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Serological Evidence of *Coxiella burnetii* Infection in Wild Animals in Japan

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ABSTRACT: One hundred and thirty-four (26%) of 511 sera from 11 wild animal species in eight prefectures in Japan had antibody titers to *Coxiella burnetii* by the enzyme-linked immunosorbent assay. High prevalences were observed in Japanese black bears (*Ursus thibetanus*) (78%), Hokkaido deer (*Cervus nippon yesoensis*) (69%), Japanese hares (*Lepus brachyurus*) (63%), Japanese deer (*Cervus nippon centralis*) (56%), and to some extent in Japanese monkeys (*Macaca fuscata*) (28%). A low prevalence (13%) was observed in nutrias (*Myocastor coypus*). Japanese serows (*Capricornis crispus*), wild rats (*Muroides* sp.), raccoon dogs (*Nyctereutes procyonoides viverrinus*), wild pigs (*Sus scrofa leucomystax*), and masked palm civets (*Paguma larvata*) had no detectable antibodies to *C. burnetii*. Thus, six of 11 wild animal species in Japan were exposed to *C. burnetii*. Based on the high prevalences in some species, they may be a potential source of infection to both domestic animal and human populations.

Key words: Enzyme-linked immunosorbent assay, *Coxiella burnetii*, wild animals, Q fever.

Q fever is a zoonotic disease caused by *Coxiella burnetii*. Among humans, *C. burnetii* is considered an occupational hazard and has caused epidemics in abattoir and livestock workers, scientific workers, and pet owners (Marrie, 1990). Acute infection of the disease usually is associated with the sudden onset of influenza-like symptoms such as fever, headache, myalgias, and anorexia (McDade and Fishbein, 1988). Chronic infection can create serious hepatitis and endocarditis which appear years after infection and later may pose a life-threatening complication (Raoult et al., 1987; Peter et al., 1988).

Among domestic animals, *C. burnetii* infection usually is subclinical except for occasional mild respiratory signs and reproductive failures. Transmission usually is through aerosols, but arthropod vectors may be important among animals (Aitken, 1989).

There is little information on *C. burnetii* infection in wild animals, and it is not known whether the disease in wild animals actually poses a threat to both humans and domestic animals.

In Japan, there are no records of *C. burnetii* infection in wild animals. We recently found antibodies to *C. burnetii* in Japanese people and domestic animals (Khin Khin Htwe et al., 1992 a, b) which encouraged us to further investigate the disease in the wild. Our objective was to determine the prevalence of *C. burnetii* antibodies among wild animals of Japan.

Serum samples were aseptically collected from 11 species of live-trapped wild animals in eight prefectures in Japan. They included three species of wild ruminants of different habitats. The Japanese serow (*Capricornis crispus*), known locally as the Nihon kamoshika, prefers high altitude niches. The Japanese deer (*Cervus nippon centralis*) or shika, prefers low altitude forested lands. The Hokkaido deer (*Cervus nippon yesoensis*) or ezoshika inhabits only the island of Hokkaido. Other species sampled were the Japanese monkey (*Macaca fuscata*), wild rat (*Muroidea* sp.), Japanese raccoon dog (*Nyctereutes procyonoides viverrinus*), nutria (*Myocastor coypus*) (an introduced species in Japan), Japanese wild pig (*Sus scrofa leucomystax*), Japanese black bear (*Ursus thibetanus*), masked palm civet (*Paguma larvata*), and Japa-

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nese hare (*Lepus brachyurus*). Sample collection was conducted from June 1991 until August 1992 except for Japanese serows which were collected in 1981 and 1982. Wild animals were handled carefully to minimize stress during blood collection and were released back into the wild after the procedure.

Two prefectures in the Northern part of Japan (Hokkaido, 42° to 45°N, 140° to 145°E; and Iwate, 39° to 41°N, 141° to 142°E) and six prefectures in central Japan (Aichi, Fukui, Gifu, Hyogo, Mie, and Shiga; 34° to 37°N, 134° to 138°E) were sampled.

The COX ELISA-Coxiellosis (Q Fever) Antibody Test Kit, prepared by Dr. Lang (University of Guelph, Ontario, Canada), was used. The enzyme-linked immunosorbent assay (ELISA) was conducted according to Lang (1988) except that *o*-phenylenediamine (OPD) was used as the substrate.

A 1:1,600 dilution of conjugated peroxidases Protein A and G (Zymed Laboratory, Inc., San Francisco, California, USA) was used consistently throughout the study. This was the optimum dilution to elicit >0.1 optical density (OD) without a nonspecific reaction to either known positive human or bovine sera.

Falcon pro-bind microassay plates (Becton Dickinson Labware, Rutherford, New Jersey, USA) were coated with 50 μl per well of antigen diluted at 1:50 with distilled water. We added 50 μl per well of 5% trichloroacetic acid in distilled water. The plates then were transferred to an electric shaker for 30 sec and stored overnight at 4 C or kept at 37 C for 1 hr before use.

