JOURNAL OF CLINICAL MICROBIOLOGY, May 1995, p. 1253–1257 Vol. 33, No. 5
0095-1137/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Potential Sources of Sporadic Human Infection with *Yersinia enterocolitica* Serovar O:8 in Aomori Prefecture, Japan

HIDEKI HAYASHIDANI,[1]* YOSHIMITSU OHTOMO,[2] YASUNOBU TOYOKAWA,[2] MASAAKI SAITO,[3] KEN-ICHI KANEKO,[1] JUNKO KOSUGE,[1] MASAHIKO KATO,[1] MASUO OGAWA,[1] AND GEORG KAPPERUD[4,5]

Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183,[1] Department of Bacteriology, Aomori Prefectural Institute of Public Health and Environment, 1-1-1 Higashi-Tsukurimichi, Aomori, Aomori 030,[2] Adult Diseases Examination Center, Hirosaki Medical Association, 2-7-1 Noda, Hirosaki, Aomori 036,[3] Japan, and Department of Bacteriology, National Institute of Public Health, 0462 Oslo,[4] and Department of Food Hygiene, The Norwegian College of Veterinary Medicine, 0033 Oslo,[5] Norway

Received 7 September 1994/Returned for modification 3 December 1994/Accepted 10 February 1995

In November 1992 and June and August 1993 rectal contents from 204 small mammals living in the wild were collected and examined for the presence of *Yersinia enterocolitica* serovar O:8 to clarify the source of human infections caused by this microbe in the Tsugaru Region of Aomori Prefecture, Japan. Serovar O:8 was isolated from 10 (5.2%) of 193 wild rodents trapped in June 1993 (9 of 107) and August 1993 (1 of 23) but not from animals trapped in November 1992 (0 of 63). This serovar was not isolated from 11 moles. From May to September 1993, 12 human patients were found to have become ill and to be infected with *Y. enterocolitica* O:8. The patients lived in the same districts where the wild rodents harboring serovar O:8 were trapped. Two different patterns by restriction enzyme analysis of the virulence plasmid were observed. One pattern obtained by restriction enzyme analysis of the virulence plasmid was observed in 20 isolates from 11 human patients and 9 wild rodents, and the other was observed in 2 isolates from 1 human patient and 1 wild rodent. These findings indicate that wild rodents seem to play an important role as a source of human *Y. enterocolitica* O:8 infection.

Yersinia enterocolitica is known to be an important human enteric pathogen (12, 23, 28, 35). Serovar O:8, biovar 1, is the most pathogenic among the serovars of *Y. enterocolitica* (5, 13, 26). The geographical distribution of this serovar is very limited. It has been isolated from humans and animals only in North America and Nigeria (1, 7, 15, 23, 25, 26, 36). In 1989, an isolate of this serovar was isolated from small rodents living in the wild in mountainous areas in Japan (17). Furthermore, in 1990 an isolate of the serovar was recovered from a human patient in the Tsugaru Region of Aomori Prefecture, Japan (16). After that, human patients infected with serovar O:8 have been sporadically reported in this area (33). Little is known about the natural reservoirs of this serovar because of its limited distribution. Furthermore, the source of human infections has not yet been identified. The present study was performed to clarify the source of sporadic human infection with *Y. enterocolitica* O:8 in the Tsugaru Region of Aomori Prefecture.

MATERIALS AND METHODS

Specimens. In November 1992 and June and August 1993, 204 small wild mammals living in the wild, including 96 large Japanese field mice (*Apodemus speciosus*), 88 small Japanese field mice (*Apodemus argenteus*), 9 Anderson's red-backed voles (*Eothenomys andersoni*), and 11 Japanese shrew moles (*Urotrichus talpoides*), were trapped in the Tsugaru Region (40°36′N, 140°28′E) of Aomori Prefecture, Japan, which is located in northern area of Honshu Island. The animals were trapped at six investigation points located in the same areas where cases of *Y. enterocolitica* O:8 infection in humans had been recorded (Fig. 1). All trapping stations were located near streams used as drinking water supplies by the local population in the mountainous area (150 to 500 m above sea level). In August 1993, the investigation was carried out at points E and F only. All animals trapped were immediately transported to the laboratory and were dissected. The rectal contents were collected from each animal and were examined for the presence of *Yersinia* spp.

