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Intestinal Carriage of *Yersinia pseudotuberculosis* by Wild Birds and Mammals in Japan

HIROSHI FUKUSHIMA* and MANABU GOMYODA

Public Health Institute of Shimane Prefecture, Nishihamasada, Matsue, Shimane 690-01, Japan

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Fecal specimens were obtained from wild birds and mammals in the eastern part of Shimane Prefecture, Japan, an area where serotype 1b, 2b, 3, and 4b strains of *Yersinia pseudotuberculosis* were found to be prevalent in humans. Each of 869 animals, including 259 wild birds and 610 wild mammals, was screened for yersiniae. A total of 37 strains of *Y. pseudotuberculosis* were isolated from 34 (5.6%) mammals, including 23 raccoon dogs (*Nyctereutes procyonoides*), eight deer (*Cervus nippon*), two hares (*Lepus brachyurus*), and one marten (*Martes melampus*), and from two (0.8%) birds, including one eastern spot-billed duck (*Anas poecilorhyncha*) and one wigeon (*Anas penelope*). The *Y. pseudotuberculosis* isolates collected belonged to virulence plasmid-positive (serotypes 1b, 2b, 3, 4b, and 6) and virulence plasmid-negative (serotype 5a) strains, the most predominant serotype being 4b. The close relationship between the regional distributions of *Y. pseudotuberculosis* in wild animals and humans suggests that wild animals are an important source of infection.

Yersinia pseudotuberculosis is recognized as an important causal agent of sporadic and epidemic human enteric disease and has a wide distribution in both wild and domestic animals (18). In Europe and North America (10, 15), wild mammals and birds are thought to be the principal reservoirs of infection-causing *Y. pseudotuberculosis*. However, the relationship between wildlife reservoirs and human infection with *Y. pseudotuberculosis* is not well understood. In Japan, 14 cases of epidemic infection with *Y. pseudotuberculosis* were reported during the last 13 years (6, 11, 18). In five cases, contaminated streams, wells, or springs were suspected reservoirs of yersiniae. We (2, 4) noted that in two sporadic cases, *Y. pseudotuberculosis* was transmitted to humans through water or soil contaminated by feces from domestic and wild animals infected with this species. Evidence of the mode of transmission of *Y. pseudotuberculosis* to humans through environmental substances such as water and soil has stimulated interest in a possible natural reservoir for this species.

We present herein our findings of a 5-year detailed systematic survey of *Y. pseudotuberculosis* and environmental yersiniae in wild mammals and birds present in the eastern part of Shimane Prefecture, Japan, where the epidemiology of human yersiniosis has been studied for 13 years.

MATERIALS AND METHODS

Specimens. Fecal and cloacal specimens were obtained from 610 wild mammals and 259 birds caught by 10 hunters during the hunting season (November to February), collected for ecological investigations of deer on the Shimane Peninsula during November to April, and submitted to one animal hospital for diagnosis of morbidity or mortality in the eastern part of Shimane Prefecture, Japan, in October 1986 through February 1990 (see Table 1). Fecal or cloacal swabs were preserved in Cary-Blair transport medium (Nitsusui, Tokyo, Japan), kept refrigerated during subsequent storage, and then brought to our laboratory.

Isolation and identification. Direct and cold enrichment culture methods and identification of yersiniae were as described in our previous report (3). Identification of yersiniae was done by the method of Wauters et al. (19), and serological grouping of *Y. pseudotuberculosis* and other *Yersinia* spp. was done as described in our previous reports (2, 3).

***Y. pseudotuberculosis* isolates from humans and wild mice.** The 55 strains of *Y. pseudotuberculosis* isolated from patients and wild mice in our laboratory were used for a comparison of serotype distributions of *Y. pseudotuberculosis* in the same distinct geographic area (see Table 2). Forty-five strains of serotypes 1b (14 strains), 2b (1 strain), 3 (2 strains), and 4b (28 strains) were isolated from stools of patients since 1978 (5). Nine strains of serotypes 1b (two strains) and 4b (seven strains) and an untypeable strain were isolated from wild mice from 1986 to 1989 (3).

