

Fig. 1. Locations of Hokkaido, Hyogo, Osaka and Wakayama.

raccoons captured on the same day and at the same site. These juveniles, whose body weights were 2.05 and 2.3 kg, had high VN titers of 1:2560 and 1:5120, respectively. In Hyogo, serum samples from 2 juveniles, whose body weights were 0.95 and 0.90 kg, showed VN titers (1:1280 and 1:1280) higher than those of other sera (Table 2).

JEV infection in wild boars: The seroprevalence of JEV in 36 wild boars in Wakayama was investigated. The results indicated that 30 (83.3%) had VN antibodies against JEV (Table 1). There were no significant differences between males (87.0%) and females (76.9%). Wild boars are generally thought to be adults when their weights are over approximately 50 kg and are thought to be juveniles when their weights are less than 50 kg. Based on these weights, there were no significant differences in JEV infection between adults (90.9%) and juveniles (80.0%). Furthermore, JEV seroprevalence was compared between 37 raccoons and 30 wild boars captured from November 2007 to February 2008. Wild boars (86.7%; 26 out of 30) were significantly more seropositive than raccoons (59.5%; 22 out of 37; Fig. 2).

JEV infection in raccoon dogs: Nineteen raccoon dogs were examined for JEV infection. The results showed that 12 (63.2%) had VN antibodies against JEV (Table 1). No significant differences in JEV infection were observed between males (61.5%) and females (66.7%).

DISCUSSION

In this study, a seroepidemiologic survey of wild animals in the Kinki district was conducted. We found that 40.7%, 64.5% and 69.1% of raccoons in Hyogo, Osaka and Wakayama, respectively, possessed VN antibodies against JEV. On the other hand, raccoons in Hokkaido did not have VN antibodies against JEV. In Hokkaido, cases of JEV infection occur infrequently [14], and a serosurvey of pigs in 2007 also showed that JEV has not spread in Hokkaido [6]. Therefore, the data obtained for raccoons in Hokkaido indicated that our VN test is specific for JEV, and the high seroprevalence of JEV in wild animals suggests that JEV-infected mosquitoes remain in the Kinki region.

The survey of JEV infection in wild boars was conducted in Hiroshima from November 2004 to February 2005 and confirmed that 17 (68.0%) of the 25 tested wild boars were seropositive [4]. As wild boars are closely related to the domestic pig, they are thought to play a similar role as an amplifier for JEV. In Wakayama, 30 (83.3%) of the 36 tested wild boars were seropositive (Table 1), and wild boars (86.7%) were significantly more seropositive than raccoons (59.5%) in the same season (November 2007 to February 2008) (Fig. 2). Wild boars may thus play an important role in the recent infection cycle of JEV. Further studies are required in order to assess their ability to act as an amplifier for JEV.

In Wakayama, 12 (63.2%) of the 19 raccoon dogs had VN antibodies against JEV (Table 1). This is very similar to the results for raccoons (69.1%) in Wakayama, indicating that many species of wild animals in Wakayama may be infected with JEV. The diseases induced in these animals by JEV are unknown, but JEV infection in these animals indicates that JEV has been highly prevalent in the area.

Because of improvements in agricultural methods, decreases in the number of mosquito vectors, isolation of pig farms from urban areas and vaccination programs, there are fewer than 10 cases of JE in Japan each year. However, our data, as well as data from JEV surveillance in pigs [6], indicated that JEV remains epidemic. As wild boars may become viremic for JEV, as in domestic pigs, they may be an important source of JEV in these areas. In addition, there were few raccoons in Japan before 2000, but they now represent one of the major mammals in some areas. In North

Table 2. Virus-neutralizing titer to JEV in raccoons, wild boars and raccoon dogs

Species	Place	Virus-neutralizing titer against JEV										Total	
		<1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560		1:5120
Raccoon	Hyogo	32	6	4	3	2	3	1	0	2	1	0	54
	Osaka	22	13	10	6	4	4	3	0	0	0	0	62
	Wakayama	21	10	13	4	7	3	3	1	1	3	2	68
	Hokkaido	20	0	0	0	0	0	0	0	0	0	0	20
Wild boar	Wakayama	6	8	5	6	1	3	1	1	0	0	0	36
Raccoon dog	Wakayama	7	2	5	5	0	0	0	0	0	0	0	19

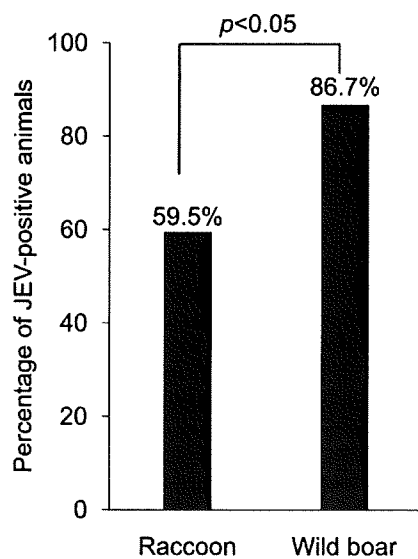


Fig. 2. Comparison of JEV infection in raccoons and wild boars captured in the same season (November 2007 to January 2008) in Wakayama. The numbers indicate the percentages of JEV-positive animals. Wild boars were significantly more seropositive than raccoons ($p < 0.05$).

America, raccoons have changed the epidemic pattern of rabies and are the most important reservoirs of canine distemper virus. Further study will be required to clarify whether the rapid increase of raccoons in Japan will influence the epidemiological pattern of JEV.

In most mammals, levels of viremia sufficient to infect mosquitoes do not develop. However, in the infection cycle of West Nile virus in the U.S.A., mammals such as the golden hamster (*Mesocricetus auratus*) [17], fox squirrel (*Sciurus niger*) [12], eastern cottontail rabbit (*Sylvilagus floridanus*) [16] and eastern chipmunk (*Tamias striatus*) [11] become sufficiently viremic for mosquito infection. In Wakayama, many wild animals were highly seropositive for JEV. Therefore, it is necessary to define whether some wild animals play a role as amplifiers for transmission of JEV to humans. Further analysis, such as PCR of sera or experimental infection using these wild animals, is needed in order to determine whether such species are amplifiers of JEV.

In conclusion, JEV infects a variety of wild animals living near human residences, indicating that JEV is still endemic in Japan, thus maintaining human infection risks. In addition, wild animals living near human habitats may be good sentinels for zoonoses such as JEV.

ACKNOWLEDGMENTS. The authors would like to thank Dr. Tomohiko Takasaki (National Institute of Infectious Disease) for kindly providing JEV and Dr. Go Abe and Dr. Yohei Matoba (Raccoon Researchers Group) for collecting sera from raccoons. This work was supported by a grant for

Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labour and Welfare of Japan (H20-Shinko-Ippan-003) and by a grant from the Innovation Center of Yamaguchi University.

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