An Occurrence of *Salmonella* Typhimurium Infection in Sika Deer (*Cervus nippon*)

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ABSTRACT. Seven sika deer (*Cervus nippon*) in a herd of 30 deer in a park died. Upon examination of three dead deer, *Salmonella* Typhimurium was isolated from the organs and intestinal contents. Histopathological examination revealed catarrhal enteritis and focal necroses in the liver. Immunohistochemically, *Salmonella* antigen of O4 was detected in the enteric lesions. The case was diagnosed as *S. Typhimurium* infection in the sika deer. Because of the importance of *Salmonella* in public health, fecal and soil samples were continuously collected from the paddock. However, no *Salmonella* was isolated from any samples collected after medication of the deer and thorough disinfection of the immediate environment.—KEY WORDS: public health, *Salmonella* Typhimurium, sika deer.

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Salmonellosis is one of the most common zoonotic infections. Companion animals including birds are considered as an important reservoir of the disease [6, 11, 14, 15]. *Salmonella* infection in zoo animals is also very important to public health, because these animals are kept in close contact with humans [7]. The authors encountered an occurrence of *Salmonella* Typhimurium infection in sika deer (*Cervus nippon*) kept in a park where many people visit. There have been a few reports on tuberculosis in sika deer [4, 9, 12], which is also one of the most important zoonosis. However, no report is available on *Salmonella* infection in this species. This paper deals with the first report of *S. Typhimurium* infection in sika deer and some related control measures against the infection.

The outbreak was seen in a park in Nagano Prefecture, where 30 sika deer consisting 10 adult males, 9 adult females and 11 young deer less than 2 years old, were kept. The paddock, 1,740 m², is surrounded by wire-fence and there is a cabin, 50 m², for feeding and drinking. Rice straws and concentrate were given in the cabin and grass, hay and vegetables were given on the paddock ground by the fence.

During the period from 18 October to 22 November 1997, a total of 7 deer; 3 adult males, 3 adult females and 1 young deer; died suddenly or within a few days after the onset of clinical signs such as general weakness, anorexia and slight diarrhea. Three adult deer were examined. Case 1 is a male deer which died on 4 November after showing clinical signs, Case 2 is a female deer which died suddenly on 12 November and Case 3 is a male deer which died on 22 November after showing clinical signs. As the incidence was seen during the breeding season, the male deer of Case 3 had a traumatic injury on the dorsolumbar part of the body which was attributed to aggressive behavior among males. Another adult female deer died suddenly on 21 October was diagnosed as gastric mucormycosis associated with proliferation of *Clostridium perfringens* [16].

Necropsy was carried out on 2 dead adult sika deer, Cases 1 and 2, and their mucous membranes of the abomasum, small intestines and cecum were reddish and partially hemorrhagic. The contents of the small intestine and cecum were watery and reddish. The intestinal lymph nodes were moderately enlarged. Petechial hemorrhages were seen on the pericardium and endocardium.

For histopathological examination, pieces of the liver, spleen, heart, lungs, kidneys, cerebrum, cerebellum, forestomach, abomasum and intestines were fixed in 10% formalin. Their paraffin sections were prepared and stained with hematoxylin and eosin (HE). Severe catarrhal enteritis associated with slight hemorrhages was observed in the small intestine. A large number of lymphocytic infiltrations was found in the lamina propria and submucosa of the small intestine. The mucous epithelium of the large intestine was slightly necrotic and cellular infiltrations were found in the mucous membrane. A small number of focal necroses was seen in the liver, and some viable-appearing Kupffer cells were observed along the sinusoid (Fig. 1). After pretreatment with a 0.1% solution of actinase, formalin-fixed, paraffin wax-embedded sections of the intestines were stained by an avidin-biotin complex immunoperoxidase method with *Salmonella* antisera for O4 (Denka, Tokyo) and an immunostaining kit (Nissui, Tokyo) for immunohistochemical examination. *Salmonella* antigen of O4 was detected in the enteric lesions of the small intestine.

For bacteriological examination, the liver, heart, spleen, lungs, kidneys, cerebrum, and cerebellum of the 2 necropsied deer were cultured on plates of blood agar, chocolate agar, heart infusion agar, desoxycholate hydrogen sulfide (DHL) agar, sabouraud agar, and mannitol salt agar at 37°C for 48 hr. The intestinal contents of the 2 necropsied deer and one rectal swab collected from an adult male diseased deer (Case 3) on 21st November were cultured in H₂O tetrathionate broth at 37°C for 24 hr. Subcultures

Fig. 1. A focal necrosis and hemorrhagic lesion seen in the liver (Case 1). Viable-appearing Kupffer cells were also observed along the sinusoid. HE stain, $\times 260$.

from these Hajna tetrathionate broth were made on DHL agar plates supplemented by 25 mg of novobiocin (Sigma Chemical Co., U.S.A.) per liter. Isolates were examined biochemically for *Salmonella* and serotyped with *Salmonella* antisera for O and H (Denka, Tokyo). Colonies suspected for *Salmonella* were isolated from the heart, lungs, spleen, and intestinal contents of Case 1, and from the liver and intestinal contents of Case 2. The pathogen was also isolated from the rectal swab of Case 3. Serologically, these isolates possessed *Salmonella* antigens of O:4, 5, H1:i, H2:1, 2, and finally they were identified as *S. Typhimurium*.

