5種類の人畜共通伝染病に関する野生ニホンカモシカの抗体調査

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Serologic Studies on Five Selected Zoonoses in Wild Japanese Serows (*Capricornis crispus*)

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ABSTRACT. The prevalence of antibodies against *Toxoplasma gondii, Brucella abortus, Leptospira interrogans* (serovars; *icterohaemorrhagiae, autumnalis, hebdomadis, australis* and *canicola*), *Chlamydia psittaci* and Japanese encephalitis virus was determined in serum samples collected from hunter-killed wild Japanese serows in Gifu Prefecture during the winter months of 1981 to 1984. Seropositive rates for each agent were 10.7% (36/335) for *Chlamydia psittaci,* 10.4% (42/404) for *Leptospira interrogans* and 5.4% (41/765) for *Toxoplasma gondii,* in descending order. Antibodies to *Brucella abortus* and Japanese encephalitis virus were not detected in 718 and 208 serum samples, respectively.The results obtained suggest that wild Japanese serows have serologic evidence of infection with some of the zoonosis agents tested.—KEY WORDS: epidemiology, Japanese serow, wild animal, zoonosis.

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The importance of wild animals has been recognized in the epidemiology of a wide variety of infectious diseases. Many wild animal species can act as reservoirs of the diseases for other wild or domestic animals and even humans. With the continuing expansion of human activities, the wildlife habitat is constantly shrinking and overlapping that of humans and domestic animals. Since the chance of contact of humans and domestic animals with many species of wild animals is increasing, clarification of the situation of infectious diseases in wild animals became very important.

From these viewpoints, many studies about prevalence of infectious diseases among wild animals have been conducted in other countries [1, 4, 31]. However, as far as we know, detailed epidemiological investigations along these lines have not been carried out in Japan. We had an opportunity to collect blood samples from wild Japanese serows (*Capricornis crispus*), which is the only wild bovine ruminant in Japan. The present study was undertaken to determine the prevalence of antibodies to some zoonosis agents such as *Toxoplasma gondii, Brucella abortus, Leptospira interrogans, Chlamydia psittaci* and Japanese encephalitis virus.

MATERIALS AND METHODS

Japanese serows: The serows were killed by hunters with permission of the Agency of Cultural Affairs of the Japanese Government in the eastern mountainous areas of Gifu Prefecture during the winter months of 1981 to 1984. The carcasses were transported to the Department of Veterinary Anatomy, Gifu University and detailed necropsies were performed on each animal by Dr. Sugimura and his colleagues. The age of serows was determined according to the eruption of teeth and wear of incisors [20].

Sera: At autopsy, blood samples were taken from the heart and partially from the thoracic and abdominal cavities of 766 serows comprising 393 males and 373

Table 1. Prevalence of antibody[a] to *Toxoplasma gondii* in Japanese serows

Date of collection	No. of sera tested	No. of positive sera[b]	Positive rate	Antibody titer						
				<16	16	32	64	128	256	512
1981	363	21	5.8	326	16	13	5	3	0	0
1982	51	4	7.8	41	6	4	0	0	0	0
1983	196	5	2.6	188	3	4	0	0	1	0
1984	155	11	7.1	127	17	5	2	3	0	1
Total	765	41	5.4	682	42	26	7	6	1	1

a) Detected by latex agglutination test using commercially available antigen.
b) Titers of ≥32 are taken as positive.

Table 2. Prevalence of antibody to *Toxoplasma gondii* in Japanese serows by age and sex

Age (years)[a] or sex		No. of sera tested	No. of positive sera	Positive rate
Age	≤1	95	3	3.2
	1.5~2.5	118	10	8.5
	3~5	264	13	4.9
	≥6	288	15	5.2
Sex	Male	393	22	5.6
	Female	372	19	5.1
Total		765	41	5.4

a) Age groups based on dental eruption and wear.

females. After centrifuging, many serum samples were found to contain some amounts of hemoglobin. The sera were stored at −20°C until tested.

