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Occurrence of Yersiniosis and Listeriosis in wild boars in Japan

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ABSTRACT: From December 1994 to February 1995, 131 wild boars (*Sus scrofa leucomysta*) living in a mountainous area in Japan were examined for yersiniosis and listeriosis. Of 131 wild boars, 76 (58%) were males and 55 (42%) were females. Four *Yersinia* spp. including *Y. pseudotuberculosis, Y. enterocolitica, Y. frederiksenii,* and *Y. aldovei,* were isolated from 49 (37%) of 131 wild boars. *Yersinia pseudotuberculosis* was isolated from five (4%) of 131 wild boars. All *Y. pseudotuberculosis* isolates were serotype 4b and harbored virulence plasmids. *Yersinia pseudotuberculosis* was isolated only from boars under 2-yr-old. No human pathogenic *Y. enterocolitica* was isolated. *Listeria monocytogenes* was isolated from two (1%) of the wild boars and both isolates were serotype 4b. These findings indicated that wild boar could be a reservoir of *Y. pseudotuberculosis* and *L. monocytogenes* in Japan.

Key words: Isolation, *Listeria monocytogenes,* occurrence, survey, *Sus scrofa* leucomysta, *Yersinia pseudotuberculosis,* wild boar.

The Japanese wild boar (*Sus scrofa leucomysta*) is widely distributed in mountainous areas in Japan. Wild boar are hunted in the winter season, from November to February. Some Japanese people prefer to consume wild animal meat as a specialty food. Kanai et al. (1997) examined retail boar meat for the presence of zoonotic bacteria and isolated species of genera such as *Yersinia, Listeria, Salmonella,* and *Erysipelothrix.* However, the occurrence of these zoonotic bacteria in natural population of wild boar has not yet been investigated. In the present study, we examined free-ranging wild boars living in Japan for the presence of *Yersinia* spp. and *Listeria* spp.

From December 1994 to February 1995, 131 wild boars were captured by a snare for human consumption in the mountainous areas of Shimane Prefecture (35°28′N, 133°03′E), in the western area of Honshu Island, Japan. Of 131 wild boars, 76 (58%) were males and 55 (42%) were females. After wild boars were euthanized, they were immediately dissected and their rectal contents were collected. The wild boars' ages were determined on the basis of tooth eruption and wear by the method of Hayashi et al. (1977). Feces were preserved in Cary and Blair transport medium (BBL, Cockeysville, Maryland, USA), kept refrigerated during subsequent storage, and then brought to Tokyo University of Agriculture and Technology, (Tokyo, Japan). All samples were tested within 3 days after collection. Approximately 1.0 g feces from each animal was suspended in 9.0 ml of phosphate-buffered saline (PBS; pH 7.2). The PBS suspensions were incubated at 4 C for 4 wk. According to alkali treatment method by Aulisio et al. (1980), 0.5 ml of PBS suspension was added to 4.5 ml of 0.5% KOH in 0.5% NaCl. After 1 min of exposure to alkali, 0.1 ml of sample suspension was spread onto irgasan-novobiocin agar plates (Fukushima and Gomyoda, 1991) and MacConkey agar with 1% sorbitol (Difco, Detroit, USA). All plates were incubated at 25 C for 48 hr. Colonies morphologically similar to those of *Yersinia* spp. were subcultured for biochemical examination. Identification of yersiniae was performed by the methods of Wauters et al. (1988). Serotyping of *Y. pseudotuberculosis* was accomplished by slide agglutination with rabbit O antisera against serovars 1a, 1b, 2a, 2b, 3, 4a, 4b, 5a, 5b, and 6 prepared according to the methods of Tsubokura et al. (1970). *Yersinia pseudotuberculosis* strains used for immunization and absorption were provided by M. Tsubokura (Tottori University, Tottori, Japan). Serotyping of *Y. enterocolitica* strains was accomplished using slide agglutination with commercial rabbit an-

TABLE 1. Isolation of *Yersinia* spp. from wild boars by age.

Age (year)	Number of animals examined	Number of *Yersinia* isolates (%)				
		Total	*Y. pseudotuberculosis*[a]	*Y. enterocolitica*[b]	*Y. frederiksenii*	*Y. aldovei*
0–1	16	8 (50)	2 (13)	6 (38)	1 (6)	1 (6)
1–2	49	20 (41)	3 (6)	16 (33)	4 (8)	2 (4)
2–3	31	8 (26)	0 (0)	8 (26)	2 (7)	1 (3)
3–4	11	2 (18)	0 (0)	2 (18)	0 (0)	0 (0)
>4	4	1 (25)	0 (0)	1 (25)	0 (0)	0 (0)
Unknown	20	8 (45)	0 (0)	8 (40)	0 (0)	0 (0)
Total	131	47 (36)[c]	5 (4)	41 (31)	7 (5)	4 (3)

[a] All *Y. pseudotuberculosis* isolates were serotyped as 4b.
[b] No human pathogenic *Y. enterocolitica* was isolated.
[c] Of 47 *Yersinia*-positive samples, two samples yielded three different species, six had two species, and 39 had one species.