Human patients. In 1993, 12 cases of infection with *Y. enterocolitica* O:8 were recorded in humans in the Tsugaru Region (Fig. 1; Table 1). Infections in humans occurred during May through September 1993. All patients became ill and were hospitalized. We interviewed the doctors in charge of the patients about the epidemiological factors related to the infections. Of 12 patients, all showed symptoms of abdominal pain and diarrhea, 11 had temperatures of 38 to 40°C, 5 had cough, 4 had nausea, 4 had pharyngeal pain, 3 had vomiting, 3 were dehydrated, 2 had bloody urine, and 2 had headaches. Serovar O:8 organisms were isolated from the feces of the patients. All patients recovered within 2 weeks. There was no secondary infection. One patient was a visitor who had been staying at a house located in the mountainous area in this region for a month before she became ill. Eleven patients were residents of this region, six were from urban areas, four were from the mountainous area, and one was from a rural plain area. Moreover, of 12 patients, 4 used the mountain stream water for their drinking water, 1 used well water, and the others used city water. Two patients had eaten the pork just before they became infected with this bacterium. Two families of three patients had a dog. We had no way of knowing exactly the patients' movements which allowed them to come into contact with the infection, such as traveling or lodging in a rodent-infested area, just before they became ill. A common epidemiological factor associated with the infection among the patients, such as occupation, school, food, or residence, could not be found. Therefore, those cases of infection were determined to be sporadic. It was not possible to elucidate the infection route or the source of those sporadic human infections with *Y. enterocolitica* O:8.

Isolation and identification. The rectal contents (0.5 g) of animals were suspended in 4.5 ml of phosphate-buffered saline (PBS; pH 7.6), and 0.1 ml of a 10-fold dilution in PBS was plated onto irgasan-novobiocin (IN) agar plates (9). All PBS suspensions were incubated at 4°C for 3 weeks and were then subcultured onto IN agar plates after alkali (KOH) treatment (3).

All plates were incubated at 25°C for 48 h. Colonies morphologically similar to those of *Yersinia* spp. were subcultured for biochemical examination. Biochemical characteristics were examined on triple sugar iron medium (Eiken, Tokyo, Japan), lysine indole motility medium (Nissui, Tokyo, Japan), and urea broth (Eiken). If reactions of typical *Yersinia* spp. were seen, additional biochemical tests were performed to identify the species of each isolate by the method of Wauters et al. (39). Serotyping of *Y. enterocolitica* isolates was accomplished by

* Corresponding author. Mailing address: Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Saiwai-cho 3-5-8, Fuchu, Tokyo 183, Japan. Phone: 81-423-67-5775. Fax: 81-423-60-8830. Electronic mail address: eisei@cc.tuat.ac.jp.

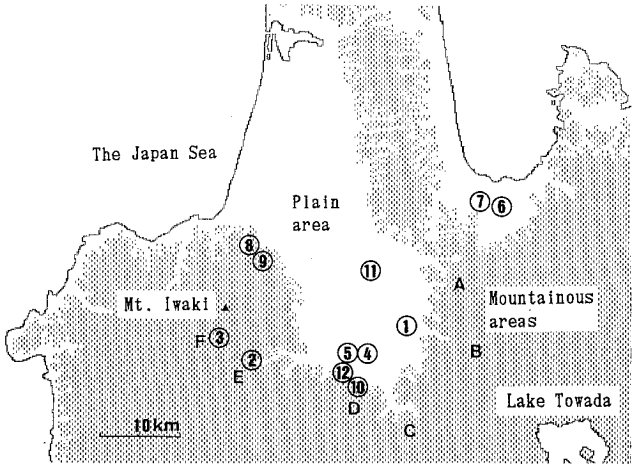

FIG. 1. Map of trapping stations (points A to F) and residences of human patients (patients 1 to 12) infected with *Y. enterocolitica* O:8 in 1993 in the Tsugaru Region of Aomori Prefecture, Japan. Patient numbers (1 to 12) correspond to the numbers in Table 1.

slide agglutination with commercial rabbit anti-*Y. enterocolitica* O:1, O:2, O:3, O:5, O:8, and O:9 sera (Denka-Seiken, Tokyo, Japan) and rabbit anti-*Y. enterocolitica* O:7 and O:19 sera made in our laboratory by the method of Winblad et al. (40).

Assay of virulence-associated properties. To evaluate potential pathogenicity, *Y. enterocolitica* serovar O:3, O:5, O:8, and O:9 isolates were examined for their temperature-dependent calcium requirements by the method of Gemski et al. (11), temperature-dependent autoagglutination by the method of Laird and Cavanaugh (27), pyrazinamidase activity by the method of Kandolo and Wauters (20), and the presence of a virulence plasmid by the method of Kado and Liu (19).