Assay of virulence-associated properties. All isolates identified as *Y. pseudotuberculosis* were examined for three virulence-associated properties. The first or second cultures from Irgasan-novobiocin agar or Kligler slant medium were examined for autoagglutination at 37°C (14) by using tryptic soy broth. The stock preparations (the third cultures) were examined for calcium dependency at 37°C on magnesium oxalate agar (9) and the presence of a virulence plasmid. The presence or absence of a virulence plasmid was kindly determined by Seiji Kaneko (Tokyo Metropolitan Research Laboratory of Public Health, Tokyo, Japan) by the method of Kaneko and Maruyama (13).

RESULTS

Isolation of *Y. pseudotuberculosis* and other yersiniae. A total of 37 strains of *Y. pseudotuberculosis* were isolated from 34 mammals (5.6%) belonging to a variety of families and two birds (0.8%) (Tables 1 and 2). Thirty-five isolates from mammals belonged to serotypes 1b, 2b, 3, 4b, 5a, and 6, and two isolates from birds belonged to serotypes 1b and 4b. The most predominant serotype was 4b. Thirty-five strains, except for two strains of serotype 5a isolated from a deer and a raccoon dog, harbored a 40- to 50-MDa virulence plasmid and were positive for virulence-associated properties. No pathogenic *Y. enterocolitica* was isolated. A total of 732 strains of environmental yersiniae were isolated from

* Corresponding author.

TABLE 1. Isolation of *Y. pseudotuberculosis* from wild animals

Animal	No. of animals examined	No. (%) of animals positive
Wild mammals	610	34 (5.6)
Deer (*Cervus nippon*)	215	8 (3.7)
Hare (*Lepus brachyurus*)	139	2 (1.4)
Wild Boar (*Sus scrofa*)	51	0
Marten (*Martes melampus*)	34	1 (2.9)
Weasel (*Mustela sibirica*)	1	0
Raccoon dog (*Nyctereutes procyonoides*)	164	23 (14.0)
Fox (*Vulpes vulpes*)	6	0
Wild birds	259	2 (0.8)
Mallard (*Anas platyrhynchos*)	74	0
Teal (*Anas crecca*)	60	0
Eastern spot-billed duck (*Anas poecilorhyncha*)	24	1 (4.2)
Wigeon (*Anas penelope*)	22	1 (4.5)
Falcated teal (*Anas falcata*)	15	0
Pintail (*Anas acuta*)	4	0
Shoveller (*Anas clypeata*)	1	0
Tufted duck (*Aythya fuligula*)	3	0
Eastern Bewick's swan (*Cygnus columbianus*)	1	0
Night heron (*Nycticorax nycticorax*)	2	0
Kamchatkan black-headed gull (*Larus ridibundus*)	20	0
Japanese pheasant (*Phasianus versicolor*)	13	0
Copper pheasant (*Phasianus soemmerringii*)	6	0
Eastern turtledove (*Streptopelia orientalis*)	3	0
Common snipe (*Gallinago gallinago*)	2	0
Falcon (*Falco tinnunculus*)	1	0
Eastern carrion crow (*Corvus corone*)	3	0
Pale ouzel (*Turdus pallidus*)	1	0
Dusky thrush (*Turdus naumanni*)	3	0
Brown-eared bulbul (*Microscelis amaurotis*)	1	0
Total	869	36 (4.1)

424 of 610 wild mammals (69.5%, 626 strains) and from 85 of 259 wild birds (32.8%, 106 strains). There were 289 *Y. enterocolitica* isolates, 8 sorbose-positive strains and 226 sorbose-negative strains of *Y. moralletii*, 58 *Y. frederiksenii* strains, 48 *Y. intermedia* strains, 42 *Y. kristensenii* strains and 7 *Y. aldvae* strains. The predominant serotype was O:14 of *Y. enterocolitica* biotype 1 (127 strains). Of 32 *Y. enterocolitica*-like strains, 10 were esculin negative, 11 were esculin positive and indole negative, and 11 were Voges-Proskauer negative.

