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Occurrence of *Yersinia enterocolitica* in Wild Animals

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Yersinia species were isolated from 16 of 495 small wild animals and from 1 of 38 foxes. The animals were trapped in seven regions of Hokkaido, Japan. Of the 17 strains isolated, 9 were *Yersinia enterocolitica* O6; 2 were *Y. enterocolitica* O5A; 1 was *Y. enterocolitica* O4; 1 was *Y. enterocolitica* O9; 1 was *Yersinia pseudotuberculosis* IVB; and 3 were sucrose-negative strains. *Yersinia pestis* was not isolated. The O6 organism was most prevalent in large red-back mice (*Clethrionomys rufocanus bedfordiae*) and showed significant differences in its mode of distribution according to region. Incidence of the O6 organism in the ileum of the animal was threefold that in the cecum, and the organism was recovered at approximately 10^5 cells per g of cecal contents per *C. rufocanus bedfordiae* animal.

In 1979 Brenner (5) proposed that *Yersinia enterocolitica* be divided into three species: *Y. enterocolitica*, *Yersinia intermedia*, and *Yersinia frederiksenii*. Since these new names are not on the *Approved Lists of Bacterial Names* (12) and have not yet been published in the *International Journal of Systematic Bacteriology*, we continue to use *Y. enterocolitica*. Kaneko et al. (9) cultured *Y. enterocolitica* of various serogroups, including the O3 organism, which is known as a human pathogen, from over 20% of the house rats examined. Other investigators (1, 2, 4, 10, 11) isolated the pathogen from wild animals; however, their findings were inconclusive as to the regional prevalence and intestinal distribution of the pathogen. In this study, we discuss the occurrence of *Y. enterocolitica* in small wild animals and foxes.

MATERIALS AND METHODS

Specimens examined. From July to September 1978, 495 small wild animals, including 238 old world woods mice (*Apodemus speciosus*), 65 Geisha mice (*Apodemus argenteus*), 176 large red-backed mice (*Clethrionomys rufocanus bedfordiae*), 5 small red-backed mice (*Clethrionomys rutilus*), 9 big-clawed shrews (*Sorex unguiculatus*), and 2 Asiatic chipmunks (*Tamias sibiricus*), were trapped in regions of Hokkaido, Japan. Figure 1 shows a map of these regions. The contents of the ileum and cecum of each were sampled during anesthetization. In February 1979, 38 foxes (*Vulpes vulpes*) were trapped in Abashiri and Kushiro, and the contents of their ilea and livers were sampled during anesthetization.

Procedures. Direct and enrichment culture methods and identification of isolated strains were as described in previous reports (8, 9). Biotyping of isolated *Y. enterocolitica* strains was made by the scheme of Wauters as described in a previous report (7).

Quantitative direct culture. The sampled specimens were kept at −80°C after preparation of the direct and enrichment cultures. Specimens found to be positive by the direct culture method were submitted to the quantitative direct culture method. Plated samples were made of the cecal contents, and these were suspended in physiological saline to concentrations of 1 and 0.1%. The selective media used were the same as those described in a previous report (9).

RESULTS

Regional distribution of the organism in animal species. *Y. enterocolitica* was isolated from 15 animals, and *Y. pseudotuberculosis* was isolated from 1 of 495 small animals. From 1 of 38 foxes, *Y. enterocolitica* was isolated. In Table 1 the number of animals sampled and those which were positive in the seven regions are shown. The organism was isolated in three of the five regions where small animals were trapped and one of the two regions where foxes were trapped. In Table 2 the number of positive animals in each region is shown. The organisms were isolated from small rodents of *C. rufocanus bedfordiae*, *A. speciosus*, *A. argenteus*, and *V. vulpes*, and not from *C. rutilus*, *T. sibiricus*, or *S. unguiculatus*. The O6 organism was isolated from 2 of 18 *C. rufocanus bedfordiae* in Hidaka, 4 of 28 in Rumoi, and none of the 53 animals examined in Kamikawa. The O6 organism was isolated from 3 of 19 *A. argenteus* and none of the 39 *A. speciosus* animals in Hidaka.