Mouse pathogenicity test. Four-week-old specific-pathogen-free ICR mice (Shizuoka Agricultural Cooperative Association Laboratory Animals, Hamamatsu, Japan) in groups of five each were inoculated intravenously with various amounts (to 10^4 CFU) of viable cells of two O:8 strains (YE93008 and NY9306089) and were observed for 4 weeks. The mean lethal dose was calculated by the method of Reed and Muench (32).

Restriction endonuclease analysis of virulence plasmid DNA. Restriction endonuclease analysis of plasmid DNA (REAP) from *Y. enterocolitica* O:8 was performed to compare isolates from humans and the wild rodents. REAP was carried out by the method of Kapperud et al. (24) by using the restriction endonuclease *Cfo*I (Boehringer Mannheim GmbH, Mannheim, Germany). Electrophoresis was performed for 17 h at 70 V in a vertical 4% polyacrylamide gel by using a Mini-Protean II slab gel cell (Bio-Rad, Richmond, Calif.).

RESULTS

Isolation of *Yersinia* spp. Five species of *Yersinia* spp. were isolated from 116 (60.1%) of 193 rodents and 4 (36.4%) of 11 moles (Table 2). *Yersinia* spp. were recovered from 56 (58.3%) of 96 *A. speciosus*, 55 (62.5%) of 88 *A. argenteus*, 5 (55.6%) of 9 *E. andersoni*, and 4 (36.4%) of 11 *U. talpoides* animals. *Y. enterocolitica* was isolated from 62 (32.1%) of 193 rodents, but it was not isolated from moles. Other *Yersinia* species, including *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii*, and *Y. aldovae*, were isolated from 70 (36.3%) of 193 rodents. *Y. pseudotuberculosis* was not isolated from rodents or moles. There was no significant difference in the isolation rates of *Yersinia* spp. among the animal species examined. Three O:3 strains, 10 O:8 strains and 1 O:9 strain were isolated from the rodents. The O:8 bacteria were isolated from 2 (2.1%) of 96 *A. speciosus*, 7 (8.0%) of 88 *A. argenteus*, and 1 (11.1%) of 9 *E. andersoni* animals. The 10 O:8 strains showed positive reactions for the virulence-associated properties such as calcium dependency, autoagglutination, and the presence of the 40- to 50-MDa plasmid and were negative for pyrazinamidase activity. All three O:3 strains and the one O:9 strain had negative reactions for the virulence-associated properties. The 50% lethal doses of two O:8 isolates from a human patient and a wild rodent were $<10^{0.1}$ and $10^{0.5}$, respectively.

Seasonal distribution of *Y. enterocolitica* O:8. Serovar O:8 was isolated from 9 (8.5%) of 105 rodents trapped in June 1993 and 1 (4.3%) of 23 rodents trapped in August 1993. However, isolates of this serovar were not isolated from the 63 rodents captured in November 1992. A significant difference in the isolation rate of *Y. enterocolitica* O:8 from rodents captured in November 1992 and the rate in June 1993 was observed ($P < 0.05$). No difference in the isolation rate of *Yersinia* spp. was observed among the different months of investigation.

Regional distribution of *Y. enterocolitica* O:8. Serovar O:8 was isolated from rodents at five of six trapping stations. No difference in the isolation rate of *Yersinia* spp. was observed among the different trapping stations (Table 3).

Comparison of REAP patterns. All of the *Y. enterocolitica* O:8 strains isolated from wild rodents and human patients harbored a 45-MDa plasmid. The genes associated with virulence are coded on this plasmid.

Figure 2 compares the *Cfo*I digestion patterns of the plasmid DNAs of isolates from humans and wild rodents. Two different REAP patterns were observed; both occurred among isolates from humans as well as rodents. One REAP pattern (REAP 1) was observed in 20 isolates from 11 humans and 9 wild rodents, and the other pattern (REAP 2) was observed in 2 isolates from 1 human and 1 wild rodent (Table 1).

DISCUSSION

In the present study, *Y. enterocolitica* O:8 was isolated from 5.2% of the wild rodents examined. All isolates were obtained in June and August 1993 and in the same area where the human cases of infection occurred. Our finding that *Y. enterocolitica* O:8 was isolated from rodents trapped at five of the six trapping stations suggests that this serovar is widely distributed in the mountainous area of the Tsugaru Region. Moreover, the same REAP patterns were detected in strains from wild rodents as well as from human patients. These results suggest that small rodents living in the wild may be a source of infection for humans in this region.

