Characteristics of Yersinia enterocolitica and Related Species Isolated from Human, Animal, and Environmental Sources

MEHDI SHAYEGANI,* ISOBEL DEFORGE, DORIS M. MCGLYNN, AND TIMOTHY ROOT

Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201

Received 9 December 1980/Accepted 1 April 1981

During a 4-year period, 4,448 human, animal, and environmental samples collected in New York State were tested for the presence of Yersinia enterocolitica or related species. A total of 339 isolates were identified as Yersinia and characterized according to source, species, biogroup, serogroup, and, in some instances, phage type. Four new biogroups of Y. intermedia were characterized. Of 149 human isolates, 120 (80.5%) were identified as Y. enterocolitica, and 29 were identified as either Y. intermedia (12.1%), Y. frederiksenii (5.4%), or Y. kristensenii (2.0%). Of the other 190 isolates, recovered from animals and the environment. 54 (28.4%) were Y. enterocolitica and 136 were either Y. intermedia (62.6%), Y. frederiksenii (4.7%), Y. kristensenii (3.7%), or an undescribed Yersinia species (0.5%). Two established human pathogenic strains of Y. enterocolitica were recovered: 59 isolates (37 from an outbreak) of "American strain" (serogroup O:8, biogroups Niléhn 2, Wauters 1, and Knapp and Thal 2, phage type X_z) and 11 isolates of "Canadian strain" (serogroup O:3, biogroups Niléhn 4, Wauters 4, and Knapp and Thal 1, phage type IX_{b}). This was the first documented isolation of the Canadian strain in the United States. Isolates of other strains implicated in human disease (serogroups 0:4,33, 0:5, 0:6,31, 0:7,8, and 0:8) were also recovered from both human and nonhuman sources.

Yersinia enterocolitica, a causative agent of human acute and chronic gastroenteritis, septicemia, wound infections, and other clinical syndromes (7), was first described and named Bacterium enterocoliticum in this laboratory in the 1930s (29). Since then, recovery of this organism has been reported throughout the world, and a distinct geographical distribution of certain pathogenic strains has been demonstrated, particularly in Europe, Canada, Japan, and South Africa (7, 22).

Because the organisms classified as Y. enterocolitica show different patterns of biochemical reactions, three schemes for dividing the species into biogroups were developed by Niléhn (24), Wauters (G. Wauters, Ph.D. thesis, University of Louvain, Louvain, Belgium, 1970), and Knapp and Thal (20). Recently Brenner (9), Brenner et al. (10-12), Bercovier et al. (3, 4), and Ursing et al. (34) proposed dividing these biogroups into a total of four named species and two additional unnamed groups, based on deoxyribonucleic acid hybridization and biochemical reactions. The name Y. enterocolitica was retained for biochemically typical strains. The atypical Y. enterocolitica-like strains were assigned to three new species, Y. intermedia (10), Y. frederiksenii (34), and Y. kristensenii (4), and two unnamed

groups, X1 and X2 (3).

Epidemiological study of human Yersinia infections has implicated water, animals, food, and other environmental sources as reservoirs of the organism, but the mode of transmission and the role of the atypical strains is not yet clear (2, 8, 13, 19).

In the present study, Yersinia strains isolated in New York State were characterized, and correlations were made between strains isolated from humans and those isolated from environmental sources on the basis of their similar serogroups and biogroups.

MATERIALS AND METHODS

Specimens: (i) Yersinia screening requested. Specimens submitted to be screened for the presence of Yersinia and received in our laboratory between October 1976 and December 1980 made up one part of this study. Included in this group were specimens collected during an outbreak of foodborne illness in 1976, which had been shown to be caused by Y. enterocolitica (6, 30). Fecal specimens from humans or animals were submitted to our laboratory in either phosphate-buffered saline, pH 7.6, or 30% glycerol buffer. Milk, water, and other nonfecal specimens were received on ice. In some cases, bacterial isolates on agar slants were sent for identification, confirmation, or both.

(ii) Other human fecal specimens. New studies were designed to survey fecal specimens obtained from certain human populations and received in our laboratory for reasons other than *Yersinia* screening.