Organism O5A was isolated from one *C. rufocanus bedfordiae* and one *A. speciosus* trapped in Rumoi; O9 from one *A. speciosus* in the same region; O4 from one *V. vulpes* in Kushiro; a sucrose-negative strain (S strain) from two *C. rufocanus bedfordiae* and one *A. speciosus* in Kamikawa and Rumoi; and a *Y. pseudotuber-*

culosis IVB organism was isolated from only one *A. speciosus* trapped in Kamikawa.

Distribution of the organism in the intestine. Isolation of the organisms from the intestines and liver of positive cases is shown in Table 2. The O4 organism was isolated from the liver. Isolation of organisms O5A, O9, IVB, and S strain occurred almost equally in the ilea and ceca of the rodents. The O6 organism was more prevalent in the ileum than in the cecum of *C. rufocanus bedfordiae*.

Quantitative direct culture. In three O6-positive and one S strain-positive *C. rufocanus bedfordiae* and in 1 IVB-positive *A. speciosus*, the organism was isolated by direct culture. Of these, the contents of the cecum of 2 O6- and 1 S strain-positive animals were submitted to a quantitative direct culture. The organisms were counted at 1.3×10^3 O6, 9.1×10^4 O6, and 5.0×10^5 S strain cells per g of cecal content.

Biochemical characters of the isolates. In Table 3 the biochemical characters of the isolates are shown, with the exception of *Y. pseudotuberculosis* IVB. All of the strains from small animals gave typical reactions for *Y. enterocolitica* (sucrose positive; rhamnose, raffinose, and melibiose negative). All of them belonged to biovar 1. The strain isolated from the fox produced acid from these three carbohydrates and also belonged to biovar 1.

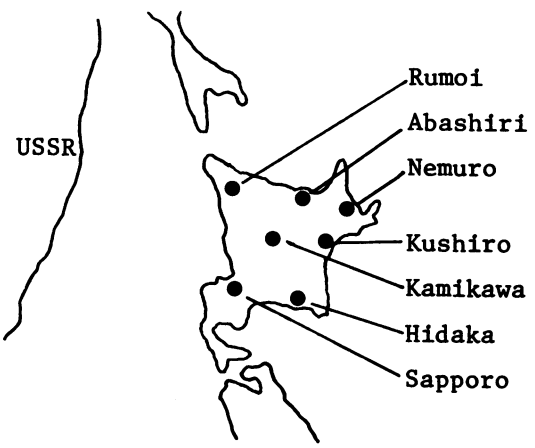

FIG. 1. *Regions examined in Hokkaido, Japan.*

DISCUSSION

Outbreaks of human infection with the O3 organism were reported in five communities in Japan (3, 14–16). Human infection with the O6 organism has not yet been reported in Japan. The O6 organism, however, is the third most predominant human pathogen in Ontario, Quebec, and the eastern provinces of Canada according to Toma et al. (13). Kaneko et al. (9) reported that the O6 organism was most commonly isolated from rats. In this study, it was also found to be most prevalent in *C. rufocanus bedfordiae*. Bercovier et al. (4) reported that the O6 organism was most prevalent among typical *Y. enterocolitica* strains isolated from small animals. Kapperud (10) also reported that the O6 organism was most prevalent in small wild rodents, and he suggested that it was a member of the normal flora of these animals. In this study, however, a significant difference according to region was found in the prevalence of the O6 organism in animals from the area between Kamikawa and Rumoi ($P = 0.0123$). The organism recovered from the intestine was either undetectable or observed in approximately 10^5 cells per g. These facts seem to discredit the concept that the O6 organism is a member of the normal flora of small wild rodents.