The 50% lethal doses of the O:8 isolates that originated from human patients and wild rodents were very low and were nearly the same as those of isolates from wild rodents reported previously (17).

Kapperud et al. (24) examined the REAP patterns of serovar O:8 isolates from North America and recognized six banding patterns with the enzymes *Bam*HI and *Eco*RI. The present study identified two REAP patterns among the Japanese isolates with the enzyme *Cfo*I. While REAP 1 was represented among the North American isolates, REAP 2 has so far only been detected in Japan (unpublished data). Further studies are needed to clarify the genetic relationship between the isolates from Japan and North America. Recently, the pulsed-field gel electrophoresis method was applied to examine the genetic relationships among *Yersinia* strains (6, 18). Clearer genetic relationships between isolates from humans and those from wild rodents might be obtained by the PFGE method because this method has a high discriminatory power.

Serovar O:8 has frequently been isolated from human patients in the United States (4, 5, 13, 25, 29, 36). Although this serovar has previously been recovered from pigs, cows, and wild animals (1, 7, 37), its natural reservoir has not been identified. Virulent *Y. enterocolitica* isolates have not been isolated from wild rodents (9, 22), although a few reports on the isolation of serovar O:3 biovar 4 from rats associated with swine

TABLE 1. Origins and REAP patterns of *Y. enterocolitica* serovar O:8 isolates infecting human patients and wild animals and medical conditions and epidemiological aspects of the patients

Patient no.	Strain no.	REAP pattern	Source (age [yr], sex, or species)	Isolation mo (1993)	Location of house or trapping station point[a]	Resident or visitor	Symptoms and signs[b]	Remarks
1	YE93005	1	Human patient (54, female)	May	Urban area	Resident	F (38), D, AP, BU	
2	YE93008	1	Human patient (3, male)	June	Mountainous area	Resident	D, AP, C, PP	
3	YE93009	1	Human patient (6, female)	June	Mountainous area	Resident	F (40), D, AP	Consumption of stream water
4	YE93010	1	Human patient (5, female)	June	Urban area	Resident	F (40), D, AP, N, V, C, PP, DH	
5	YE93011	1	Human patient (4, female)	July	Urban area	Resident	F (39.2), D, AP, N, PP, HA, DH	
6	APCC Y9311	1	Human patient (3, male)	Aug.	Urban area	Resident	F (38.2), D, AP	
7	APCC Y9312	1	Human patient (6, male)	Aug.	Urban area	Resident	F (40), D, AP, N, V, C, DH	Consumption of pork
8[c]	YE93017	1	Human patient (4, female)	Aug.	Mountainous area	Resident	F (39.3), D, AP, BU	Consumption of stream water and contact with a household dog
9[c]	YE93019	1	Human patient (2, male)	Aug.	Mountainous area	Resident	F (38.8), D, AP, PP, C	Consumption of stream water and contact with a household dog
10	APCC Y9313	1	Human patient (2, female)	Aug.	Mountainous area	Visitor	F (39), D, AP, V, C	Consumption of stream water
11	APCC Y9314	2	Human patient (5, female)	Aug.	Rural plain area	Resident	F (38), D, AP, HA	Consumption of pork and well water and contact with a household dog
12	YE93024	1	Human patient (11, male)	Sept.	Urban area	Resident	F (38.3), D, AP, N	
13	NY9306005	2	Wild rodent (*A. argenteus*)	June	A			
14	NY9306039	1	Wild rodent (*A. speciosus*)	June	C			
15	NY9306061	1	Wild rodent (*E. andersoni*)	June	D			
16	NY9306062	1	Wild rodent (*A. argenteus*)	June	D			
17	NY9306071	1	Wild rodent (*A. speciosus*)	June	E			
18	NY9306073	1	Wild rodent (*A. argenteus*)	June	E			
19	NY9306089	1	Wild rodent (*A. argenteus*)	June	E			
20	NY9306101	1	Wild rodent (*A. argenteus*)	June	F			
21	NY9306104	1	Wild rodent (*A. argenteus*)	June	F			
22	NY9308023	1	Wild rodent (*A. argenteus*)	Aug.	E			