Phosphate buffered saline with 0.05% polyoxyethylene sorbitan monolaureate (Tween 20, Nacalai Tesque, Inc., Kyoto, Japan) (PBS Tween) was used as a washing solution. The solution also served as diluent to both the serum and enzyme conjugates. The plates were washed three times after each antigen coating, serum distribution, and conjugate binding.

In separate microassay plates, serum samples including positive and negative control sera were serially diluted (1:100 to 1:3,200) with PBS Tween, and 50 μl per well of the diluted serum was distributed to the washed antigen-coated plates. The plates then were shaken for 30 sec in an electric shaker and kept at 37 C for 1 hr.

After washing, 50 μl peroxidase conjugate (1:1,600 dilution) was pipetted into each well. The plates again were shaken and incubated in a wet chamber incubator at 37 C for 1 hr.

The plates were washed for the last time and 100 μl per well of substrate (50 mM citrate buffer at pH 5.1, 0.4 mg/ml of OPD and 0.012% hydrogen peroxide) was added to each well. After 30 min at 37 C, 50 μl per well of 4 N sulfuric acid was added to arrest the reaction. Immediately, OD was determined with a BioRad Microplate Reader model 450 (Nippon Bio-Rad Laboratories, Tokyo, Japan) at 420 nm.

Since no data were available on binding of Protein G and Protein A with immunoglobulins of wild animals, all serum samples first were tested with Protein G. Samples showing low or no reaction (<0.1 OD) were retested with Protein A.

The cut-off value was determined based on the bimodal distribution of antibody titer per species. Usually, two peaks of antibody titer distribution were observed in every species; a peak in a lower dilution and a peak in a higher one. The tapering end of the first peak, which was the start of the second peak, was considered the cut-off point for seropositive samples. Those species with only one peak at the low dilution were considered *Coxiella*-antibody negative.

Immunobinding of Protein G (absorbance range of 0.0 to 1.0 OD) was observed in all species examined except the black bear. However, the bear immunoglobulins had no reaction with Protein G but did react with Protein A when peroxidase conjugated Protein A was used.

One hundred thirty-four (26%) of the

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511 serum samples tested had antibodies to Q fever. Seropositive animals were found in all prefectures except Aichi.

The highest antibody prevalence was encountered in black bears (28 of 36, 78%). High prevalences also were recorded in Hokkaido deer (42 of 61, 69%), Japanese hares (5 of 8, 63%), Japanese deer (40 of 72, 56%), and to a lesser extent in the monkeys (15 of 54, 28%). A low prevalence was observed in nutria (4 of 32, 13%). No antibodies to *C. burnetii* were found in 117 serows, 54 wild rats, 37 raccoon dogs, 30 wild pigs, or 10 masked palm civets. There were no noticeable differences by sex in any species.

The cut-off titer varied by animal species. Animals with a high antibody prevalence, usually had a 1:100 cut-off titer, as was the case for black bears, Hokkaido deer, Japanese hares, and Japanese deer. For animals with a lower antibody prevalence such as monkeys and nutria, the cut-off titer was 1:400. The cut-off titer was difficult to determine in the Japanese serow, wild pig, raccoon dog, wild rat, and masked palm civet because only a single peak of occurrence was observed in these animals at very low serum titers. These species then were considered *C. burnetii*-antibody negative.

Infection of wild animals with *C. burnetii* long was thought to occur in Japan, but there were no data to support this speculation. Our data provide the first evidence that infection in wild animals does occur in Japan.

Four of the 11 species tested had an antibody prevalence of >50% to *C. burnetii*. If these seropositive hosts excrete the organism, they may be sources of *C. burnetti* for humans and domestic animals in Japan; but without more detailed studies this is speculative.

Based on the high prevalence of *Coxiella*-antibodies in black bears (78%), we propose that the animal may harbor a natural focus of *C. burnetii*. Babudieri (1959) cites earlier studies in which a co-existing host-parasite relationship of *Coxiella* infection was proposed to occur in other wild animal species.

The high prevalence of *C. burnetii* in Hokkaido deer (69%), Japanese hares (63%) and Japanese deer (56%) might be associated with the feeding habits of these animals, especially when one considers the presence of *C. burnetii* infection in Japan's livestock (Omori et al., 1952; Kitaoka, 1954; Yoshiie et al., 1990; Khin Khin Htwe et al., 1992b). Enright et al. (1971) associated feeding on contaminated sheep and cattle pastures with a high prevalence of *C. burnetii* antibody in wild animals.

The absence of infection in five species could be explained by three possibilities: geographic isolation, innate resistance to infection, or failure of the ELISA test to detect antibodies. Of these five species, only the Japanese serow occupied a geographically isolated location in its favored habitat of high-altitude, icy mountain tops. The raccoon dog and wild pig shared the same environment as seropositive animals. Resistance of seronegative species to infection with *C. burnetii* is possible, but can be proven only by experimental infection. In the absence of any species-specific anti-immunoglobulin conjugated to peroxidases, the ELISA was done with peroxidase conjugated Protein A and G. Although this was satisfactory for seropositive species, the binding may have been inadequate for seronegative species. Thus, the reason for seronegativity in these five species is still unknown and can be elucidated only by further study using experimental infections and more detailed studies on the mechanics of the ELISA test.

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