeonbuk	93	9 (9.68%)	9 (9.68%)	75 (80.64%)	7 (7.53%)	86 (92.47%)
Jeonnam	37	3 (8.11%)	6 (16.21%)	28 (75.68%)	2 (5.41%)	35 (94.59%)
Total	196	18 (9.18%)	20 (10.21%)	158 (80.61%)	13 (6.63%)	183 (93.37%)

Table 2. Comparison of ELISA and real-time PCR results of *C. burnetii* in 196 serum samples from wild Korean water deer

		Real-time PCR		Total
		Positive	Negative	
ELISA	Positive	6	12	18
	Intermediate	1	19	20
	Negative	4	154	158
Total		11	185	196

Hilden, Germany). To amplify a 70-bp fragment, forward primer AAA ACG GAT AAA AAG AGT CTG TGG TT, reverse primer CCA CAC AAG CGC GAT TCA T and probe 6-carboxyfluorescein (FAM)-AAA GCA CTC ATT GAG CGC CGC G-6-carboxytetramethylrhodamine were used [15]. Real-time PCR was performed, as described by Schneeberger *et al*. [15]. Purified DNA (AmpliRun® COXIELLA BURNETII DNA CONTROL, Vircell, Spain) was used as a positive control.

As shown in Table 1, 18 (9.18%) of 196 sera had antibodies against *C. burnetii*. By region, 9 (9.68%) out of 93 samples from Jeonbuk province, 2 (5.71%) out of 35 samples from Gyeonggi province, 4 (12.90%) out of 31 samples from Chungnam province and 3 (8.11%) out of 37 samples from Jeonnam province were seropositive for *C. burnetii* antibodies. Moreover, 13 (6.63%) of 196 sera were real-time PCR positive for *C. burnetii*. By region, 7 (7.53%) out of 93 samples from Jeonbuk province, 1 (2.86%) out of 93 samples from Gyeonggi province, 3 (9.68%) out of 31 samples from Chungnam province and 2 (5.41%) out of 37 samples from Jeonnam province were detected as real-time PCR positive for *C. burnetii*. However, no statistical differences were observed among the regions.

Results of the correlation of detection between ELISA and real-time PCR from 196 sera are shown in Table 2. There were four discordant samples that were positive by real-time PCR but negative with ELISA. It has been reported that most of the animals infected with *C. burnetii* are asymptomatic [9, 10], appeared as healthy and excrete the microorganism, which serves as a significant source of infection to humans. In pet cat, 4 (1.3%) out of 310 cases were PCR-positive and were negative by ELISA, reported in Japan [9]. These results indicate that Korean water deer can be a reservoir of Q fever in humans.

In this study, positive reactions for ELISA and real-time PCR occurred in all 4 provinces, which were located in the western and central regions of the Republic of Korea. These results suggest that Q fever is present in the Korean water deer population, even though the Republic of Korea (33–38°N and 125–131°E) lies in a temperate zone with four distinct seasons. While there is no information on the occurrence of Q fever in Korean water deer, the warm summer season provides a suitable environment for ticks, which act as vectors and are found in habitats throughout the country.

To confirm the reactivity of water deer IgG used in Q fever ELISA kit, bovine IgG and water deer serum (IgG) were tested and used as coating material for ELISA kit, and HRP labeled anti-ruminant IgG conjugate was used as a secondary antibody in the kit. The results confirmed that water deer IgG reacts with HRP labeled anti-ruminant IgG conjugate.

Despite the presence of Q fever in Korea, little is known about its current incidence and geographic distribution in domestic and wild animals. A 2006 epidemiological study of livestock in Korea showed that 25.6% of dairy cattle with no clinical history of reproductive disorders were seropositive for *C. burnetii,* using an indirect microimmunofluorescence antibody assay [8]. Recently, 13 of 1,000 (1.3%) cattle, 10 of 604 (1.7%) elk and 0 of 30 (0%) Sika deer on farms had antibodies against *C. burnetii* [5]. Because of scare information on the prevalence of Q fever in farmed and wild animals in Korea, it is difficult to compare these results to the results of this study. Several studies of Q fever infection in wild animals, including wild ruminants, birds and rodents, have reported a high prevalence of *C. burnetii* in wildlife in Europe and Japan [2, 3, 9, 13]. On the basis of the results of present and previous studies in the Republic of Korea, Korean water deer have significantly high seroprevalence than domestic Korean cattle and sheep [5]. Therefore, Korean water deer could play a role as a wild reservoir in the epidemiology of Q fever in Korea. Human Q fever is more often transmitted by domestic animals, such as cattle and goats, than wild animals. However, the Korean water deer population has

recently become so widespread that the animals are common even in urban areas, resulting in increased contact with humans and domestic animals. Moreover, Korean water deer are the most common rescued wild animal in Korea.

In conclusion, this is the first description of Q fever in wild Korean water deer in the Republic of Korea by the detection of antibodies and genomes from *C. burnetii*. Based on overall high seroprevalence (9.18%) and PCR positive (6.63%), Korean water deer may serve as a wild reservoir of Q fever. Moreover, Q fever in wild Korean water deer is an important threat to the health of the Korean water deer population, as well as humans in the Republic of Korea. Further studies are required for the continuous monitoring of vector ticks and of Korean water deer in the Republic of Korea.

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