Ten isolates of *S. Typhimurium* isolated from the feces, three dead deer and soil samples were examined for antibiotic sensitivity tests with 12 disks of ampicillin, cloxacillin, pivmecillinam, streptomycin, kanamycin, fradiomycin, oxytetracycline, erythromycin, cefazolin, cefuroxime (Showa, Tokyo), enrofloxacin (Bayer AG, Leverkusen) and oxolinic acid (Tanabe, Tokyo) with a sensitivity test agar (Nissui, Tokyo). They were highly sensitive to ampicillin, pivmecillinam, oxytetracycline, cefazolin, enrofloxacin and oxolinic acid, moderately sensitive to kanamycin, streptomycin, fradiomycin and cefuroxime, and resistant to cloxacillin and erythromycin according to the manufacturer's criteria.

Parasitologically neither parasitic ova nor coccidia oocysts were detected from the intestinal contents of the 2 necropsied deer.

For epizootiological surveys, a total of one hundred fecal samples from surviving deer, 50 soil samples from the

paddock ground and 30 wiped samples from mangers, floor and walls in the cabin were collected during the period from October 1997 to August 1998. For wiped samples, sterilized absorbent cotton and saline were used. These samples were cultured in Hajna tetrathionate broth and subcultures were made on DHL agar. EEM broth (Eiken, Tokyo) was used prior to Hajna tetrathionate broth culture for soil and wiped samples. *S. Typhimurium* was isolated from only one out of 10 fecal samples on 22 October, while the pathogen was detected from 8 out of 15 fecal samples on 21 November (Table 1). Among *Salmonella* positive feces, 7 samples were from adult deer. Though all of the soil samples collected from the paddock ground on 22 October were negative for *Salmonella*, 4 out of 6 soil samples collected from inside of the paddock on 21 November showed positive for *S. Typhimurium* (Table 1).

Analysis of plasmids was made for 12 isolates of *S. Typhimurium* isolated from the feces on 22 October, dead deer of Cases 1–3 and soil samples on 21 November and 15 December according to the modified method of Kado and Liu [5]. A standard strain of *S. Typhimurium*, which has 90 kb of plasmid, was used. Then all of them possessed the same size of plasmids around 100 kb.

Results obtained from the pathological and bacteriological examinations indicated that epizootic infection of *S. Typhimurium* among the deer was evident. Ampicillin (10 mg/kg/day), to which the isolate is highly sensitive, was medicated to all the surviving deer mixed with concentrate from the end of November for 10 days. Since the soil of the paddock was contaminated by the pathogen, the deer were fed only in the cabin provisionally, in which the mangers and floor were cleaned with disinfectants containing benzalkonium chloride or sodium hypochlorite. Moreover, feed residues on the paddock ground were removed with superficial soil and slaked lime was spread there repeatedly. After the medication and control measures mentioned above were taken, all of fecal samples became negative, though 2 soil samples still remained positive to *Salmonella* on 15 December. Both fecal and soil samples showed negative on April 1998. For fundamental sanitation, a concrete made feeding place was constructed in the paddock by the fence on April 1998, where grass, hay and vegetables were previously given.

Although there are some reports on salmonellosis in red deer (*Cervus elaphus*) [8] and white-tailed deer (*Odocoileus virginianus*) [13], this is the first report of the infection in sika deer.

Only one fecal sample was positive for *S. Typhimurium* among 10 fecal and 10 soil samples collected from the paddock on 22 October, while many of those samples became positive for the pathogen on 21 November. Furthermore, the initial isolates of *S. Typhimurium* derived from one fecal sample showed the same plasmid and quite similar antibiotic sensitivity patterns as those of *S. Typhimurium* isolated from Cases 1, 2 and 3, and soil samples collected on 21 November and 15 December. These findings possibly indicate that there was a carrier deer in

Table 1. Isolation of *S. Typhimurium* from the surviving deer and surrounding environmental samples collected during the period from October 1997 to August 1998

Samples	Oct. 22	Nov. 21	Dec. 15	Feb. 16	Apr. 20	May 26	Aug. 10
Fecal samples							
adult deer	1/10 ^{a)}	7/10	0/10	0/10	0/10	0/10	0/10
young deer	NT ^{b)}	1/5	0/5	0/5	0/5	0/5	0/5
Soils from the paddock							
inside	0/6	4/6	2/6	NT	0/6	0/6	0/6
outside	0/4	0/2	0/2	NT	0/2	0/2	0/2
Wiped samples in the cabin							
manger	NT	0/3	NT	0/3	0/3	0/3	0/3
floor and walls	NT	0/3	NT	0/3	0/3	0/3	0/3

a) Number of samples positive for *S. Typhimurium* per number of samples examined. b) Not tested.

the herd.

