Serological Survey of *Rickettsia japonica* Infection in Dogs and Cats in Japan

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Antibodies against *Rickettsia japonica* in 20 of 1,207 dogs and 5 of 584 cats in Japan were detected using immunofluorescence. Some antibody-positive animals were detected in Niigata and Kagawa Prefectures, areas in which Japanese spotted fever in human patients has never been identified. Some animals were positive for antibodies against other new *Rickettsia* species.

Rickettsiae belong to the order *Rickettsiales* and are obligate intracellular, gram-negative bacteria. Several species cause disease in humans and other animals and have a worldwide distribution. The genus *Rickettsia* comprises the spotted-fever group rickettsiae and the typhus group (TG) rickettsiae (14). In Japan, *Rickettsia japonica*, classified within the spotted-fever group, is the causative agent of Japanese spotted fever (JSF) (12). In 1984, the first JSF patient was reported in Tokushima Prefecture, and since then most JSF patients have been identified in the western part of Japan (12). Recent epidemiological studies clarified both the vectors and the reservoirs for JSF in Japan. *Dermacentor taiwanensis* and *Haemaphysalis flavas* are confirmed vectors of *R. japonica* (10), and *Haemaphysalis longicornis* may also be a potential vector of the pathogen (17). The isolation of *R. japonica* from wild mice indicated that mice are a mammalian reservoir of the pathogen (19). Furthermore, dogs are also thought to be a mammalian reservoir of *R. japonica*, as antibodies against *R. japonica* have been detected in canine serum (7, 9). Dogs and cats are often exposed to a large number of tick species, depending on the distribution of these arthropod vectors in the environment (15, 16), and likely have an increased risk of tick bites compared to humans due at least in part to their activity in woodland and bush areas. Thus, dogs and cats can serve as valid indicators of the prevalence of JSF in a given area. In this study, the prevalence of antibodies against *R. japonica* in dogs and cats were surveyed in 35 prefectures in Japan.

Sera were collected from 1,207 dogs and 584 cats, all of which were domestic, examined in animal hospitals located within 35 prefectures (Hokkaido, Aomori, Akita, Miyagi, Fukushima, Tochigi, Ibaraki, Saitama, Chiba, Kanagawa, Niigata, Fukui, Nagano, Yamanashi, Shizuoka, Aichi, Gifu, Mie, Osaka, Kyoto, Nara, Wakayama, Hyogo, Tottori, Okayama, Yamaguchi, Kagawa, Tokushima, Kochi, Fukuoka, Nagasaki, Kumamoto, Miyazaki, and Okinawa) between July 2005 and July 2006. All the animals examined had experienced outdoor activity. The clinical status and epidemiological information, including the sex, age, and breed of each animal, the level of tick infestation, any history of travel, the clinical history, and the main location of activity, were recorded by the veterinarians treating these animals.

The sera were evaluated by indirect immunofluorescence analysis (IFA) using the *R. japonica* (strain Aoki) antigen, which was provided by H. Fujita. The bacteria were cultivated at 32°C in a tissue culture flask containing L929 cell monolayers. Heavily infected L929 cells were harvested and centrifuged at 10,000 × g for 10 min, and the pellet was suspended in phosphate-buffered saline with 3% fetal bovine serum. The suspension was applied to each well of the 18-well microscope slides. After the slides had been air dried, the cells were fixed in acetone for 15 min at room temperature. The detection of antibodies was carried out as described previously (18). The serum samples were screened at a 1:20 dilution in phosphate-buffered saline (pH 7.2) with 0.5% Tween 20. Fluorescein isothiocyanate-labeled rabbit anti-canine immunoglobulin G (Fc) conjugates (Rockland Inc., Gilbertsville, PA) or fluorescein isothiocyanate-labeled rabbit anti-feline immunoglobulin G (Fc) conjugates (Rockland Inc., Gilbertsville, PA) were used as the secondary antibodies for the IFA. Reactive antibodies were then detected using a fluorescence light microscope. End point titers were determined for those samples that reacted with the *R. japonica* antigen at the screening dilution. In this study, antibody titers of 1:40 or above were considered positive, as in a previous survey (7). The samples that were positive for antibodies against *R. japonica* were also examined for titers of antibodies against other domestic *Rickettsia* species, including *R. helvetica* (strain IP-1; H. Fujita), *R. tamurae* (strain AT-1; H. Fujita), and *R. asiatica* (strain IO-1; H. Fujita). The method was exactly the same as that described above.