In the first group, three groups of human fecal specimens received in our laboratory (in 30% glycerol buffer) for routine Salmonella and Shigella screening were also screened for the presence of Yersinia. These groups consisted of 300 specimens received during the winter and spring of 1977 (30), 1,287 received during 1978, and 188 received during February through December 1980.

In the second group, fecal specimens from patients with signs and symptoms of gastroenteritis were collected in phosphate-buffered saline, pH 7.6, during 1978 by six geographically separate clinical laboratories in New York State: Albany Medical Center Hospital (229 specimens), Binghamton General Hospital (85 specimens), The Children's Hospital of Buffalo (33 specimens), Champlain Valley Physicians Hospital (19 specimens), Rome Hospital (217 specimens), and Automated Biochemical Laboratories, Inc., in Spring Valley (25 specimens).

In the third group, fecal swabs from 97 normal children, aged 1 to 5 years, and from seven normal adult staff members from daycare centers in Rensselaer County were placed in Stuart transport medium and delivered to our laboratory within 1 to 2 h after collection. These specimens were tested for the presence of Salmonella, Shigella, and Campylobacter fetus, as well as for Yersinia. This was a joint study with Richard Rothenberg, Director of the Bureau of Disease Control of the New York State Department of Health, and Gerald Eichner, of the Albany Medical Center Hospital.

(iii) Animal and environmental specimens. Yersinia strains were also isolated from animal and environmental specimens collected between October 1976 and December 1980. Most samples from animals were fecal specimens, but 28 were bovine cervical specimens, and a small number were of rodent or avian tissue or intestinal contents. W. E. Mellon, veterinarian of Oneida County, obtained most of the animal and raw milk samples. Surface water samples were collected from various locations in the state with the cooperation of the New York State water laboratories in Albany and Syracuse and other agents of the Department of Health.

Other human, animal, food, water, and soil samples were obtained and tested by our laboratory staff when there was a possible epidemiological link between the samples and human yersiniosis.

Isolation. On receipt, each fecal specimen or fecal swab was plated on Endo, salmonella-shigella, and deoxycholate-citrate agars. After plating, a portion of each specimen or the entire swab was placed in phosphate-buffered saline, refrigerated at 4° C, and 3 weeks later transferred to the same three types of plates. To facilitate differentiation of *Yersinia* colonies, the deoxycholate-citrate agar was also prepared with the addition of 0.5% sucrose. The formula used is similar to BBL Microbiology Systems' DCLS agar, which was also found to be satisfactory. The typical small *Yersinia* colonies appear dark red on this medium because of the fermentation of sucrose. This has been used in addition to the three above-mentioned primary plating media in this study since November 1979.

Milk and water samples were not plated when initially received. Milk samples were placed in cookedmeat medium and refrigerated at 4°C. Water samples were filtered through a 0.45- μ m membrane filter (Millipore Corp.), which was then placed in cooked-meat medium and refrigerated. After 3 weeks of cold enrichment, these samples were inoculated on the same plating media as the fecal specimens.

All plates, whether inoculated directly or after 3 weeks of cold enrichment, were placed in a 25° C incubator for 48 h.

Identification. Small, round, translucent colonies were transferred to triple sugar iron (TSI) and urea agars. Isolates acid in both the butt and the slant of the TSI and urease positive, and isolates with typical colonial morphology giving either acid only in the butt of the TSI or urease-negative reactions, were further examined by biochemical testing.

Because of the number of biochemical tests required by the recently proposed species designation of this group of organisms and the various biogrouping schemes, each isolate was tested in 36 different biochemicals.

Five tests were performed at 25°C: lecithinase, methyl red, oxidation-fermentation lactose, o-nitrophenyl- β -D-galactopyranoside, and Voges-Proskauer. Twenty-seven tests were performed at 35 to 37°C: arginine dihydrolase, esculin, gas from glucose, indole production, lysine decarboxylase, nitrate reduction, ornithine decarboxylase, phenylalanine deaminase, Simmons citrate, urease, and 17 carbohydrates (adonitol, α -methyl-D-glucoside, L-arabinose, dulcitol, D-glucose, isoinositol, lactose, maltose, D-mannitol, D-melibiose, D-raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, D-trehalose, and D-xylose). Motility tests were performed both at 25 and 35 to 37°C. The hydrogen sulfide test was read from the TSI, and Pathotec strips were used for the oxidase test.

