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## Short Communication

# A Serological Survey of *Leptospira* spp., Antibodies in Wild Raccoons (*Procyon lotor*) in Osaka, Japan

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## Abstract

**Background and objective:** Leptospirosis can cause a wide range of symptoms with an acute febrile disease in humans and animals. Recently, in Japan, there has been an increase in the number of feral raccoons (*Procyon lotor*) and they have been implicated as a source of zoonotic pathogens, including *Leptospira* spp. However, there are only a few reports on the prevalence of *Leptospira* spp. in wild animals, although 7 serovars of *Leptospira* spp. have been reported. The objective of this study was to examine seroprevalence of *Leptospira* spp. in raccoons. **Methodology:** The seroprevalence of *Leptospira* spp. antibodies was determined in 40 raccoons (*Procyon lotor*) in Osaka, Japan, from April-June, 2011. *Leptospira* spp., antibodies were detected in 23 raccoons (57.5%) using the Microscopic Agglutination Test (MAT). Antibodies against *Leptospira interrogans* serovar hebdomadis were most frequently detected (30.0%), followed by those against *Leptospira interrogans* serovar autumnalis (17.5%), *Leptospira kirschneri* serovar grippothyphosa (15.0%), *Leptospira interrogans* serovar copenhageni (7.5%) and *Leptospira interrogans* serovar australis (2.5%). **Results:** *Leptospira interrogans* serovars hebdomadis and australis were detected in all areas examined in Osaka. *Leptospira kirschneri* serovar grippothyphosa and *Leptospira interrogans* serovar copenhageni were detected only in animals from Southern Osaka, suggesting a regional distribution. Raccoons are environmentally adaptable omnivores and are expanding their range into urban areas and forests in Japan. **Conclusion:** Therefore, it is important to elucidate the potential risks that they may pose to public health by conducting surveys throughout Japan for zoonotic pathogens, such as *Leptospira* spp. that they may carry.

**Key words:** Japan, leptospirosis, raccoon, serological survey, urban area

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Leptospira* spp. can cause a wide range of symptoms, commonly presenting as an acute febrile disease in humans and animals. These bacteria are often transmitted through open skin, eyes or mucous membranes by contact with water contaminated with urine from infected animals<sup>1</sup>. In recent years, some serovars which were not protected by commercial vaccine were detected from companion animals in Japan as well as other urban areas<sup>2-4</sup>. The pet animals live together with human, hence might increase a risk of human leptospirosis. Wild animals are implicated as a source of infection in humans and domestic animals, thus, it is important to conduct epidemiological surveys of *Leptospira* spp. in wild animals to better understand and control the disease<sup>1</sup>. In Japan, 7 serovars of *Leptospira* spp. have been reported according to the domestic animal infectious disease control law. However, there are only a few reports on the prevalence of *Leptospira* spp. in wild animals in the country<sup>1,5,6</sup>.

Presently, there has been an increase in the number of feral raccoons (*Procyon lotor*) in Japan because of abandonment of pet raccoons imported to Japan from North America<sup>7</sup>. According to the Ministry of the Environment, the number of exterminations and captures increased from 9 in 1991 to >10,000 in 2006 and >14,000 in 2009. Raccoons living near houses cause damage to buildings and agriculture and have caused significant human injuries from attacks<sup>7</sup>. Additionally, raccoons have been implicated as a source of zoonotic pathogens, including *Leptospira* spp.<sup>8</sup>. The high prevalence of leptospirosis in raccoons have been reported at elsewhere<sup>9,10</sup> and the species could be potential risk for the spread of infection. However, in Japan, the serological study of leptospirosis of raccoons was quite limited in serovar at particular area<sup>11,12</sup>. Therefore, further study is required to determine the existence and distribution of raccoon leptospirosis. In this study, it is aimed to determine the seroprevalence of *Leptospira* spp. in raccoons in Osaka, Japan.