tisera against O3, O5, O8, and O9 (Denkaseiken Company, Tokyo, Japan). All isolates identified as *Y. pseudotuberculosis* and *Y. enterocolitica* were subjected to the autoagglutination test (Laird and Cavanaugh, 1980), were examined for calcium dependency at 37 C on magnesium oxalate agar (Gemski et al., 1980), and were tested for the presence of the virulence plasmid by using the modified method of Kado and Liu (1981). In brief, bacterial cells were grown overnight in 5 ml of brain heart infusion broth at 25 C, harvested by centrifugation, and resuspended in 1 ml of TAE buffer (40 mM Tris-acetate, 2 mM EDTA, pH 7.9). The cells were then lysed by the addition of 2 ml of freshly prepared lysing solution (3 g SDS, 0.6 g Tris, 6.4 ml 2N NaOH in 100 ml of distilled water), incubated at 55 C for 1 hr, followed by 6 ml phenol-chloroform (1:1, V/V). After centrifugation, the supernatant was subjected to agarose gel electrophresis for plasmid DNA screening and size-determination.

Isolation of *Listeria* was performed after incubation at 4 C for 4 wk as described above. A loopful of suspension was plated on Palcam agar (Merck, Darmstadt, Germany) and Oxford agar (Oxoid, Basingstoke, UK). All plates were incubated at 37 C for 48 hr. Colonies morphologically similar to those of *Listeria* spp. were subcultured for biochemical examination. Identification of *Listeria* spp. to the genus level relied on Gram staining, catalase production, umbrella-shaped motility at 25 C, esculin hydrolysis, and nitrate reduction. Species were determined by using the fermentation of D-xylose, L-rhamnose, and D-mannitol and the CAMP test with *Staphylococcus aureus* and *Rhodococcus equi.* Serotyping of *L. monocytogenes* isolates was accomplished using antisera according to Seeliger and Jones (1986). *L. monocytogenes* strains for serotyping were provided by Tokyo Metropolitan Research Laboratory of Public Health (Tokyo, Japan).

Yersinia pseudotuberculosis, Y. enterocolitica, Y. frederiksenii, and *Y. aldovei,* were isolated from 47 (36%) of 131 wild boars (Table 1). No recognized human pathogenic *Y. enterocolitica* was isolated. Five *Y. pseudotuberculosis* isolates were serotype 4b. All *Yersinia pseudotuberculosis* isolates showed positive reactions for virulence-associated properties, such as calcium dependency and autoagglutination, and harbored a 40- to 50-mDa virulence plasmid.

Listeria spp. were isolated from two (<2%) of 131 boars. Both isolates were identified as *L. monocytogenes* serotype 4b. One was isolated from a 1 to 2-yr-old boar and the other was isolated from a boar of unknown age.

Although *Y. pseudotuberculosis* and *L. monocytogenes* have been detected from wild animals and birds (Lovett, 1989; Schiemann, 1989), neither zoonotic bac-

teria had ever been isolated from a wild boar living in Japan prior to this study. Wild Japanese boars, therefore, may harbar *Y. pseudotuberculosis* and *L. monocytogenes.*

Yersinia pseudotuberculosis is known to be a foodborn pathogen and has been isolated from numerous domestic and free-living animals (Schiemann, 1989; Tsubokura et al., 1989). In Japan, *Y. pseudotuberculosis* has been isolated from wild animals such as raccoon dogs (*Nyctereutes procyonoides*), Japanese deer (*Cervus nippon*), Japanese hare (*Lepus brachyurus*), Japanese marten (*Martes melampus*), large Japanese field mice (*Apodemus speciosus*), and black-faced buntings (*Emberiza spodocephala*) (Fukushima et al., 1990; Fukushima and Gomyoda, 1991; Hamasaki et al., 1989). The prevalence of *Y. pseudotuberculosis* from the wild boars in the present study is relatively high. Fukushima et al. (1987) reported that the predominant *Y. pseudotuberculosis* serotype isolated from human patients in Shimane prefecture, was 4b. This is the same area where the wild boars were captured, and all were *Y. pseudotuberculosis* serotype 4b. Therefore, wild boars could be a national reservoir of *Y. pseudotuberculosis* in this area.

Yersinia pseudotuberculosis was isolated only from boars <2-yr-old. Sato and Komagane (1991) reported that children are more sensitive to *Y. pseudotuberculosis* than adults. Fukushima (1991) determined that young large Japanese field mice are more sensitive to *Y. pseudotuberculosis* than are adult mice. Young wild boar may be more important carrier of *Y. pseudotuberculosis* than the older animals.

Listeria monocytogenes was isolated at a high rate from pork by Johnson et al. (1990). Kanai et al. (1997) reported that *L. monocytogenes* from five (5.0%) of 100 samples of raw retail boar meat. Of five *L. monocytogenes* isolates in their investigation, three were serovar 1/2c and two were 4b. The same serovars were isolated from retail boar meat and boar feces. Thus, *L. monocytogenes* contaminated retail wild boar meats may have originated from free-ranging wild boars. However, the infectivity and pathogenicity of *L. monocytogenes* from wild boar is unknown. Because wild boars harbored *Y. pseudotuberculosis* and *L. monocytogenes* at a relatively high rate, appropriate care should be taken in slaughter and handling of wild boar meat.

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