Serologic tests: Prevalence of antibodies to the following 5 pathogens was investigated: *T. gondii*; The latex agglutination test [11] was used with commercially available antigen (Eiken Chemical Co., Tokyo). Titers higher than 1:32 were referred to as positive. *B. aboruts*; Both the agglutination and complement fixation (CF) tests recommended by the Brucella Committee in Japan were conducted using antigens provided by the Institute of Animal Health, Japan [8]. *L. interrogans*; Antibodies to 5 *L. interrogans* serovars; *icterohaemorrhagiae, autumnalis, hebdomadis, australis* and *canicola*, were examined by the latex agglutination test with commercially available antigens (Denka-Seiken Co., Tokyo). The screening test at the serum dilution of 1:10 was conducted by a modified microplate method in which agglutination was read microscopically. The specificity of the microplate method was confirmed beforehand by comparing with that of the manufacturer's indicated method. Serum showing agglutination with any one of the 5 antigens at the serum dilution of 1:10 was considered positive and as to the positive sera, further tests to determine corresponding serovars were performed by the manufucturer's indicated test procedure using the tube test. *C. psittaci*; The CF test with a commercially available antigen (Denka-Seiken Co., Tokyo) was used, and a titer of 1:10 or more was referred to as positive. Japanese encephalitis virus; The hemagglutination inhibition (HI) test using the JaGAr-01 strain was employed [15].

Statistical analysis: Significance of serological test results in relation to sex, age and years collected were analyzed by means of the chi-square test.

RESULTS

Prevalence of antibody to T. gondii: As shown in Table 1, a total of 765 serows were

Table 3. Prevalence of antibody[a] to *Leptospira interrogans* in Japanese serows

Serovars	No. of sera tested	No. of positive sera[b]	Positive rate	No. of sera showing highest titer to	Antibody titer					
					<10	10	20	40	80	160
L. icterohaemorrhagiae	404	40	9.9	19	364	9	13	10	8	0
L. autumnalis	404	35	8.7	15	369	4	11	13	5	2
L. hebdomadis	404	30	7.4	14	374	1	11	9	7	2
L. australis	404	30	7.4	13	374	4	9	7	9	1
L. canicola	404	31	7.7	5	373	8	18	4	1	0
One of the 5 serovars	404	42	10.4	42	362	4	9	8	16	5

a) Detected by latex agglutination test using commercially available antigens.
b) Titers of ≥10 are taken as positive and 36 out of 42 positive sera reacted with 2 or more serovars.

Table 4. Prevalence of antibody to *Leptospira interrogans* in Japanese serows by date collected, serow age and sex

Date collected, age or sex		No. of sera tested	No. of positive sera	Positive rate
Date	1981	105	15	14.3
	1982	51	5	9.8
	1983	141	10	7.1
	1984	107	12	11.2
Age	≤1	57	4	7.0
	1.5~2.5	69	6	8.7
	3~5	126	15	11.9
	≥6	152	17	11.2
Sex	Male	207	28	13.5[a]
	Female	197	14	7.1[a]
Total		404	42	10.4

a) Significant ($P<0.05$).

examined, and 41 of them, or 5.4%, were seropositive. The positive rates ranged from 2.6 to 7.8% by years, and a statistical difference ($p<0.05$) was observed between the rates of 1983 and 1984. High antibody titers of 1:128 or more were found in 8 serows (1.0%).

Prevalence of antibody titer in relation to age and sex is shown in Table 2. Seropositive rates varied from 3.2 to 8.5% by age groups, but no statistical differences were present among them. Similarly, no difference was obtained between the positive rates of male and female.

Prevalence of antibody to B. abortus: Of 718 serum samples tested, no positive case which reacted to both the agglutination and CF tests, was detected.