## MATERIALS AND METHODS

Serum samples obtained from 40 raccoons (*Procyon lotor*) captured in Osaka from April-June, 2011 were investigated with the cooperation of the Osaka local government. Each animal was captured and euthanized in accordance with the Invasive Alien Species Act. The section of Animal Protection and Livestock Management in Osaka Prefecture conducted the euthanasia of captured animals. Raccoons were captured from residential areas adjacent to forests or mountains in Osaka. Ten animals were captured from the Northern area, 10 from the Eastern area, 10 from the Western area and

20 from the Southern area. After euthanasia with an excessive quantity of an anesthetic, blood was collected from the jugular vein using a syringe and processed by centrifugation (1,180×g, 15 min). The serum was decanted and stored at 4°C until use. All experimental procedures using the animals and their samples were performed in accordance with the guidelines regulating animal use and ethics issued by the Animal Experiment Committee of Osaka Prefecture University.

Sera were tested using the Microscopic Agglutination Test (MAT) and a panel of 7 reference serotypes, as indicated by the standard method described by the world health organization<sup>13</sup>. Seven serovars were used: *Leptospira interrogans* serovars canicola, copenhageni, hebdomadis, autumnalis, australis and pomona, as well as *L. kirschneri* serovar grippothyphosa. The antibody titers for *L. interrogans* serovar copenhageni were considered as those for *L. interrogans* serovar icterohaemorrhagiae because serovar copenhageni belongs to serovar icterohaemorrhagiae. The threshold for antibody detection by MAT was set at a titer of 1:10 or higher.

## RESULTS

Among the 40 raccoons tested, at least one of the 7 antibodies was detected in 23 raccoons (57.5%) (Table 1). Serovars against *L. interrogans* serovar hebdomadis were most frequently detected (30.0%), followed by those against *L. interrogans* serovar autumnalis (17.5%), *L. kirschneri* serovar grippothyphosa (15.0%) and *L. interrogans* serovars copenhageni (7.5%) and australis (2.5%). Serovars against *L. interrogans* serovars canicola and pomona were not detected in any of the raccoons. Among the 23 raccoons in which antibodies were detected, only one serovar was detected in 18 (78.3%) raccoons. The range of the MAT titer was 1:10-1:320 for *L. interrogans* serovar hebdomadis, 1:20-1:160 for *L. interrogans* serovar australis and 1:20-1:320 for *L. kirschneri* serovar grippothyphosa and *L. interrogans* serovars hebdomadis and autumnalis. *Leptospira interrogans* serovars hebdomadis and autumnalis were detected throughout Osaka, whereas *L. kirschneri* serovar grippothyphosa and *L. interrogans* serovar copenhageni were detected only in raccoons from Southern Osaka (Fig. 1).

## DISCUSSION

In this survey, in addition to the serovars that were examined in other studies, it was examined *L. kirschneri* serovar grippothyphosa and *L. interrogans* serovar pomona. There have been limited investigations of antibodies against

Table 1: Reaction patterns and distribution of captured area in the Microscopic Agglutination Test (MAT) in sera from each raccoons (*Procyon lotor*, n = 23) with titers  $\geq 1:10$  to one or more leptospiral serovars

