

Occurrence of Yersiniosis and Listeriosis in wild boars in Japan

Authors: Hayashidani, Hideki, Kanzaki, Nobuo, Kaneko, Yuji, Okatani, Alexandre Tomomitsu, Taniguchi, Takahide, et al.

Source: Journal of Wildlife Diseases, 38(1) : 202-205

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-38.1.202>

The BioOne Digital Library (<https://bioone.org/>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<https://bioone.org/subscribe>), the BioOne Complete Archive (<https://bioone.org/archive>), and the BioOne eBooks program offerings ESA eBook Collection (<https://bioone.org/esa-ebooks>) and CSIRO Publishing BioSelect Collection (<https://bioone.org/csiro-ebooks>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Occurrence of Yersiniosis and Listeriosis in wild boars in Japan

Hideki Hayashidani,^{1,3} Nobuo Kanzaki,² Yuji Kaneko,² Alexandre Tomomitsu Okatani,¹ Takahide Taniguchi,¹ Ken-ichi Kaneko,¹ and Masuo Ogawa¹ ¹ Department of Veterinary Medicine and ² Department of Ecoregion Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan, ³ Corresponding author (e-mail: eisei@cc.tuat.ac).

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 <i>Yersinia</i> isolates (%) | | | | |
|------------|----------------------------|--|---|---------------------------------------|-------------------------|-------------------|
| | | Total | <i>Y. pseudotuberculosis</i> ^a | <i>Y. enterocolitica</i> ^b | <i>Y. frederiksenii</i> | <i>Y. aldovei</i> |
| 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 electrophoresis 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 pro-

duction, 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 harbor *Y. pseudotuberculosis* and *L. monocytogenes*.

Yersinia pseudotuberculosis is known to be a foodborne 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.

LITERATURE CITED

- AULISIO, C. C. G., I. J. MEHLMAN, AND A. C. SANDERS. 1980. Alkali method for rapid recovery of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from foods. Applied and Environmental Microbiology 39: 135–140.
- FUKUSHIMA, H. 1991. Susceptibility of wild mice to *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*. Zentralblatt für Bakteriologie 275: 530–540.
- , AND M. GOMYODA. 1991. Intestinal carriage of *Yersinia pseudotuberculosis* by wild birds and mammals in Japan. Applied and Environmental Microbiology 57: 1152–1155.
- , ———, AND S. KANEKO. 1990. Mice and moles inhabiting mountainous areas of Shimane Peninsula as source of infection with *Yersinia pseudotuberculosis*. Journal of Clinical Microbiology 28: 2448–2455.
- , K. HOSHINA, R. NAKAMURA, Y. ITO, AND M. GOMYODA. 1987. Epidemiological study of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in Shimane Prefecture, Japan. Contributions to Microbiology and Immunology 9: 103–110.
- GEMSKI, P., J. R. LAZERE, AND T. CASEY. 1980. Plasmid associated with pathogenicity and calcium dependency of *Yersinia enterocolitica*. Infection and Immunity 27: 682–685.
- HAMASAKI, S., H. HAYASHIDANI, K. KANEKO, M. OGAWA, AND Y. SHIGETA. 1989. A survey for *Yersinia pseudotuberculosis* in migratory birds in coastal Japan. Journal of Wildlife Diseases 25: 401–403.
- HAYASHI, Y., T. NISHIDA, AND K. MOCHIZUKI. 1977. Sex and age determination of the Japanese wild boar (*Sus scrofa leucomystax*) by the lower teeth. Japanese Journal of Veterinary Science 39: 165–174.
- JOHNSON, J. L., M. P. DOYLE, AND R. G. CASSENS. 1990. *Listeria monocytogenes* and other *Listeria* spp. in meat and meat products. Journal of Food Protection 53: 81–91.
- KADO, C. I., AND S. T. LIU. 1981. Rapid procedure for detection and isolation of large and small plasmids. Journal of Bacteriology 145: 1365–1373.
- KANAI, Y., H. HAYASHIDANI, K. KANEKO, M. OGAWA, T. TAKAHASHI, AND M. NAKAMURA. 1997. Oc-

- currence of zoonotic bacteria in retail game meat in Japan with special reference to *Erysipelothrix*. *Journal of Food Protection* 60: 328–331.
- LAIRD, W. J., AND D. C. CAVANAUGH. 1980. Correlation of autoagglutination and virulence of yersiniae. *Journal of Clinical Microbiology* 11: 430–432.
- LOVETT, J. 1989. *Listeria monocytogenes*. In *Foodborne bacterial pathogens*, M. P. Doyle (ed.). Marcel Dekker, New York, New York, pp. 283–310.
- SATO, K., AND M. KOMAGANE. 1991. *Yersinia pseudotuberculosis* infection in children due to untreated drinking water. *Contributions to Microbiology and Immunology* 12: 5–10.
- SCHIEHMANN, D. A. 1989. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. In *Foodborne bacterial pathogens*, M. P. Doyle (ed.). Marcel Dekker, New York, New York, pp. 601–672.
- SEELIGER, H. P. R., AND D. JONES. 1986. *Listeria*. In *Bergey's manual of systematic bacteriology*. Vol. 2, P. H. A. Sneath, N. S. Maine, M. E. Sharpe, and J. G. Holt (eds.). Williams & Wilkins, Baltimore, Maryland, pp. 1235–1245.
- TSUBOKURA, M., K. ITAGAKI, AND K. KAWAMURA. 1970. Studies on *Yersinia (Pasteurella) pseudotuberculosis* I. Sources and serological classification of the organisms isolated in Japan. *Japanese Journal of Veterinary Science* 32: 227–233.
- , K. OTSUKI, K. SATO, M. TANAKA, T. HONGO, H. FUKUSHIMA, T. MARUYAMA, AND M. INOUE. 1989. Special features of distribution of *Yersinia pseudotuberculosis* in Japan. *Journal of Clinical Microbiology* 27: 790–794.
- WAUTERS, G., M. JANSSENS, A. G. STEIGERWALT, AND D. J. BRENNER. 1988. *Yersinia mollaretii* sp. nov. and *Yersinia bercovieri* sp. nov., formerly called *Yersinia enterocolitica* biogroups 3A and 3B. *International Journal of Systematic Bacteriology* 38: 424–429.

Received for publication 2 August 1999.