[a] Trapping station points are given in Fig. 1.
[b] F, fever; the values in parentheses are body temperatures (in degrees Celsius); D, diarrhea; AP, abdominal pain; N, nausea; V, vomiting; C, cough; PP, pharyngeal pain; HA, headache; DH, dehydration; BU, bloody urine.
[c] Patients 8 and 9 were a sister and a brother living in the same home.

have been published (2, 21). In the present study, serovar O:8 was isolated from 10 wild rodents belonging to the species *A. argenteus*, *A. speciosus*, and *E. andersoni*. To our knowledge, this is the first reported isolation of *Y. enterocolitica* O:8 from *A. speciosus* in the world. Iinuma et al. (17) have previously recovered serovar O:8 from *A. argenteus* and *E. andersoni* in Japan.

Fukushima (8) challenged *A. speciosus* orally with *Y. enterocolitica* O:3. He suggested that *A. speciosus* is not a reservoir for serovar O:3 since this variant was unable to colonize the intestinal tracts of the mice. However, Hayashidani et al. (14) reported that serovar O:8 was able to colonize the intestinal tracts of three wild rodent species (*A. argenteus*, *A. speciosus*, and *E. andersoni*) after oral challenge, and eventually caused their death. The period in which the organism was excreted in feces was 10 to 14 days for *E. andersoni* and *A. speciosus* and 35 to 49 days for *A. argenteus*. We conclude that all three species appear to be an important natural reservoir for *Y. enterocolitica* O:8 in the study area.

Human infections with *Y. enterocolitica* have been reported throughout the year (10, 30, 41). In Japan, the infection is most common in the warm months of the year. In fact, almost all human cases of infection caused by serovar O:8 in the Tsugaru Region have been recorded during the summer months (33).

TABLE 2. Isolation of *Yersinia* spp. from rodents and moles in the Tsugaru Region of Aomori Prefecture, Japan

Animal species	Investigation and yr	No. of animals examined	No. (%) of *Yersinia*-positive animals					
			Total	Y. enterocolitica				Non-*Y. enterocolitica* species[a]
				Total	O3	O8	O9	Other[b]
Rodents								
Apodemus speciosus	Nov. 1992	45	25 (55.6)	7 (15.6)	1			6	20 (44.4)
	June 1993	38	24 (63.2)	17 (44.8)		2		15	8 (21.1)
	Aug. 1993	13	7 (53.8)	4 (30.8)				4	3 (23.1)
Subtotal		96	56 (58.3)	28 (29.2)	1	2		25	31 (32.3)
Apodemus argenteus	Nov. 1992	13	9 (69.2)	6 (46.2)	1			6	6 (46.2)
	June 1993	65	41 (63.1)	20 (30.8)		6		15	28 (43.1)
	Aug. 1993	10	5 (50.0)	4 (40.0)	1	1		3	1 (10.0)
Subtotal		88	55 (62.5)	30 (34.1)	2	7		24	35 (39.8)
Eothenomys andersoni	Nov. 1992	5	2 (40.0)	1 (20.0)				1	2 (40.0)
	June 1993	4	3 (75.0)	3 (75.0)		1	1	1	2 (50.0)
	Aug. 1993								
Subtotal		9	5 (55.6)	4 (44.4)		1	1	2	4 (44.4)
Total of rodents		193	116 (60.1)	62 (32.1)	3	10	1	51	70 (36.3)
Moles, *Urotrichus talpoides*	Nov. 1992	3	2 (66.7)						2 (66.7)
	June 1993	7	2 (28.6)						2 (28.6)
	Aug. 1993	1	0 (0.0)						0 (0.0)
Total		11	4 (36.4)						4 (36.4)
Total of small animals		204	120 (58.8)	62 (30.4)	3	10	1	51	74 (36.3)

[a] *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii*, and *Y. aldovae.*
[b] Nongroupable with antisera O3, O5, O8, and O9.

In contrast, *Y. pseudotuberculosis* infections prevail during the cold months in Japan (10, 34). Fukushima et al. (9) examined the prevalence of yersiniae in wild rodents and moles from mountainous areas of Shimane Peninsula. They suggested that wild rodents, especially young individuals born during the cold season when the rodents breed, are an important reservoir of *Y. pseudotuberculosis* and may transmit the bacterium to humans. In our study, *Y. enterocolitica* O:8 was isolated from rodents during the warm months but not in the cold months. Furthermore, all isolates were obtained from adult rodents. Further studies should be carried out to identify the ecological or behavioral factors influencing the presence of serovar O:8 in the small rodent populations.