Captured area	Serovar						
	Canicola	Copenhageni	Hebdomadis	Autumnalis	Australis	Pomona	Grippothyphosa
Izumi, Kuroishi	-a	-	10	-	-	-	20
Sennan, Shinke	-	-	-	-	-	-	40
Kishiwada, Kamishirahara	-	20	-	20	-	-	-
Sennan, Shindachimutsuo	-	10	20	-	-	-	-
Izumisano, Oki	-	-	-	-	-	-	80
Hannan, Ishida	-	-	20	-	-	-	-
Izumi, Hakata	-	-	10	-	-	-	-
Kaizuka, Sobura	-	-	-	-	-	-	320
Kaizuka, Okawa	-	-	-	-	-	-	40
Kaizuka, Sobura	-	-	-	-	-	-	20
Takatsuki, Daizoji	-	-	-	160	-	-	-
Nose, ManoOhara	-	-	-	20	-	-	-
Hirakata, Sonenji	-	-	40	-	-	-	-
Ibaraki, Izuhara	-	-	10	-	-	-	-
Kawachinagano, Takihata	-	40	-	20	40	-	-
Kashiwara, Hondo	-	-	80	-	-	-	-
Kawachinagano, Amami	-	-	-	40	-	-	-
Kawachinagano, Hino	-	-	320	-	-	-	-
Yao, Korigawa	-	-	-	20	-	-	-
Taishicho, Tamuro	-	-	20	20	-	-	-
Tondabayashi, Tomigaoka	-	-	20	-	-	-	-
Tondabayashi, Tomigaoka	-	-	20	-	-	-	-
Tondabayashi, Tomigaoka	-	-	40	-	-	-	-

The serovar used in the study were *Leptospira interrogans* serovar canicola, *L. interrogans* serovar copenhageni, *L. interrogans* serovar hebdomadis, *L. interrogans* serovar autumnalis, *L. interrogans* serovar australis, *L. interrogans* serovar pomona, *L. kirschneri* serovar grippothyphosa, a: Negative result : A MAT-positive result was set at a titer of 1:10

*L. kirschneri* serovar grippothyphosa from raccoons in Japan and to the best of this knowledge, this is the first time that the antibodies have been detected in raccoons in Japan. In a previous survey conducted in Hokkaido, the serovar grippothyphosa was investigated, however, no antibodies against it were detected<sup>12</sup>. In previous clinical cases investigated by MAT using a single serum sample, a threshold titer of 1:800 was used to determine a positive result, although a titer of 1:100 is recommended by the National Veterinary Services Laboratories (NVSL) and WHO<sup>13,14</sup>. The detection limit set in the manual of the National Institute of Infectious Diseases in Japan is 1:40 and 1:10 has been recommended by another study, in addition to various other recommendations<sup>15,16</sup>. The titer value of  $\geq 1:100$  was considered to be positive for leptospirosis<sup>17</sup>.

In this study, the epidemiological investigation was conducted during a non-epidemic season, therefore, the threshold for antibody detection by MAT was set at a titer of  $\geq 1:10$ . In Japan, leptospirosis shows seasonality in occurrence, occurring mostly from August-October<sup>18</sup>. Therefore, it is possible that at the time of investigation, the antibody titers were low. The purpose of this survey was to determine the prevalence of antibodies against *Leptospira* spp. in the raccoon population, therefore, the titer threshold was set to a low value.

In North America, leptospirosis in dogs caused by *L. interrogans* serovar pomona and *L. kirschneri* serovar grippothyphosa is considered to be a serious disease and a vaccine against both these strains has been produced<sup>8</sup>. In Japan, a vaccine for canine leptospirosis, including serovars pomona, grippothyphosa, autumnalis and australis was recently produced. In this study, antibodies against *L. kirschneri* serovar grippothyphosa were detected at a relatively high frequency only in raccoons from Southern Osaka. Thus, this study suggests the possibility that *L. kirschneri* serovar grippothyphosa is regionally distributed in the Southern part of Osaka Prefecture. Further studies investigating antibodies against *L. kirschneri* serovar grippothyphosa are required.

In Japan, the most common serovars detected were canicola and icterohaemorrhagiae. However, in recent years, leptospirosis in dogs caused by *L. interrogans* serovar hebdomadis, which is not a notifiable infectious agent has increased<sup>2</sup>. In this study, raccoons presented with a high seroprevalence of *L. interrogans* serovar hebdomadis, which leads to the possibility that raccoons are a source of this zoonotic pathogen and should be investigated further<sup>8</sup>.