Prevalence of antibody to L. interrogans: Table 3 shows the prevalence of antibody to the 5 serovars of *L. interrogans*. Fourty-two of 404 sera, or 10.4%, were positive for at least one of the 5 serovars tested, with 36 of them reacting to more than one serovar. On these 42 positive sera, end agglutinin titers to each reacted antigen were obtained to determine the infecting serovars. However, many of these sera had similar titers to more than one serovar. Sera showing the highest titers to serovar *icterohaemorrhagiae* were the most common (19 sera), followed by those to *autumnalis* (15 sera), *hebdomadis* (14 sera), *australis* (13 sera) and *canicola* (5 sera), in descending order. As shown in Table 4, no statistical differences in the antibody prevalence were observed for the dates collected and also for serow age groups. However, a significant difference was found between the results of males (13.5%) and females (7.1%), at the 5% level.

Table 5. Prevalence of antibody[a] to *Chlamydia psittaci* in Japanese serows

Date of collection	No. of sera tested	No. of positive sera[b]	Positive rate	Antibody titer			
				<8	8	16	32
1981	158	17	10.8	141	12	5	0
1982	39	4	10.3	35	3	1	0
1983	71	9	12.7	62	8	1	0
1984	67	6	9.0	61	3	3	0
Total	335	36	10.7	299	26	10	0

a) Detected by CF test using commercially available antigen.
b) Titers of ≥8 are taken as positive.

Prevalence of antibody to C. psittaci: Of 335 serum samples tested, 36 (10.7%) were found to be seropositive (Table 5). The positive rates persisted at almost similar levels throughout the 4 years studied. Antibody titers were relatively low, such as 1:16 or less. There were no differences in positive rates by age and sex (the data were not illustrated).

Prevalence of antibody to Japanese encephalitis virus: Antibodies to the virus were checked in 208 serows for 2 years (1981 and 1982). None of them showed positive.

DISCUSSION

The Japanese serow has been protected as a special natural monument. However, in recent years, to prevent forest damage by their overbreeding, the Agency of Cultural Affairs of the Japanese Government has permitted limited killing of serows within specific quotas and locations since 1979. We took this opportunity to learn the prevalence of some infectious diseases among wild Japanese serows, and we have already reported partial data from our bacteriological and serological investigations [10, 21–23].

In general, the collection of good serum samples without hemoglobin from hunter-killed wild animals was difficult, so many sera showed an anticomplemental effect in the CF test. As a result, the number of serum samples tested was much decreased. However, these sera have shown almost no effect in the agglutination tests. In particular, the results of the toxoplasma latex agglutination test were detected clearly without interference from hemoglobinated sera. Hence, we should pay attention to selection of serologic methods in wild animal investigations.

The seropositive rate (5.4%) for *T. gondii* in serows obtained in the present study does not seem to be high, but of the seropositive cases, 8 individuals showed antibody titers of 1:128 or more, suggesting recent or current infection at the time of capture (Table 1).

Since the herbivores may be infected by any grazing pasteur accidentally contaminated with toxoplasma oocysts excreted from infected felidae [3], it is likely that infected felidae may be present in or near the serow's habitat. The reason for the lower seropositive rate of 2.6% obtained in 1983 is unknown. Many wild animals are known to be affected by toxoplasmosis [27], but very few reports are available. In one serologic survey of wild ruminants, Kocan *et al.* [12] reported a seropositive rate of 23% (25/110) in the moose of Alaska. Though the positive rate of the serows reported here was lower, the toxoplasmosis may well be one of the infectious diseases occurring among the serow population.

A high incidence of abortion due to *B. abortus* occurred in pregnant cows in almost all areas of Japan, including Gifu Prefecture from 1955 to 1965 [14]. However, the prevalence decreased rapidly thanks to an eradication program, and no outbreak has been reported since 1980. It is very interesting to determine whether this disease occurred among the serow population as well, but no serologic evidence for the presence of the disease was found among 718 serows tested.

In the other countries, brucellosis has been encountered in many species of wild ruminants such as bison, elk, moose, caribou, deer and antelope, showing clinical signs similar to those seen in cattle [5, 24, 28, 29]. Since the serows, like other ruminants, are thought to be susceptible to *B. abortus,* serow's habitat may well be free of any reservoirs of this bacterium.

