Seroprevalence of Leptospirosis in Korean Municipal Zoo Animals

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ABSTRACT. From 2002 to 2005, we collected 118 serum samples from 34 species belonging to 13 families of zoo animals in Korea and determined the prevalence of antibodies for 18 serovars of Leptospira spp. using the microscopic agglutination test (MAT). Twenty-nine (25%) of the serum samples tested were positive for one or more of the serovars. There were no significant differences in relation to genders: 23% and 26% of positives occurring in male and female animals, respectively (P>0.05). However, the seroprevalence for the Leptospira spp. was significantly higher (P<0.05) in herbivores (45%) than in either carnivores (17%) or omnivores (17%). Among the 5 serovars detected in this study, the most common was sejroe (n=27; 87% of all positive reactions). All positive reactions showed low titers (≤1:200) and the positives were most frequently detected in 1:25 (58%) and 1:50 (23%) serum dilutions. The highest antibody titer (1:200) was observed for the serovars sejroe (n=1) and bratislava (n=1). We conclude that the exposure of zoo animals to Leptospira spp. is relatively common in Korea and produces low MAT titers, with sejroe being the most commonly encountered serovar.

KEY WORDS: leptospirosis, seroprevalence, zoo animals.

Leptospirosis, caused by spirochetes of the genus Leptospira, is a zoonotic disease affecting domestic animals, wildlife, and humans worldwide [10]. The incidence of leptospirosis in wildlife is an important epidemiologic factor in the maintenance and spread of this disease to livestock and humans. In captive situations, animals are forced to live in close proximity, which may create opportunities for the spread of leptospiriosis. Indeed, studies have reported fetal infection and seropositivity against leptospires in zoo animals [11, 13]. In addition, zoological parks may potentially be sources of human leptospirosis [12]. In this study, we investigated the prevalence of antibodies against 18 Leptospira spp. in Korean zoo animals.

From 2002 to 2005, we collected blood samples from 118 zoo animals, including 34 species belonging to 13 families, which had been transferred to the Wild Animal Hospital in Daejeon Zoo Land (36°17’N, 127°23’E) for routine health checkups. We studied 56 male and 62 female animals, consisting of 64 carnivores, 31 herbivores, and 23 omnivores. All animals were apparently healthy as well as not vaccinated to the leptospiriae. Serum samples were stored at −20°C until examination.

Microscopic agglutination tests (MATs) were performed with 18 live Leptospira antigens of the following serovars: australis (Ballico), bratislava (Jez-Bratislava), autumnalis (Akiyami A), ballum (S102), bataviae (Van Tienen), canicola (Honda Utrecht IV), grippotyphosa (Andaman), hebdomadis (Hebdomadis), copenhageni (M 20), icterohaemorrhagiae (RGA), javanica (Veldrat Batavia 46), szwajizak (Szwajizak), pomona (Pomona), pyrogenes (Salinem), hardjo (Hardjoprajitno), sejroe (M 84), tarassovi (Perepelitsin), and patoc (Patoc 1). Fifty microliters of twofold-diluted serum samples ranging from 1:12.5 to 1:400 were prepared on a microtiter plate, and 0.05 ml of the live antigens were added to each well, respectively. The serum samples were incubated overnight at 4°C and examined under a dark field microscope [16]. Serum samples with agglutination of at least 50% of the leptospires at a ≥1:25 titer were considered positive [1]. For serum samples showing positive reactions against more than two serovars, the serovars that gave lower dilution steps than the highest recorded titer were considered as cross-reactions and excluded from further analyses. And if a serum sample had equal titers for more than 2 serovars, the source of infection was not assigned to one serovar or the other. Chi-square tests were used to analyze the differences in seroprevalence between the genders, and among the different eating habits (i.e., carnivores, herbivores, and omnivores). A P-value less than 0.05 was considered to be statistically significant.

Twenty-nine (25%) of the 118 serum samples showed leptospirotic titers of ≥1:25 against one or more serovars. Although the seroprevalence was higher for females (26%) than for males (23%), the difference was not statistically significant (P>0.05). The highest seroprevalence (45%) was detected in the herbivore group compared to the carnivore (17%) and omnivore (17%) groups (P<0.05; Table 1). The seroprevalence results in this study are different from those of a previous study of zoo and wild animals in Korea.
that did not show antibodies to *Leptospira* spp. except in two of 26 free-living rats (*Rattus rattus*) [14]. The difference in the number of samples tested and the difference in the MAT cutoff levels between the studies may be responsible for this discrepancy. Free-living rodents are virtually the only mammals able to come into contact with captive animals in zoos, and they have been reported to be the main reservoirs of pathogenic leptospires in nature; therefore, they may be a source of infection for other animals [15]. Hence, we speculate that free-living rodents harboring leptospires might infect zoo animals by coming near their cages. As shown in Table 1, the seroprevalence of herbivores for leptospires was higher than that of carnivores or omnivores. One possible explanation for this result is that herbivores may have more chances for contact with free-living rodents than animals in the other groups. These findings are similar to a previous study that showed a difference in the leptospiral infection rate according to the lifestyle of the species [8].

Among the 29 seropositive samples, 27 samples had titers for a single serovar and the remaining 2 samples had titers for 2 different serovars: 1 sample was cross-reacted with *autumnalis* and *sejroe* serovars at 1:25 serum dilution, and the other with *copenhageni* and *sejroe* serovars at 1:100. The predominant serovar was *sejroe* (n=27; 87% of all positive reactions). All positive reactions had low titers (≤ 1:200) and were most frequent at 1:25 (58%) and 1:50 (23%) serum dilutions. The highest antibody titer (1:200) was observed against the serovars *sejroe* (n=1) and *brat-
islava (n=1). Positive reactions were recorded for the autumnalis, bratislava, copenhageni,icterohaemorrhagiae, and sejroe serovars (Table 2).

It is difficult to clarify the infecting serovars without isolates, however, the MAT data can give a general impression about which serovars are present in herds. Although a higher cutoff value of the MAT was necessary for defining cases of leptospirosis [2], a lower cutoff value has been widely used in wild animals [1, 6, 7, 14]. Hence, titers of 1:25 or higher were considered as a positive reaction in the present study. According to our results, sejroe was the most prevalent serovar among those tested, in support of studies in which sejroe was reported as the most commonly detected serovar in humans and animals [3, 9].

Although the seroprevalence of leptospires in domestic animals or small rodents in Korea have been reported [4, 5], to our knowledge, few seroprevalence studies have involved zoo animals. In conclusion, the exposure of zoo animals to Leptospira spp. seems to be relatively common and produces low MAT titers, with sejroe being the most frequently encountered serovar.

REFERENCES