To determine the species of certain atypical strains, the following seven biochemical tests were incubated for as long as 2 weeks at both 25 and 35 to 37°C: α methyl-D-glucoside, lactose, D-melibiose, D-raffinose, L-rhamnose, Simmons citrate, and sucrose.

Use of 34 of these tests has been previously described (30); however, two of the carbohydrates (α -methyl-D-glucoside and D-melibiose) and the seven biochemicals tested at both temperatures have recently been added to the battery of tests. All organisms isolated before the addition of these tests have been retested in the two carbohydrates and, if necessary, in the seven other tests.

Species of the Yersinia isolates were determined according to the recently proposed taxonomy of Brenner (9), Brenner et al. (10-12), Bercovier et al. (3, 4), and Ursing et al. (34) on the basis of biochemical reactions of sucrose, rhamnose, raffinose, melibiose, and α -methyl-D-glucoside. The characters are as follows: for Y. enterocolitica, sucrose positive, does not ferment any of the other four carbohydrates; for Y. intermedia, ferments all five carbohydrates; for Y. frederiksenii, ferments sucrose and rhamnose but not the other three; and for Y. kristensenii, does not ferment sucrose or any of the other four sugars. The X1 and X2 strains are also sucrose negative. The X1 strain is negative in all five sugars, as is Y. kristensenii, and can be differentiated from this species by a negative ornithine decarboxylase reaction. The X2 strain can be identified by the above five sugar reactions: it ferments only rhamnose.

Biogrouping. Biogroups of the isolates were determined according to the schemes of Niléhn (24), Wauters (Ph.D. thesis, 1970), and Knapp and Thal (20). Isolates that differed by one or two reactions from the scheme patterns were assigned to the biogroup they most closely resembled.

Serogrouping. Isolates were serogrouped in our laboratory by slide agglutination. In the early part of the study, antisera O:1 through O:21 (prepared by B. H. Hudson and T. Quan; supplied by P. Carter of the Trudeau Institute at Saranac Lake, N.Y.) and antisera O:22 through O:34 (provided by S. Toma of the National Reference Center for *Yersinia*, Public Health Laboratory, Ontario Ministry of Health, Toronto, Canada) were used. Antisera O:1 through O:34 (prepared in our laboratory) were used in the latter part of the study.

Thirty representative strains were serogrouped by H. H. Mollaret of the *Yersinia* Center, Institut Pasteur, Paris, France. The Oneida County outbreak isolates were serogrouped by James C. Feeley of the Bureau of Epidemiology, Centers for Disease Control, Atlanta, Ga.

Phage typing. Thirty representative isolates of Y. enterocolitica and related species of various serogroups and biogroups were phage typed by H. H. Mollaret at the Institut Pasteur. Ten of the 11 isolates of serogroup O:3 were phage typed by S. Toma.

RESULTS

All specimens. During a period of 4 years, 4,448 human, animal, and environmental samples were tested for Y. *enterocolitica* (Table 1). This organism and the related species were isolated from 3.2% of the human specimens (2.1% when isolates of Y. *enterocolitica* serogroup O:8 from the 1976 foodborne outbreak are excluded), from 6.6% of the animal specimens, from 2.5% of the raw milk samples, and from 23.6% of the surface water samples.

Human specimens. Table 2 shows Yersinia species isolated from specimens received for Yersinia screening and survey studies (as described in Materials and Methods). Of 22 Y. enterocolitica isolated from a survey study, one was serogroup O:8, four were serogroup O:6,31, and seven were other serogroups. Ten were nongroupable. All fecal swab specimens from children in daycare centers were negative for Salmonella, Shigella, and C. fetus.