Leptospirosis is widespread among many animal species including cattle, horses, swine, dogs, cats and rats in Japan [17, 30]. Although all parasitic serovars are potential pathogens to all species of domestic animals [26], in this experiment, we examined the antibodies against only 5 major serovars; *icterohaemorrhagiae, autumnalis, hebdomadis, australis* and *canicola,* which are known to be the main causative serovars of human leptospirosis in Japan [25]. Fourty-two (10.4%) out of 404 sera reacted with any one of the 5 antigens at a serum dilution of 1:10. Though an attempt was made to determine the corresponding serovars by obtaining and comparing end agglutinin titers, many of the sera had similar titers to more than one serovar. Therefore, it needs further cross-agglutinin-absorption-agglutination tests between cross-reacted sera [26]. Moreover, the isolation of leptospira organisms from serows is required to determine the serovars of current pathogens. Although we could not determine serovars of leptospires in this study, it may be said that the similar serovars of leptospires which infect human and domestic animals in Japan are also prevalent among serow population.

The positive rate (10.4%) of leptospirosis in serows was comparable with 7 to 20% of wild white-tailed deer in the United States [5, 6, 18, 28]. A recent survey run by the Japanese Veterinary Medical Association [9] showed that 13.8% (63/457) of dogs in Japan were seropositive. Although there has been no serologic survey on humans and domestic animals living near areas from where serows were captured, it can be presumed that the reservoirs of leptospires exist in the serow's habitat. The percentage of seropositive males was significantly higher than in females (Table 4). Similar data were obtained in dogs [17]. The reason for the fact mentioned above is not sure, but it may be attributable to the male's sexual behavior and also male's habit of wandering more widely in areas where infected rodents or contaminated inanimate vehicles such as soil and water are present. On the other hand, it may be possible to expect that the infected serows serve as reservoirs of leptospires.

Chlamydiosis occurred epizootically among goats and cattle in Japan in the 1950s. At the time, Omori *et al.* [16] investigated serologically bovine sera collected from 8 districts throughout Japan with a CF antibody positive rate of 35.0% (202/577). In their studies, the sera collected from Chubu district, where the serows were captured, have been shown to have CF antibody at the rate of 34.7% (35/101). However, in the following 20 years, very little serologic work has been done on the domestic animals in Japan. Recently, Fukushi *et al.* [7] confirmed the CF antibody positive rate of 30.2% in 1,048 bovine sera collected from Aichi and Gifu Prefectures in the Chubu district. The present study showed a seropositive rate of 10.7% without any difference due to age, sex or dates of collection. These and our own data together suggested that the chlamydial reservoirs were widely distributed and continuously maintained for long time in the habitats of cattle and serows in the Chubu district. As far as we know, no report regarding chlamydiosis in wild mammals has been published in Japan. But in the other countries, the disease is widely distributed among wild animal species; muskrats [19], snowshoe hares [19], koalas [2] and roe deer [1]. Thus, chlamydiosis in wild animals in Japan, like

leptospirosis, is generally suspected.

Finally, as to the Japanese encephalitis virus, we could not detect any HI antibody among 208 serows tested. Because the ecology of this virus in nature, especially in the winter season, is not clear, we are much interested in knowing whether the serows have the antibody to this virus. Though the serows tested were few, the lack of antibody prevalence indicated that the serows may not be so important for Japanese encephalitis epidemiology.

As presented here, we checked the antibodies to the 5 pathogens, and demonstrated an antibody prevalence to *T. gondii, L. interrogans* and *C. psittaci.* The results suggested that the zoonoses caused by at least these 3 pathogens have occurred among wild Japanese serows. However, none of these serows manifested signs of these diseases at autopsy. Only the cases of parapoxvirus infection which occurred among serows in 1984, presented the clinical signs [23]. We have also investigated serologically the prevalence of the Ibaraki disease, the Akabane disease and rotavirus infection, in which rotavirus infection was most prevalent. The data will be published elsewhere.

In the present study, we could recognize an important role of wild animals in epidemiology of some zoonoses in Japan. Thus, it needs more examinations to clarify the situation of infectious diseases prevalent in wild Japanese serows and also other wild animals species. The authors are now pursuing further studies in this regard.