In our study, environmental yersiniae (nonvirulent *Y. enterocolitica*, *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii*, and *Y. aldovae*) were isolated from 109 (56.5%) rodents and 4 (36.1%) moles. Such bacteria have frequently been isolated from small wild mammals (9, 12, 15). Thus, *Yersinia* spp. appear to be a normal component of the intestinal flora of these animals.

Keet (26) isolated serovar O:8 from a mountain stream in the United States and linked this finding to a case of human infection. Aulisio et al. (4) recovered serovar O:8 from water used in the preparation of a suspected food source (soybean curd [tofu]) during an outbreak in the United States. Many of the human patients lived in mountainous areas and used untreated or imperfectly treated stream water for their drinking water (33). Because the mountain stream water which the patients used for their drinking water was not examined, the significance of stream water as the vehicle for human infection with *Y. enterocolitica* O:8 is being investigated. Serovar O:8 may also be transmitted from wild rodents to humans by rodent fleas, because serovar O:21, which is closely related to serovar O:8 in many ways (biochemical profile, H antigens, animal virulence), has been isolated from fleas from wild rodents in the United States (31).

TABLE 3. Regional distribution of *Yersinia* spp. in the Tsugaru Region of Aomori Prefecture, Japan

Trapping station point[a]	No. of animals examined	No. (%) of *Yersinia*-positive animals					
		Total	*Y. enterocolitica*				Non-*Y. enterocolitica* species[b]
			Total	O3	O8	O9	Other[c]
A	36	21 (58.3)	8 (22.2)		1		7	15 (41.7)
B	16	10 (62.5)	3 (18.8)				3	8 (50.0)
C	20	12 (60.0)	7 (35.0)		1	1	5	7 (35.0)
D	35	18 (51.4)	10 (28.6)	1	2		8	10 (28.6)
E	60	36 (60.0)	21 (35.0)	2	4		16	18 (30.0)
F	37	23 (62.2)	13 (35.1)		2		12	16 (43.2)
Total	204	120 (58.8)	62 (30.4)	3	10	1	51	74 (36.3)

[a] Trapping station points are shown in Fig. 1.
[b] *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii*, and *Y. aldovae*.
[c] Nongroupable with antisera O3, O5, O8, and O9.

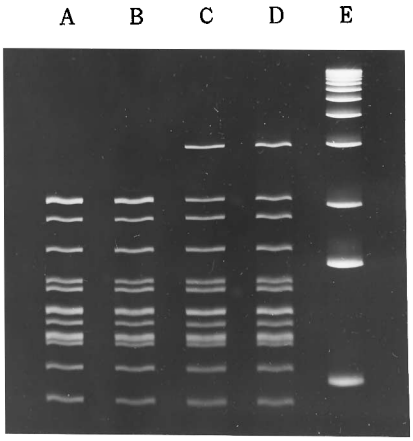

FIG. 2. *Cfo*I digestion patterns of plasmid DNA from *Y. enterocolitica* serovar O:8 from humans and wild rodents. Lanes: A, NY9306005 (wild rodent); B, APCC Y9314 (human patient); C, NY9306101 (wild rodent); D, YE93008 (human patient); E, 1-kb DNA ladder.

From 1991 to 1993 we tried to isolate serovar O:8 from 120 samples of retail pork and beef in the Tsugaru Region, but this serovar could not be isolated from them (unpublished data). However, serovar O:8 was isolated from retail pork in Taiwan (38), and two patients in our study had eaten pork just before they became ill. Therefore, pork may be a potential source of human infection with *Y. enterocolitica* O:8 in urban areas where city water is used. Moreover, further surveys should be carried out to isolate serovar O:8 from dogs in this region, because two families of three patients had dogs, although this serovar has never been isolated from a dog.