Among the 1,207 dogs and 584 cats examined, 20 dogs (1.7%) and 5 cats (0.9%) had antibodies against *R. japonica*. Profiles of the positive animals are shown in Table 1. The end point titers of antibodies against *R. japonica* in the samples from the positive animals ranged from 1:40 to 1:320. Among the 25 animals that were positive for antibodies against *R. japonica*, 14 dogs and 5 cats also had antibodies against one or more of the other *Rickettsia* antigens (Table 1). Eight dogs (D1 to D8) showed the highest titers of antibodies against *R. japonica*, suggesting that these animals may indeed be infected...
with *R. japonica*. Furthermore, dogs D1 to D8 lived in seven prefectures, including Niigata, Mie, Osaka, Wakayama, Kagawa, Tokushima, and Fukuoka, and JSF patients have already been identified in six of these seven prefectures. In the present study, two dogs (D1 and D4) in Niigata Prefecture and another dog (D7) in Kagawa Prefecture, where JSF has not previously been reported, had significant titers of antibodies to *R. japonica*. These three dogs did not have histories of travel to other prefectures, indicating that *R. japonica* may exist in both Niigata and Kagawa Prefectures.

Another 14 animals (D9 to D17 and C1 to C5) were positive for antibodies against *R. japonica*, with end point titers ranging from 1:40 to 1:320; however, their sera also cross-reacted with other antigens at no more than one dilution. The *Rickettsia* species that stimulated the antibodies in these samples could not be determined because there may be cross-reactivity between closely related species (6). Because of the limited amounts of sera, cross-absorption analysis to determine the stimulant (6, 11) was not performed.

The remaining three positive animals (D18 to D20) showed titers of antibodies against *R. helvetica, R. tamurae, or R. asiatica* at least two dilutions higher than those of antibodies against *R. japonica*, indicating that the positive results for antibodies against *R. japonica* in these three animals may be attributable to the cross-reactivity of other *Rickettsia* antigens. For example, D18 and D19, from Shizuoka and Wakayama Prefectures, respectively, showed the highest titers of antibodies against *R. tamurae*, with titers ranging from 1:80 to 1:320. The antibodies titers in these two animals were considered to have been stimulated by *R. tamurae*. Another dog (D20) in Yamaguchi Prefecture also showed a positive titer of antibodies against *R. japonica*; however, the highest titers recorded were those of antibodies against *R. helvetica* and *R. tamurae* (1:640), suggesting that the dog may be infected with *R. helvetica* or *R. tamurae*, although there have been no reports of the existence of these *Rickettsia* species in Yamaguchi Prefecture.

Recently, new *Rickettsia* species have been isolated or detected in Japan. *R. helvetica* was previously known to exist only in European countries (2), but *R. helvetica* is now widespread in Japan, from Hokkaido, the northern island, to Kyusyu, a southern island (1, 5, 10). Indeed, the first human case of *R. helvetica* infection was reported in the Fukui Prefecture (13). In the present study, some dogs and cats were positive for *R. helvetica* antibodies, although the species that stimulated the antibodies in these samples could not be determined. Furthermore, other *Rickettsia* species, including *R. tamurae* (3) and the closely related species “Candidatus Rickettsia taraschevichiae,” were also detected in ticks isolated in Japan (5, 8). *R. asiatica* was also isolated from *Ixodes ovatus* (4). Despite the wide variety of *Rickettsia* species in Japan, little information is available on their pathogenesis and epidemiology, including vectors and reservoir animals for these *Rickettsia* species. Additionally, cross-reactivity with TG rickettsiae should be considered, although the numbers of patients with TG rickettsia infection are presently very low in Japan.