In urban and residential areas, animals living in close proximity to humans, such as mice and brown rats have been considered important reservoirs of disease-causing agents for

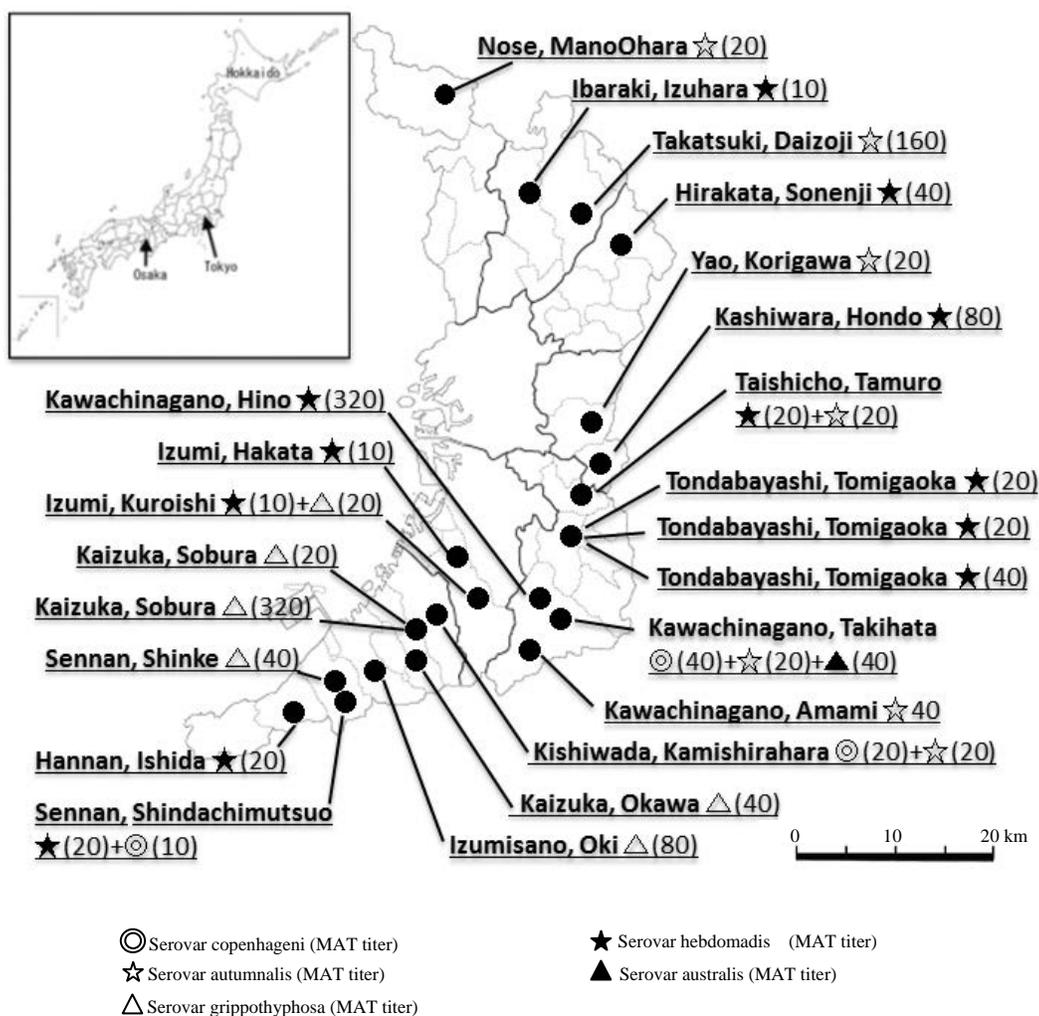


Fig. 1: Distribution of anti-*Leptospira* Microscopic Agglutination Test (MAT) antibodies from opportunistically sampled nuisance-culled raccoons (*Procyon lotor*) in Osaka Prefecture, Japan

humans and dogs<sup>11</sup>. The habitat area for raccoons is extending to urban areas in addition to forests in Japan. Therefore, it is important to continue to survey *Leptospira* spp. in raccoons, as well as in mice and other wild animals, to evaluate the potential impact on public health.

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