REFERENCES

1. **Adesiyun, A. A., D. E. Agbonlahor, L. H. Lombin, and J. K. P. Kwaga.** 1986. Occurrence of virulence markers in species of *Yersinia* isolated from animals in Nigeria. Vet. Microbiol. **12:**289–294.
2. **Aldova, E., J. Cerny, and J. Chmela.** 1977. Finding *Yersinia* in rats and sewer rats. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **239:**208–212.
3. **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. Appl. Environ. Microbiol. **39:**135–140.
4. **Aulisio, C. C. G., J. T. Stanfield, S. D. Weagant, and W. E. Hill.** 1983. Yersiniosis associated with tofu consumption: serological, biochemical and pathogenicity studies of *Yersinia enterocolitica* isolates. J. Food Prot. **46:**226–230.
5. **Black, R. E., R. J. Jackson, T. Tsai, M. Medvesky, M. Shayegani, J. C. Feeley, B. S. Kenneth, K. I. E. Macleod, and A. M. Wakelee.** 1978. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. N. Engl. J. Med. **298:**76–79.
6. **Buchrieser, C., S. D. Weagant, and C. W. Kaspar.** 1994. Molecular characterization of *Yersinia enterocolitica* by pulsed-field gel electrophoresis and hybridization of DNA fragments to ail and pYV probes. Appl. Environ. Microbiol. **60:**4371–4379.
7. **Doyle, M. P., M. B. Hugdahl, and S. L. Taylor.** 1981. Isolation of virulent *Yersinia enterocolitica* from porcine tongues. Appl. Environ. Microbiol. **42:**661–666.
8. **Fukushima, H.** 1991. Susceptibility of wild mice to *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **275:**530–540.
9. **Fukushima, H., M. Gomyoda, and S. Kaneko.** 1990. Mice and moles inhabiting mountainous areas of Shimane Peninsula as source of infection with *Yersinia pseudotuberculosis*. J. Clin. Microbiol. **28:**2448–2455.
10. **Fukushima, H., K. Hoshina, R. Nakamura, Y. Ito, and M. Gomyoda.** 1987. Epidemiological study of *Yersinia enterocolitica* and *Yersinia psudotuberculosis* in Shimane Prefecture, Japan. Contrib. Microbiol. Immunol. **9:**103–110.
11. **Gemski, P., J. R. Lazera, and T. Casey.** 1980. Plasmid associated with pathogenicity and calcium dependency of *Yersinia enterocolitica*. Infect. Immun. **27:**682–685.
12. **Gilmour, A., and S. J. Walker.** 1988. Isolation and identification of *Yersinia enterocolitica* and the *Yersinia enterocolitica*-like bacteria. J. Appl. Bacteriol. Suppl. **65:**213–236.
13. **Gutman, L. T., E. A. Ottesen, T. J. Quan, P. S. Noce, and S. L. Kazt.** 1973. An inter-familial outbreak of *Yersinia enterocolitica* enteritis. N. Engl. J. Med. **288:**1372–1377.
14. **Hayashidani, H., E. Kitahara, and M. Ogawa.** 1994. Infectivity and pathogenicity of *Yersinia enterocolitica* serovar O:8 to wild rodents in Japan. J. Vet. Med. B **41:**504–511.
15. **Hurvell, B.** 1981. Zoonotic *Yersinia enterocolitica* infection: host range, clinical manifestations, and transmission between animals and man, p. 145–159. *In* E. J. Bottone (ed.), *Yersinia enterocolitica*. CRC Press, Inc., Boca Raton, Fla.
16. **Ichinohe, H., M. Yoshioka, H. Fukushima, S. Kaneko, and T. Maruyama.** 1991. First isolation of *Yersinia enterocolitica* serotype O:8 in Japan. J. Clin. Microbiol. **29:**846–847.
17. **Iinuma, Y., H. Hayashidani, K. Kaneko, M. Ogawa, and S. Hamasaki.** 1992. Isolation of *Yersinia enterocolitica* serovar O:8 from free-living small rodents in Japan. J. Clin. Microbiol. **30:**240–242.
18. **Iteman, I., C. Baril, I. S. Girons, and E. Carniel.** 1991. Pulse field electrophoresis of the chromosome of the pathogenic yersiniae. Contrib. Microbiol. Immunol. **12:**198–202.
19. **Kado, C. I., and S.-T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145:**1365–1373.
20. **Kandolo, K., and G. Wauters.** 1985. Pyrazinamidase activity in *Yersinia enterocolitica* and related organisms. J. Clin. Microbiol. **21:**980–982.