Animal specimens. Yersinia species were recovered from 37 of 557 (6.6%) animal specimens (Table 1). Nineteen of the 21 isolates from

TABLE 1. Y. enterocolitica and related species isolated in New York State during October 1976 to December 1980

Specimen	Positive			
Source	No.	No.	%	
Humans				
Specimens	2,982	$96(62)^{a}$	3.2(2.1)	
Isolates	53	53	100	
Animals				
Cows	352	21	6.0	
Pigs	108	13	12.0	
Horses	25	0	0	
Dogs	29	1	3.4	
Cats	10	0	0	
Mice	10	1	10.0	
Birds	9	0	0	
Rats	6	1	16.7	
Bats	5	0	0	
Moles, rabbits,	3	0	0	
worms				
Environment				
Raw milk	160	4	2.5	
Surface water	622	147	23.6	
Food	67	2	3.0	
Soil	7	0	0	
Total	4,448	33 9	7.6	

^a Numbers in parentheses exclude 34 isolates from fecal specimens of serogroup O:8 from an Oneida County outbreak of 1976.

cows, 6 of the 13 isolates from pigs, the 1 isolate from dogs, and the 1 isolate from mice were all Y. enterocolitica (75% of the animal isolates). The other 10 animal isolates were related species, five Y. intermedia and five Y. kristensenii.

Surface water samples. A total of 622 water samples were tested from 43 of the 57 New York counties, and 147 *Y. enterocolitica* and related species were isolated (23.6%). Multiple isolates were recovered from some samples.

Of the 147 isolates, 23 (15.6%) were Y. enterocolitica, 113 (76.9%) were Y. intermedia, 9 (6.1%) were Y. frederiksenii, 1 (0.7%) was Y. kristensenii, and 1 (0.7%) was an undescribed Yersinia species.

Characterization of *Y. enterocolitica.* (i) **Biogroups.** The 174 isolates of *Y. enterocolitica*, 120 from humans and 54 from animals, milk, and water, were biogrouped by three schemes (Table 3). Most were Niléhn biogroups 1 and 2, Wauters biogroup 1, and Knapp and Thal biogroups 2 and 3. Human isolates could not be differentiated from animal and environmental isolates by biogrouping alone.

Five human isolates, two from fecal specimens

		No. of isolates of:				
Specimen	No. of speci- mens	Y. entero- colitica	Y. interme- dia	Y. freder- iksenii	Y. kristen- senii	
Screening requested						
Oneida County outbreak, 1976	376	44	6	2		
Fecal specimens	119	7		1		
Isolates	53	47	4	1	1	
Survey						
Fecal specimens						
1977	300	5	1		1	
1978	1,287	13	5	4		
1980	188	1	1			
Specimens from 6 hospitals	608	2			1	
Specimens from daycare centers	104	1	1			

TABLE 2. Yersinia screening and surveys of human specimens

TABLE 3. Biogroups of Y. enterocolitica isolated in
New York State during October 1976 to December
1980 (174 isolates)

No. isolated from:					
Humans	Animals	Water	Raw milk		
40	26	17	4		
63	1	5			
5		1			
12					
99	26	19	4		
5	1	3			
5		1			
11					
14		1			
66	2	3			
40	25	19	4		
	Humans 40 63 5 12 99 5 5 11 14 66 40	No. isolat Humans Animals 40 26 63 1 5 12 99 26 5 1 11 1 14 66 2 40 25 25	No. isolated from: Humans Animals Water 40 26 17 63 1 5 5 1 1 99 26 19 5 1 3 5 1 1 11 - - 14 1 - 66 2 3 40 25 19		

and one from sputum, were raffinose and lactose positive, traits which are considered to be plasmid associated (15).

(ii) Serogroups. Of the 120 human isolates, 59 were serogroup O:8, biogroups Niléhn 2, Wauters 1, and Knapp and Thal 2, phage type X_2 . All serogroup O:8 isolates from humans were of this combination of biogroups and phage type, and we refer to these isolates as the American strain. Of these, 37 had been isolated during the 1976 Oneida County outbreak (30). Median age for patients in this group was 13 years for fecal specimens. For throat, appendix, and sputum specimens, the patient ages were 13, 14, and 71, respectively. Of the others, received from 11 counties, 17 were from fecal specimens (patients 4 weeks to 14 years old); 2 were from blood and sputum of a single patient (age 43); 1 was from

a foot puncture wound (patient age 52); 1 was from a liver abscess (patient 2 months old); and 1 was from an appendix (patient age unknown).

Seven Y. enterocolitica serogroup O:8 isolates were obtained from animal and environmental sources: two from cows, two from milk, and three from water samples. However, these isolates were salicin and esculin positive and thus in different biogroups (Niléhn 1 and Knapp and Thal 3) from the American strain.