Some of the antibody-positive animals analyzed in this study had present or past illnesses (Table 1). Because there is little information available on the pathogenesis of the *Rickettsia* species that were detected in Japan, the relationships between positive antibody titers and illness are difficult to determine. Although none of these animals had fever and erythema, typ-

### Table 1. Profiles of dogs and cats with sera that reacted to *R. japonica* antigen with titers of 1:40 or more

| Animal | IFA titer of antibodies to antigen from: | Prefecture | Age (yr) | Breed | Sex | Present or past illness(es) or condition(s) |
|--------|----------------------------------------|------------|---------|-------|-----|--------------------------------------------|
| D1     | 80                                     | Niigata    | 13      | Beagle| F   | Mammary gland tumor                        |
| D2     | 80                                     | Shiba     | 2       |       | F   | None                                       |
| D3     | 40                                     | Osaka     | 12      | Mix   | M   | Diabetes mellitus                          |
| D4     | 40                                     | Niigata   | 3       | Pomeranian| F  | Otitis externa                             |
| D5     | 40                                     | Wakayama | 3       | Mix   | F   | Anemia                                     |
| D6     | 40                                     | Tokushima| 7       | Mix   | F   | Heart failure                               |
| D7     | 40                                     | Kagawa    | 9       | Mix   | M   | Testis tumor                               |
| D8     | 40                                     | Fukuoka   | 8       | Mix   | F   | None                                       |
| D9     | 320                                    | Mie       | 14      | Mix   | F   | Insect bite                                |
| D10    | 320                                    | Kochi     | 5       | Mix   | M   | Filarisis                                  |
| D11    | 320                                    | Nagasaki  | 9       | Mix   | F   | IVDP                                       |
| D12    | 80                                     | Mie       | 5       | Toy poodle| M  | Otitis externa, diarrhea                   |
| D13    | 80                                     | Hyogo     | 9       | German shepherd| M | None                                       |
| D14    | 40                                     | Chiba     | 13      | Mix   | F   | None                                       |
| D15    | 40                                     | Kanagawa  | 2       | Miniature dachshund| M | None                                       |
| D16    | 40                                     | Kagawa    | 8       | Beagle| M   | Filarisis                                  |
| D17    | 40                                     | Mie       | 11      | Great Pyrenees| M | Otitis externa                             |
| C1     | 80                                     | Hyogo     | 7       | Mix   | M   | FIV infection                              |
| C2     | 80                                     | Miyazaki  | 17      | Mix   | F   | Renal failure                              |
| C3     | 40                                     | Chiba     | 11      | Mix   | M   | Head tilt                                  |
| C4     | 40                                     | Mie       | 10      | Mix   | M   | None                                       |
| C5     | 80                                     | Mie       | 8       | Mix   | M   | Flea dermatitis                            |
| D18    | 40                                     | Shizuoka  | 2       | Cocker spaniel| M | None                                       |
| D19    | 40                                     | Wakayama | 11      | Maltese| M | Wound                                      |
| D20    | 80                                     | Yamaguchi | 6       | Akita | M | Filarisis                                  |

*Animal designations D1 to D20 represent dogs and C1 to C5 represent cats. Titters of ≥:40 are listed in bold. F, female; M, male; IVDP, intervertebral disk protrusion; FIV, feline immunodeficiency virus.*
tical symptoms related to spotted fever, some of them had severe conditions such as heart (D6) and renal (C2) failure. Other animals suffered from diabetes mellitus (D3) or canine immunodeficiency virus infection (C1). Such diseases can cause immunodeficiencies, which may result in the appearance of the clinical symptoms of *Rickettsia* infection. Further studies are required to clarify the pathogenesis of these *Rickettsia* species in domestic animals.

Finally, the present findings demonstrate *Rickettsia* antibody detection in cats in Japan, suggesting that cats may be possible carriers of the agents. Most cats have free-roaming habitats, and they suffer from tick infestation (19). As cats also live in close contact with humans, they may be important reservoir animals for tick-borne *Rickettsia*.

In conclusion, this study showed that blood or sera isolated from dogs and cats may supply much-needed information on the epidemiology of *Rickettsia* infection in Japan.

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