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REFERENCES

1. Blancou, J. 1983. Serologic testing of wild roe deer (*Capreolus capreolus L.*) from the Trois Fontaines forest region of eastern France. *J. Wildl. Dis.* 19: 271–273.
2. Cockram, F. A., and Jackson, A. R. B. 1981. Keratoconjunctivitis of the koala, *Phascolarctos cinereus,* caused by *Chlamydia psittaci. J. Wildl. Dis.* 17: 497–503.
3. Costa, A. J., Araujo, F. G., Costa, J. O., Lima, J. D., and Nascimento, E. 1977. Experimental infection of bovines with oocysts of *Toxoplasma gondii. J. Parasitol.* 63: 212.
4. Everard, C. O. R., Fraser-Chanpong, G. M., Bhagwandin, L. J., Race, M. W., and James, A. C. 1983. Leptospires in wildlife from Trinidad and Grenada. *J. Wildl. Dis.* 19: 192–199.
5. Ferris, D. H., Hanson, L. E., Rhoades, H. E., and Alberts, J. O. 1961. Bacteriologic and serologic investigations of brucellosis and leptospirosis in Illinois deer. *J. Am. Vet. Med. Assoc.* 139: 892–896.
6. Fournier, J. S., Gordon, J. C., and Dorn, C. R. 1986. Comparison of antibodies to leptospira in white-tailed deer (*Odocolieus virginianus*) and cattle in Ohio. *J. Wildl. Dis.* 22: 335–339.
7. Fukushi, H., Ogawa, H., Morikoshi, T., Okuda, Y., Shimakura, S., and Hirai, K. 1985. Chylamydial complement fixing antibodies in cows. *Res. Bull. Fac. Agr. Gifu Univ.* 50: 259–263.
8. Isayama, Y. 1971. Serodiagnosis of bovine brucellosis in Japan. Bull. Nat. Inst. Anim. Hlth. 62: 64–82 (in Japanese).
9. Japanese Veterinary Medical Association 1984. Serologic survey for zoonoses. *J. Jpn. Vet. Med. Assoc.* 37: 546–549 (in Japanese).
10. Kinjo, T., Sugiyama, Y., and Minamoto, N. 1982. Antimicrobial susceptibility of fecal *Escherichia coli* isolated from wild Japanese serows (*Capricornis crispus*). *Res. Bull. Fac. Agr. Gifu Univ.* 46: 243–248 (in Japanese with English summary).
11. Kobayashi, A., Hirai, N., Suzuki, Y., Nishikawa, H., and Watanabe, N. 1977. Evaluation of a commercial toxoplasma latex agglutination test. *Jpn. J. Parasitol.* 26: 175–177 (in Japanese).
12. Kocan, A. A., Barron, S. J., and Fox, J. C. 1986. Antibodies to *Toxoplasma gondii* in moose (*Alces alces* L.) from Alaska. *J. Wildl. Dis.* 22: 432.
13. Meyer, K. F. 1967. The host spectrum of psittacosis-lymphogranuloma venereum (PL) agents. *Am. J. Ophthalmol.* 63: 1225–1246.
14. Murase, N. 1980. Brucellosis. pp. 200–203, *In*: Veterinary Infectious Diseases (Sasahara, J. *et al.* eds.), Kindai-shuppan, Tokyo (in Japanese).
15. Nakajima, Y., and Nakamura, H. 1983. Hemagglutination inhibition test for Japanese encephali-