Four isolates of Y. enterocolitica serogroup O: 7,8 having the same biogroups as the American strain (salicin and esculin negative) were isolated, three from human sources and one from a cow cervical specimen. All four isolates were serogrouped as O:8 in our laboratory and by the Centers for Disease Control, but as O:7,8 by Institut Pasteur. The bovine isolate had been previously reported by us as belonging to serogroup O:8 (30).

Eleven isolates of Y. enterocolitica Canadian strain, serogroup O:3, biogroups Niléhn 4, Wauters 4, and Knapp and Thal 1, phage type IXb, were obtained from human sources. Eight of these isolates were from fecal specimens, two were from blood specimens, and one was from a renal abscess. The first Canadian strain was isolated and identified in our laboratory in May 1977; four were isolated in 1978; two were isolated in 1979; and four were isolated in 1980 (Table 4).

Two other Y. enterocolitica serogroup O:3 isolates were found, one from a human fecal specimen and one from water, but both were biogroups Niléhn 2 (or 3), Wauters 1, and Knapp and Thal 1 (or 2), phage type X_z , different biogroups and phage type from the Canadian strain (Table 5). This combination of biogroup and phage type has not been implicated in human disease.

TABLE 4. Canadian strain Y. enterocolitica, biogroups Niléhn 4, Wauters 4, Knapp and Thal 1, serogroup O:3, and phage type IX_b, isolated from humans in New York

Source	Date of isolation	Age of sub- ject	County
Feces	May 1977 April 1978 July 1978 August 1978 August 1980 October 1980 October 1980 October 1980	2 years 14 months Unknown 11 months 11 years 4 months 15 years Newborn	Monroe Suffolk Monroe Rockland Monroe Suffolk Sullivan Erie
Other Renal abscess Blood Blood	December 1978 January 1979 February 1979	76 years 83 years 79 years	Nassau Albany Nassau

 TABLE 5. Serogroups of Y. enterocolitica isolated

 in New York State during October 1976 to December

 1980 (174 isolates)

	No. isolated from:					
Serogroup	Humans	Animals	Water	Raw milk		
0:3 ^{<i>a</i>}	11					
O:3 ^b	1		1			
O:4,33	3	13	1			
0:5	8	1	2			
O:6,31	9	3	2	2		
0:7,8	4	1				
0:8	59	2	3	2		
O:10K ₁		2				
0:12	1					
0:12,25	2					
0:14	1		1			
0:16			1			
0:18	1					
0:22			1			
0:25	3					
O:30			1			
O:31	1		1			
0:34			1			
Nongroupable	16	5	8			

^a Serogroup O:3, biogroups Niléhn 4, Wauters 4, and Knapp and Thal 1, phage type IX_{b} .

^b Serogroup O:3, biogroups Niléhn 2 (or 3), Wauters 1, and Knapp and Thal 1 (or 2), phage type X₂.

Of 11 Y. enterocolitica serogroup O:5 isolates, 7 were O:5a (Wauters biogroup 1): three from human feces, two from water, and one each from sputum and mouse tissue. The other four were serogroup O:5b (Wauters biogroup 3), isolated from human fecal specimens. Of 16 serogroup O: 6,31 isolates, 9 were from humans, 3 were from animals, and 2 each were from milk and water.

Thirteen percent of the human isolates of Y. enterocolitica and 26% of the animal and water isolates were nonserogroupable with the 34 available antisera.

(iii) Phage types. Except to confirm the identification of Canadian strain isolates, phage typing was not useful for our isolates. Representative strains of human, cow, milk, and water isolates, serogroupable or nongroupable, were either phage type X_z (as is the American strain) or X_o .

Characterization of Yersinia-related species. (i) Biogroups. Biogrouping of Yersinia species other than Y. enterocolitica by the three schemes did not produce any useful pattern for differentiation. An interesting finding was four biogroups of Y. intermedia which did not exactly correspond biochemically to the given reactions for this species, although the biochemical reactions were tested at both 25 and 35 to 37°C for as long as 2 weeks. These reactions, plus those of a typical Y. intermedia and of one undescribed Yersinia species which we encountered. are shown in Table 6. Twelve representatives of groups 2, 3, and 4 were tested by Don J. Brenner at the Centers for Disease Control for deoxyribonucleic acid relatedness and were found to be Y. intermedia.