TABLE 2. Serotypes of *Y. pseudotuberculosis* isolated from wild animals and humans

Source	No. of *Y. pseudotuberculosis* isolates							
	Total	1b	2b	3	4b	5a[a]	6	UT
Deer	8			1	6	1		
Hare	2	1	1					
Marten	1				1			
Raccoon dog	24	3	2[b]		17	1[b]	1	
Eastern spot-billed duck	1	1						
Wigeon	1				1			
Wild mouse[c]	10	2			7			1
Human[d]	45	14	1	2	28			

[a] Plasmid-negative strain of *Y. pseudotuberculosis*.
[b] Two strains were concomitantly isolated from the same animal.
[c] Data from reference 3.
[d] Data from reference 5.

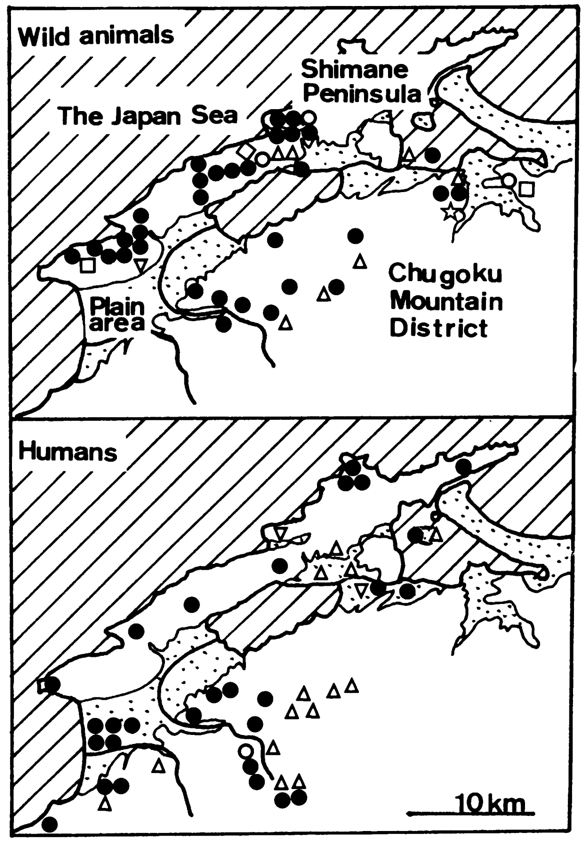

FIG. 1. Regional distribution of *Y. pseudotuberculosis* among wild animals and humans in the eastern part of Shimane Prefecture. Symbols: △, serotype 1b; ○, serotype 2b; ▽, serotype 3; ●, serotype 4b; □, serotype 5a; ☆, serotype 6; ◇, unserotypeable.

Regional distribution of isolates. Fecal specimens from wild animals and humans were collected over a wide area in the eastern part of Shimane Prefecture. The regional distributions of *Y. pseudotuberculosis* serotypes 1b and 4b were closely related between wild animals and humans (Fig. 1). Serotype 4b strains were widely isolated from deer, a marten, raccoon dogs, wild mice, an eastern spot-billed duck, and a wigeon in the same area in which serotype 4b strains were prevalent among humans. Serotype 1b strains were isolated mainly from raccoon dogs and a hare in the limited area of the Chugoku Mountain district, an area in which serotype 1b strains were prevalent among humans. Serotype 1b and 4b strains isolated from an eastern spot-billed duck and a wigeon collected on rivers or lakes were prevalent among raccoon dogs collected on a mountain about 10 km away. Environmental *Yersinia* organisms were isolated from animals over a wide area.

DISCUSSION

Wild birds (1, 8, 10, 15, 20) and mammals (1, 10, 12, 16, 17) have been investigated worldwide as a possible natural reservoir for *Y. pseudotuberculosis*; however, the role of wildlife reservoirs as the source of infection with *Y. pseudotuberculosis* is not well understood. In the present study, *Y.*

pseudotuberculosis serotype 1b, 2b, 3, and 4b strains, which are prevalent among humans in the eastern part of Shimane Prefecture, Japan, were isolated from raccoon dogs, deer, hares, and a marten in the mountainous area and from wild ducks in rivers and lakes. Thus, an epidemiologic link between humans and wild animals became evident.