Furthermore, our findings showed that the distribution of the O6 organism in the intestines of *C. rufocanus bedfordiae* differed from that in rats. Kaneko et al. (9) reported that in the rat intestine the O6 organism was predominant first in the cecum, second in the colon, and third in the rectum and that the prevalence of the O6 organism in the ileum was less than half of that

TABLE 1. *Number of wild animals examined in seven regions*

Region	No. of animals from the following species:							
	A. speciosus	*A. argenteus*	*C. rufocanus bedfordiae*	*C. rutilus*	*T. sibiricus*	*S. unguiculatus*	*V. vulpes*	Total
Sapporo	76	33	28			4		141
Nemuro	4		49	5				58
Hidaka	39	19 (3)[a]	18 (2)					76 (5)
Kamikawa	36 (1)	11	53 (1)		2	2		104 (2)
Rumoi	83 (2)	2	28 (7)			3		116 (9)
Kushiro							11 (1)	11 (1)
Abashiri							27	27
Total	238 (3)	65 (3)	176 (10)	5	2	9	38 (1)	533 (17)

[a] The number of *Yersinia*-positive cases is given within parentheses.

TABLE 2. *O group or serovar of isolates from the intestine and liver of 17 animals in four regions*

Animal species	Body region	Isolates from:						
		Hidaka (O6)[a]	Kamikawa		Rumoi			Kushiro (O4)
			IVB	S	O5A	O6	O9	S	
A. speciosus	Ileum				1			1	
	Cecum		1					1	
No. of positive cases			1		1			1	
A. argenteus	Ileum	2							
	Cecum	2							
No. of positive cases		3							
C. rufocanus bedfordiae	Ileum	2		1	1	4	1	1	
	Cecum			1		2		1	
No. of positive cases		2		1	1	4	1	1	
V. vulpes	Ileum								
	Cecum								
	Liver								1
No. of positive cases									1

[a] Organisms O6, O5A, O9, and O4 are O groups of *Y. enterocolitica*; IVB is a serovar of *Y. pseudotuberculosis*; and S indicates a sucrose-negative strain.

TABLE 3. *Biochemical characters of isolates at 22°C*

Test	Strains from small animals (12)[a]	Fox strain (1)	Sucrose-negative strains (3)
Rhamnose	−	+	−
Raffinose	−	+	−
Melibiose	−	+	−
Sucrose	+	+	−
Trehalose	+	+	+
Ornithin decarboxylase	+	+	+
Nitrate	+	+	+

[a] Number of strains tested is given within parentheses.

in the rectum. Our study demonstrated, however, that the prevalence in the ileum was threefold that in the cecum. Whether or not this phenomenon correlates with the feeding habits of herbivorous *C. rufocanus bedfordiae*, whose cecum is large enough to ferment the contents, is of particular interest.

Two *Apodemus* species were trapped in the same locality of Urakawa at the same time. Although the O6 organism was not isolated from *A. speciosus*, it has been recovered from three *A. argenteus* individuals. Further investigations are necessary to determine whether or not the two species within the same genus differ in susceptibility to infection with the O6 organism.

Alonso et al. (2) reported that isolation of *Y. enterocolitica* occurred in 5.7% of 3,533 small animals. In this study, it occurred at a significantly lower rate (3.0% of 495 small animals) than in the previous results ($P < 0.02$). Thus, the prevalence of *Y. enterocolitica* isolated in small animals in Hokkaido was lower than that in France.

It was suggested that the O4 organism might be pathogenic to foxes because it was isolated from the liver. Spread of the O4 organism by the food chain between the fox and the small animals, however, was not demonstratable in this study as there was no isolation of this organism from the small animals.

In Hidaka, Kamikawa, and Rumoi, the organisms were isolated from small animals in the forests in the heart of mountains far from human residence sections. In Nemuro and Sapporo, where the organisms were not detected in 58 and 141 animals, they were found in a cow pasture and in two forests in the hills not so far from human residence sections. It is unknown, however, whether recovery of the organism might be affected by the distance between the examined region and human residence sections or by natural features of that region. Since small animal trapping was done during 2 summer months, the duration of trapping might not have affected the recovery of the organisms. Fox trapping was done during 1 winter month in 2 cow pastures. We could not speculate as to the effects of duration of trapping and topographic features on the recovery of the organism.

According to Brenner (5), the 12 strains which did not produce acid from rhamnose, raffinose, and melibiose corresponded to *Y. enterocolitica*, and 1 fox strain corresponded to *Y. intermedia*. It seems necessary to separate the sucrose-negative organisms from *Y. enterocolitica* as their character has been shown to differ from that of sucrose-positive organisms by deoxyribonucleic acid-deoxyribonucleic acid homology (6).

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