tis virus. *Nisseiken-Tayori.* 29: 29–32 (in Japanese).
16. Omori, T., Ishii, S., and Matsumoto, M. 1960. Miyagawanellosis of cattle in Japan. *Am. J. Vet. Res.* 21: 564–573.
17. Ryu, E., Bessho, M., Iyoda, K., and Shinohara, N. 1975. Investigation on leptospiral agglutination of dogs in the Shikoku region. *J. Jpn. Vet. Med. Assoc.* 28: 369–372 (in Japanese with English summary).
18. Shotts, E. B., and Hayes, F. A. 1971. Leptospiral antibodies in white-tailed deer of the southeastern United States. *J. Wildl. Dis.* 6: 259–268.
19. Spalatin, J., Fraser, C. E. O., Connell, R., Hanson, R. P., and Berman, D. T. 1966. Agents of psittacosis-lymphogranuloma venereum group isolated from muskrats and snowshoe hares in Saskatchewan. *Can. J. Comp. Med. Vet. Sci.* 30: 260–264.
20. Sugimura, M., Suzuki, Y., Kamiya, S., and Fujita, T. 1981. Reproduction and prenatal growth in the wild Japanese serow, *Capricornis crispus. Jpn. J. Vet. Sci.* 43: 553–555.
21. Sugiyama, Y., Kinjo, T., and Minamoto, N. 1983. Isolation and antimicrobiotic susceptibility of Yersinia and Salmonella from feces of wild Japnese serows (*Capricornis crispus*). *Res. Bull. Fac. Agr. Gifu Univ.* 48: 129–135 (in Japanese with English summary).
22. Suzuki, J., Kinjo, T., and Minamoto, N. 1984. Prevalence of antibodies to some zoonoses in wild Japanese serows (*Capricornis crispus*). *Res. Bull. Fac. Agr. Gifu Univ.* 49: 253–258 (in Japanese with English summary).
23. Suzuki, Y., Sugimura, M., Atoji, Y., Minamoto, N., and Kinjo, T. 1986. Widespread of parapox infection in wild Japanese serows, *Capricornis crispus. Jpn. J. Vet. Sci.* 48: 1279–1282.
24. Thorne, E. T., Morton, J. K., and Thomas, G. M. 1978. Brucellosis in elk I. Serologic and Bacteriologic survey in Wyoming. *J. Wildl. Dis.* 14: 74–81.
25. Tomioka, S. 1978. Leptospirosis. pp. 363–368. *In*: Clinical Bacteriology (Ozawa, A. *et al.*), Kodansha, Tokyo (in Japanese).
26. Torten, M. 1979. Leptospirosis. pp. 363–421. *In*: CRC Handbook Series in Zoonoses (Steel, J. H. eds.), CRC Press, Florida.
27. Walton, B. C., and Walls, K. W. 1966. Prevalence of toxoplasmosis in wild animals from Fort Stewart, Georgia, as indicated by serological tests and mouse inoculation. *Am. J. Trop. Med. Hyg.* 13: 530–533.
28. Wedman, E. E., and Driver, F. C. 1957. Leptospirosis and brucellosis titers in deer blood. *J. Am. Vet. Med. Assoc.* 130: 513–514.
29. Witter, J. F. 1981. Brucellosis, pp. 280–287. *In*: Infectious Diseases of Wild Mammals (Davis, J. W. *et al.* eds.), Iowa State Univ. Press, Iowa.
30. Yanagawa, R., and Takashima, I. 1974. Animal leptospirosis. *J. Jpn. Vet. Med. Assoc.* 27: 211–217 (in Japanese).
31. Zarnke, R. L., and Yuill, T. M. 1981. Serologic survey for selected microbial agents in mammals from Alberta, 1976. *J. Wildl. Dis.* 17: 453–461.

要　約

5種類の人畜共通伝染病に関する野生ニホンカモシカの抗体調査：金城俊夫・源　宣之・鈴木　順（岐阜大学農学部獣医公衆衛生学教室）——岐阜県東部山岳地帯で1981年から1984年の冬期間に捕殺された野生ニホンカモシカの血清について，*Toxoplasma gondii, Brucella abortus, Leptospira interrogans, Chlamydia psittaci* 及び日本脳炎ウイルスの5種類の人畜共通伝染病の病原体に対する抗体調査を行った．抗体陽性率は，*C. psittaci* に対し10.7%（36/335），*L. interrogans* に対し10.4%（42/404）及び *T. gondii* に対し5.4%（41/765）であった．しかし，*B. abortus* および日本脳炎ウイルスに対する抗体は，それぞれ718例及び208例調べたが検出されなかった．