Four of the biogroups isolated in our study are among the eight biogroups of Y. intermedia recently reported by Brenner et al. (10). In addition, we have found another biogroup, rhamnose and raffinose positive, melibiose and α methyl-D-glucoside negative (biogroup 4, Table 6). Our strains were negative for citrate utilization at 37°C, but those tested at 25°C were positive for this test. This criterion is useful for identifying rhamnose-negative strains of Y. intermedia (10).

(ii) Serogroups. The serogroups of the Yersinia group of organisms had been established before determination of species was attempted. Therefore, the related Yersinia species were serogroupable with the same antisera as are used

TABLE 6. Biochemical patterns of five Y.intermedia types and one undescribed Yersinia

species						
	Y. intermedia type				Unde-	
Carbohydrate	1 (typi- cal)	2	3	4	5	scribed Y <i>ersinia</i> species
Rhamnose	+	_	_	+	+	+
Raffinose	+	+	+	+	+	
Melibiose	+	+	+	-	+	-
α-Methylgluco- side	+	+	-	-	-	+
Sucrose	+	+	+	+	+	+
No. of isolates	99	18	8	8	4	1

for Y. enterocolitica (Table 7). Of the 165 isolates, 101 (61%) were not groupable with the 34 antisera.

Because serogrouping of Yersinia is not species specific, some isolates of Y. enterocolitica, Y. intermedia, Y. frederiksenii, and Y. kristensenii were serogroup O:16. Also, serogroups O:3, O:4,33, O:5, O:6,31, O:14, O:18, and O:30 were found in both Y. enterocolitica and Y. intermedia.

(iii) Phage types. A few representative strains of related Yersinia species were phage typed. Y. intermedia serogroup O:10 (from human feces) and serogroup O:7 and nongroupable (both from water) were phage type X_z . An isolate of Y. kristensenii serogroup O:16,29 (isolated from a pig) was phage type X_o .

Y. enterocolitica of the same biogroups and serogroups from various sources. A

 TABLE 7. Serogroups of related Yersinia species (165 isolates)

		No. of	
Organism	Source	iso-	O serogroups ^a
Ū		lates	•••
Y. intermedia			
Type 1 ^b	Human, feces	3	4.32: 16 29: NG
- , , , , , , , , , , , , , , , , , , ,	Human vomit	Ĩ	NG
	Cow. feces	1 ī	NG
	Rat tissue	lī	NG
	Food	li	NG
	Water	91	4 33 (4) 17 (17)
	Watch		others $(15)^{\circ}$
			NG (55)
Type 2	Human soutum	1	NG
1902	Cow feces	li	13
	Water	17	4 33 (2) 5 22
	Waki	1	33 NG (12)
Type 3	Human feces	6	6 31 10 30 NG
Type o	Human, Icces		(3)
	Pig, feces	2	NG (2)
Type 4	Human, feces	7	13,16; 16; NG
			(5)
	Water	1	NG
Type 5	Water	4	NG (4)
V 6. J: 1	TT		
1. freaeriksenu	Human, Ieces	1 '	2,3; 4,16; 16 (2);
	TT		NG (3)
	Human, throat	1	16,34
	Water	9	16; NG (8)
Y. kristensenii	Human, feces	2	16; 34
	Human, foot	1	28
	Pig, feces	5	11 (2); 16; 16,29
	-		(2)
	Food	1	NG
	Water	1	12,25
Yersinia sp.	Water	1	NG

^a Numbers in parentheses indicate number of isolates; NG, nongroupable.

^b For types 1 through 5, see Table 6.

^c Serogroups 2; 3 (biogroups Niléhn 2, Wauters 1, and Knapp and Thal 2); 4; 7; 11,24; 14; 16,29 (2); 18; 20; 22; 22,31; 29; and 33 (2). number of Y. enterocolitica strains isolated from humans had the identical biogroups (with all three schemes) and serogroups as strains isolated from animal and environmental sources (Table 8). The American and Canadian strains were found only in human specimens. The isolates listed in Table 8 with the same biogroups as the American strain (Niléhn 2, Wauters 1, Knapp and Thal 2) were serogroup O:7,8. The serogroup O:8 isolates listed were of different biogroups than the American strain.