The isolates of *Y. pseudotuberculosis* serotypes 1b, 2b, 3, 4b, and 6, but not those of serotype 5a, contained a virulence plasmid and were found in raccoon dogs, deer, hares, a marten, an eastern spot-billed duck, and a wigeon. In the high or remote places among these mountains, the residents of which used unchlorinated water from a well and/or a mountain stream, many strains of serotype 1b, 2b, and 4b were isolated from patients with gastroenteritis due to *Y. pseudotuberculosis* (5). The frequency of isolation of serotypes from wild animals was exactly the same as that from humans, who were infected mostly with serotype 4b, followed by serotype 1b and then serotype 2b. Serotype 4b strains were widely prevalent among humans and wild animals regardless of areas. Serotype 1b strains, however, were prevalent mainly among humans, raccoon dogs, and a hare in the Chugoku Mountain district. We (2, 4) reported evidence of transmission of *Y. pseudotuberculosis* to humans through water contaminated by feces from wild and domestic animals infected with this species. In five cases of epidemic infection with *Y. pseudotuberculosis* in Japan (6, 11), drinking water from streams, wells, and springs in mountainous areas was considered to have caused the infection. Thus, wild animals are an important reservoir of infection with *Y. pseudotuberculosis* in a mountainous area.

Raccoon dogs harbored *Y. pseudotuberculosis* at the highest rate (14%) among the animals. This omnivorous animal belonging to a canine family is found in Japan, China, and Korea. Recently the population of raccoon dogs showed a tendency to increase in mountainous areas close to residences in Japan. Some residences in mountainous areas use drinking water from mountain streams or wells. Thus, it was strongly suggested that raccoon dogs are the most important reservoir of infection-causing *Y. pseudotuberculosis* in the mountainous area examined. Elsewhere, 0.7% of the wild mice examined in this area harbored plasmid-positive strains of *Y. pseudotuberculosis* (3). The question of whether a significant source of infection with *Y. pseudotuberculosis* in a mountainous area is raccoon dogs or wild mice remains unclear, since the population of raccoon dogs is much smaller than that of wild mice.

Y. pseudotuberculosis was harbored in carnivorous animals, such as martens; herbivorous animals, such as deer, hares, and ducks; and omnivorous animals, such as raccoon dogs, and it was also isolated from soil (1, 2) and stream water (4, 11). These findings suggest that *Y. pseudotuberculosis* infects animals by both routes, i.e., preying upon animals infected with *Y. pseudotuberculosis* and ingesting environmental substances contaminated with *Y. pseudotuberculosis*.

In the present study, *Y. pseudotuberculosis* serotype 1b and 4b strains were isolated from an eastern spot-billed duck and a wigeon collected in rivers and lakes and from wild mammals collected on the nearby mountain (Fig. 1). These ducks inhabit lakes and marshes, rivers, seas, and paddy fields and live in ponds in mountainous areas. Thus, birds with *Y. pseudotuberculosis* might have been infected through environmental substances, such as water and soil, contaminated by wild animals infected with this species in the area examined. Eastern spot-billed ducks inhabit the eastern part of Asia and also multiply in Japan, but wigeons multiply in the northern part of Eurasia and migrate to Japan, southern China, India, North America, or northern Africa, etc. Hamasaki et al. (8) isolated serotype 4b strains from migratory birds, two black-faced buntings (*Emberiza spodocephala*), obtained in coastal regions of Japan. These serotypes have been isolated from various specimens all over the world (1, 10, 15, 16, 18, 20). There is no evidence that migratory birds are significant reservoirs for the transmission of *Y. pseudotuberculosis* between southern and northern continents, although Hubbert (10) did report that the three major serotypes of *Y. pseudotuberculosis* present in the United States are found along the major flyways of migratory birds.

As environmental yersiniae were found in 34.9% of mammals and 12.4% of birds, regardless of the *Yersinia* species, environmental yersiniae are probably among the normal flora of wild animals. Serotype O:14 *Y. enterocolitica* organisms were most prevalent in wild animals, regardless of the animal species. Serotype O:14 organisms were frequently detected in surface water of rivers (7) and in wild mice and moles (3). Thus, contamination with yersiniae may be reciprocal between animals and environmental substances.

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