21. **Kaneko, K., S. Hamada, Y. Kasai, and E. Kato.** 1978. Occurrence of *Yersinia enterocolitica* in house rats. Appl. Environ. Microbiol. **36:**314–318.
22. **Kaneko, K., and N. Hashimoto.** 1981. Occurrence of *Yersinia enterocolitica* in wild animals. Appl. Environ. Microbiol. **41:**635–638.
23. **Kapperud, G.** 1991. *Yersinia enterocolitica* in food hygiene. Int. J. Food Microbiol. **12:**53–66.
24. **Kapperud, G., T. Nesbakken, S. Aleksic, and H. H. Mollaret.** 1990. Comparison of restriction endonuclease analysis and phenotypic typing for differentiation of *Yersinia enterocolitica* isolates. J. Clin. Microbiol. **28:**1125–1131.
25. **Kay, B. A., K. Wachsmuth, P. Gemski, J. C. Feeley, T. J. Quan, and D. J. Brenner.** 1983. Virulence and phenotypic characterization of *Yersinia enterocolitica* isolated from humans in the United States. J. Clin. Microbiol. **17:**128–138.
26. **Keet, E. E.** 1974. *Yersinia enterocolitica* septicemia, source of infection and incubation period identified. N.Y. State J. Med. **74:**2226–2230.
27. **Laird, W. J., and D. C. Cavanaugh.** 1980. Correlation of autoagglutination and virulence of yersiniae. J. Clin. Microbiol. **11:**430–432.
28. **Lee, L. A., J. Taylor, G. P. Carter, B. Quinn, J. J. Farmer III, and R. V. Tauxe.** 1991. *Yersinia enterocolitica* O:3: an emerging cause of pediatric gastroenteritis in the United States. J. Infect. Dis. **163:**660–663.
29. **Morse, D. L., M. Shayageni, and R. J. Gallo.** 1984. Epidemiological investigation of a *Yersinia* camp outbreak linked to a food handler. Am. J. Public Health **74:**589–592.
30. **Prentice, M. B., D. Cope, and R. A. Swann.** 1991. The epidemiology of *Yersinia enterocolitica* infection in the British Isles 1983–1988. Contrib. Microbiol. Immunol. **12:**17–25.
31. **Quan, T. J., J. L. Meek, K. R. Tsuchiya, B. W. Hudson, and A. M. Barnes.** 1974. Experimental pathogenicity of recent North American isolates of *Yersinia enterocolitica*. J. Infect. Dis. **129:**341–344.
32. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27:**493–497.
33. **Saito, M., M. Yamaguchi, Y. Toyokawa, Y. Ohtomo, S. Kaneko, and T. Maruyama.** 1994. *Yersinia enterocolitica* serotype O:8 infection in the Hirosaki district in Aomori Prefecture from 1984 to 1991. J. Jpn. Assoc. Infect. Dis. **68:**960–965.
34. **Sato, K.** 1987. *Yersinia pseudotuberculosis* infection in children. Contrib. Microbiol. Immunol. **9:**111–116.
35. **Shayegani, M., I. DeForge, D. M. McGlynn, and T. Root.** 1981. Characteristics of *Yersinia enterocolitica* and related species isolated from human, animal, and environmental sources. J. Clin. Microbiol. **14:**304–312.
36. **Shayegani, M., D. Morse, I. DeForge, T. Root, L. M. Parsons, and P. S. Maupin.** 1983. Microbiology of a major foodborne outbreak of gastroenteritis caused by *Yersinia enterocolitica* serogroup O:8. J. Clin. Microbiol. **17:**35–40.
37. **Shayegani, M., W. B. Stone, I. DeForge, T. Root, L. M. Parsons, and P. Maupin.** 1986. *Yersinia enterocolitica* and related species isolated from wildlife in New York State. Appl. Environ. Microbiol. **52:**420–424.
38. **Tsai, S., and L. Chen.** 1991. Occurrence of *Yersinia enterocolitica* in pork products from Northern Taiwan. Contrib. Microbiol. Immunol. **12:**56–62.
39. **Wauters, G., M. Janssens, A. G. Steigerwalt, and D. J. Brenner.** 1988. *Yersinia mollaretti* sp. nov. and *Yersinia bercovieri* sp. nov., formerly called *Yersinia enterocolitica* biogroups 3A and 3B. Int. J. Syst. Bacteriol. **38:**424–429.
40. **Winblad, S., B. Niléhn, and N. H. Sternby.** 1966. *Yersinia enterocolitica* (*Pasteurella* X) in human enteric infections. Br. Med. J. **2:**1363–1366.
41. **Wormser, G. P., and G. T. Keusch.** 1981. *Yersinia enterocolitica*: clinical observation, p. 83–94. *In* E. J. Bottone (ed.), *Yersinia enterocolitica*, CRC Press, Inc., Boca Raton, Fla.