In the present incidence, 6 out of 19 adult deer died, while only one young deer died among 11. One adult deer was diagnosed as gastric mucormycosis [16], and 3 adult deer were diagnosed as *S. Typhimurium* infection. During the breeding season, aggressive attacks and mating were frequently observed among adult deer [10]. It is said that one male to 20–30 females is suitable ratio for the breeding of sika deer [3]. Nevertheless, there were 10 males to only 9 females in the herd. This inadequate ratio of male to female might have made males more aggressive in their breeding behavior during the breeding season. Consequently, the adult deer had been disposed by more conspicuous stress later on 21 October than did in young deer, which probably induced *Salmonella* infection as well as mucormycosis in the herd.

Since salmonellosis is one of the most important zoonosis, there have been many reports on persistence of the pathogen. The authors confirmed that excretion of *S. Typhimurium* could last more than 3 weeks in infected bengalees and zebra finches which are very popular cage birds [14, 15]. Tanaka *et al.* [17] reported that carrier dogs with *S. Typhimurium* excreted the same serotype for 2 to 3 months or longer into feces. On the other hand, Aoki *et al.* [1] reported an outbreak of *S. Typhimurium* infection on a nursery school, where sands in the playground were the probable source of the infection. They examined the sands for *Salmonella* and reported that the pathogen could have survived for 124 days when the sands were kept at 20°C [2]. There is another report that *S. Dublin* could survive for at least 1,069 days in artificially contaminated and dried feces [18]. As the present outbreak of salmonellosis occurred in a park where many people, especially children including infants who belong to an age group with greater susceptibility than adults to the pathogen, visit to see the deer and often touch them through the fence, the surrounding environment was thoroughly disinfected and continuous examinations followed. In addition, a concrete feeder was constructed by the fence in the paddock to avoid contamination from feces and soil, where grass, hay and

vegetables were previously given. Though the pathogen was isolated from 2 soil samples in the paddock on 15 December, control measures were successful, because no *Salmonella* was isolated from any samples collected since February 1998.

REFERENCES

1. Aoki, A., Itaya, T., Saito, A., Ando, K., Masaki, H., Tokumaru, Y. and Yamamoto, T. 1994. *J. Jpn. Vet. Med. Assoc.* 47: 513–517 (in Japanese with English summary).
2. Aoki, A., Itaya, T., Saito, A., Ando, K., Masaki, H. and Tokumaru, Y. 1994. *J. Jpn. Vet. Med. Assoc.* 47: 603–605 (in Japanese with English summary).
3. Ishijima, Y. 1998. *Anim. Husbandry* 52: 219–222 (in Japanese).
4. Itoh, R., Kagabu, Y. and Itoh, F. 1992. *J. Vet. Med. Sci.* 54: 803–804.
5. Kado, C.I. and Liu, S.T. 1980. *J. Bacteriol.* 145: 1365–1373.
6. Kaufmann, A. F. 1966. *J. Am. Vet. Med. Assoc.* 149: 1655–1661.
7. Komorowski, R. A. and Hensley, G. T. 1973. *Arch. Environ. Health* 27: 110–111.
8. McAllum, H. J. F., Familton, A. S., Brown, R. A. and Hemmingsen, P. 1978. *N. Z. Vet. J.* 26: 130–131.
9. Mirsky, M. L., Morton, D. Piehl, J. W. and Gelberg, H. 1992. *J. Am. Vet. Med. Assoc.* 200: 1540–1542.
10. Miura, S. 1984. Sika deer. pp. 90–93. In: *The Encyclopedia of Animals* (Macdonald, D. W. ed.), Andromeda Oxford Ltd., Oxford (Japanese edition, 1992, Imaizumi, Y. ed., Heibonsha, Tokyo).
11. Nishio, T. 1979. *Bull. Hiroshima Inst. Publ. Health* 26: 1–25 (in Japanese).
12. Rhyan, J. C. and Saari, D. A. 1995. *Vet. Pathol.* 32: 215–220.
13. Robinson, R. M., Hidalgo, R. J., Daniel, W. S., Rideout, D. W. and Marbuger, R. G. 1970. *J. Wildl. Dis.* 6: 389–396.
14. Sato, Y. 1996. *J. Vet. Med. Sci.* 58: 1175–1179.
15. Sato, Y. and Aoyagi, T. 1996. *J. Vet. Med. Sci.* 58: 845–848.
16. Sato, Y. and Matsuura, S. 1998. *J. Vet. Med. Sci.* 60: 981–983.
17. Tanaka, Y., Katsube, Y. and Imaizumi, K. 1976. *Jpn. J. Vet. Sci.* 38: 215–224.
18. Wray, C. and Sojka, W. J. 1977. *J. Dairy Res.* 44: 383–425.