Prevalence of *Leptospira* spp. in the Kidneys of Wild Boars and Deer in JapanNobuo KOIZUMI¹⁾, Maki MUTO¹⁾, Akio YAMADA²⁾ and Haruo WATANABE³⁾¹⁾Departments of Bacteriology, ²⁾Veterinary Science and ³⁾Deputy of Director General, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku, Tokyo 162-8640, Japan

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ABSTRACT. We surveyed the prevalence of *Leptospira* spp. from 2005 to 2008 in wild boars and deer in Japan using polymerase chain reaction. Leptospiral *flaB* was detected in the kidneys of wild boars (positive ratio, 15.2%; 22 of 145) from 9 prefectures and a deer (1.1%; 1 of 94) from 1 prefecture in Japan. There was no annual change in the prevalence of positive animals during the investigation period (chi-squared test, $p=0.94$) or in the prevalence in male and female wild boars in the 2007 to 2008 season (Fisher's exact test, $P=0.45$). The *Leptospira* species harbored by these animals were deduced to be *L. interrogans* (from 22 animals) and *L. borgpetersenii* (from 1 animal).

KEY WORDS: deer, *Leptospira*, reservoir, wild boar.

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Leptospirosis, which is caused by infection with pathogenic *Leptospira*, is an important zoonotic disease distributed worldwide [2, 8]. Leptospire infect the proximal renal tubules of various mammals and are excreted in the urine of carrier animals. Any mammal can be a carrier and excretor of leptospire [5]. Transmission of *Leptospira* to mammals occurs mainly through indirect contact with water or soil contaminated with the urine of infected animals. Important reservoir animals of leptospirosis for human transmission are rats and mice that live near human habitats, domestic animals such as cattle and swine and companion animals, especially dogs. Feral animals, except rats and mice, also serve as important carriers of leptospire [2, 5]. In wild boar (*Sus scrofa*), antibodies against leptospire and leptospiral cells in kidney tissues have been detected [3, 6, 11]. Leptospiral antibodies have also been found in various species of deer [3, 4, 9, 10], and leptospirosis has frequently been reported in farmed deer in New Zealand [1]. We recently detected leptospiral DNA in the kidneys of wild boars and deer (*Cervus nippon*) in Miyazaki Prefecture, Japan [7]. The population density of wild boars and deer has recently increased in Japan. These animals migrate close to human habitats and damage agricultural crops. Thus, they may pose a risk of transmission of leptospirosis to humans in various regions of Japan if they carry leptospire. In this study, in order to assess the potential role of these feral animals as reservoirs of leptospire throughout Japan, we attempted to detect leptospiral DNA in the kidneys of wild boars and deer by polymerase chain reaction.

Kidney tissues from wild boars and deer captured in the hunting season from 2005 to 2008 were used in this study. DNA was extracted from these tissues using a DNeasy Tissue Kit (Qiagen), the leptospiral *flaB* gene was amplified by nested PCR [7] and the nucleotide sequences of the ampli-

cons were then determined.

Leptospiral *flaB* was detected in the kidneys of wild boars (positive ratio, 15.2%; 22 of 145) from 9 prefectures and 1 deer (1.1%; 1 of 94) from Hokkaido, Japan (Table 1 and Fig. 1). The sensitivity of this assay was 1 to 2 genome equivalents per reaction (data not shown). Thus, the number of leptospire was estimated to be at least 8000 cells per gram of kidney tissue, although we did not perform a quantitative analysis. Wild boars carried leptospire more frequently than deer (Fisher's exact test, $p<0.01$), and *Leptospira*-carrying wild boars are widely distributed throughout Japan (Fig. 1). Wild boars often wallow in mud for maintenance of body temperature, which may enhance the transmission of leptospire among wild boars. Because of feeding habitat differences, the nasopharyngeal mucosae of wild boars more frequently come into contact with soil than those of deer, which may also contribute to higher prevalence in wild boars. There was no annual change in the prevalence of positive animals during the investigation period (chi-squared test, $p=0.94$). As far as the sex of the wild boars (positive males, 6/36; females, 3/25; unknown, 1/5) in the 2007 to 2008 season, there was no difference in prevalence among males and females (Fisher's exact test, $p=0.45$). Leptospirosis in humans is transmitted by direct contact with infected animals or by exposure to water or soil contaminated with the urine of infected animals [5]. Leptospiral DNA was detected in the kidneys of these animals, indicating a risk of leptospirosis in humans, especially hunters and meat processing workers, and hunting dogs. Wild boars and deer migrate close to human habitats and damage agricultural crops, and the amount of damage has increased recently. These facts also suggest that there is a potential risk of leptospirosis in agricultural workers.

We determined the nucleotide sequences of the amplicons (accession numbers AB462457-AB462479) and obtained three sequence types (ST1–3) of the *flaB* gene. The *Leptospira* species harbored by these animals were deduced to be *L. interrogans* (ST1 and 2 in 22 animals) and

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Table 1. Results of *flaB*-PCR on wild boars and deer in Japan (2005–2008)

Animals	2005–2006		2006–2007		2007–2008		Total	
	No. of animals examined	Positive (%)	No. of animals examined	Positive (%)	No. of animals examined	Positive (%)	No. of animals examined	Positive (%)
Wild boars	40	6 (15.0)	39	6 (15.4)	66	10 (16.7)	145	22 (15.2)
Deer	30	1 (3.3)	31	0	33	0	94	1 (1.1)
Total	70	7 (10.0)	70	6 (8.6)	99	10 (10.1)	239	23 (9.6)

Fig. 1. Map of Japan showing the locations at which wild boars and deer were captured. The numbers and sequence types (ST 1–3) in the parentheses indicate the number of PCR-positive animals and sequence types of the *flaB* gene amplified, respectively. WB, wild boar; D, deer.

L. borgpetersenii (ST3 in 1 animal) by comparison of the nucleotide sequences of the *flaB* genes from the animals with those from reference strains (Fig. 1). The association of *Leptospira* serovars with their hosts is well known; for example, serovar Icterohaemorrhagiae is found in *Rattus* species, and serovar Hardjo is found in cattle [2]. Two *flaB* sequence types were detected in the kidneys of wild boars, which are widely distributed throughout Japan. This may indicate that leptospires with these sequence types are specifically associated with wild boars, although the serovars of leptospires were not determined in this study.

In conclusion, wild boars act as reservoir animals of lep-

tospores in many areas in Japan. Special attention should be paid to the risk of leptospirosis in hunters, meat processing workers, and hunting dogs in Japan.

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