Geographic distribution. Figure 1 shows the statewide distribution of different species of *Yersinia* according to county and source of isolation. Selected serogroups implicated in human infections are also indicated. The *Yersinia* isolates did not show any particular distribution pattern within New York State.

DISCUSSION

Y. enterocolitica and related species are found in the gastrointestinal tracts of humans and animals and in the environment. In our study, 2.1% of the human fecal specimens (excluding positive human fecal specimens from the Oneida County outbreak), 2.5% of the raw milk samples, 6.6% of the animal fecal specimens, and 23% of the water samples contained Y. enterocolitica and related species. Of the Y. enterocolitica isolates from human specimens (excluding those from the Oneida County outbreak), 18% were American strain and 9% were Canadian strain. This, as far as we know, is the first documented isolation of the Canadian strain in the United States, although isolation of Y. enterocolitica serogroup O:3 was reported on three previous occasions (5, 31, 35). Two of these reports (5, 31)

TABLE 8. Y. enterocolitica isolates of the same biogroup and serogroup recovered from humans or animals and the environment

Biogroup scheme					
Sero- group	Niléhn Wauters		Knapp and Thal	Source ^a	
0:4,33	1	1	3	Human (3), cow (13), water (1)	
O:5	1	1	3	Human (4), mouse (1), water (2)	
O:6,31	1	1	3	Human (9), cow (2), pig (1), milk (2), water (2)	
O:7,8	2	1	2	Human (3), cow cervix (1)	
O:8	1	1	3	Cow (2), milk (2), water (3)	

^a Numbers in parentheses indicate number of isolates.



FIG. 1. Representative Y. enterocolitica and related species isolated from humans and water in New York State.

did not give information on phage type, and the third (35) described the isolate as phage type IX without specifying subtype a or b. Also, the isolate in this report was described as belonging to serogroup 1 or 2 and was later reassigned to serogroup O:3 (5, 7). In our study, most serogroup O:8 and O:3 strains were isolated either from fecal specimens from children or from older persons with systemic infections. No American or Canadian strains were isolated from animals or the environment in our study. Also, no serogroup O:3, phage type VIII, the most common human isolate outside of North America, or serogroup O:9, the second most common human isolate in Europe, was recovered in our study.

The prevalence of Y. enterocolitica in specimens received in our laboratory was compared with that of other pathogenic enteric bacteria known to cause gastroenteritis. Of the human fecal specimens received by our laboratory in recent years, 1.9% have contained *Shigella* and 9% have contained *Salmonella*. In the human fecal specimens submitted from individuals with symptoms of gastroenteritis, the isolation rate for *Yersinia* was 1.6% (36 of 2,299 specimens).

In a comparable study in Montreal, Canada (21), specimens from children with gastroenteritis were tested during a 15-month period for pathogenic enteric bacteria. Salmonella was isolated from 4.4% of the specimens, Y. enterocolitica from 2.8%, and Shigella from 1.1%. Of the total specimens, 2.6% contained the known pathogenic Y. enterocolitica serogroup O:3—a very high rate of isolation, since no outbreak is mentioned. By comparison, the recovery rate in our study for the pathogenic serogroup O:8 was 0.6% if serogroup O:8 isolates from the Oneida County outbreak were excluded and 1.3% if they were included. Our recovery rate for the 11 serogroup O:3 isolates was extremely low (0.2%).

Y. enterocolitica and particularly the related species are found world-wide in animals (25, 33), dairy products (19, 27), food (18), water (26, 28), and other natural sites (2). The related species most frequently found in the environment are not considered pathogenic, although they have on rare occasions been isolated from nonintestinal specimens (8) and from infants' blood cultures (16). In our study, human isolates represented 13% of a total of 137 isolates of Y. inter*media*, which is the same percentage recovery reported by others (10). Although the number of isolates was few, 8 of 17 Y. frederiksenii and 3 of 10 Y. kristensenii isolates were from human sources, which is higher in percentage in total recovery than reported (4, 34) in other parts of the world (23 and 18%, respectively). We did not isolate the X1 and X2 strains, which have been isolated from nonhuman sources (12), or the modified Wauters biogroup 5 of Y. enterocolitica, which has been isolated from hares in Europe (3).

Even for the well-known human pathogenic strains of Y. enterocolitica, a good understanding of both the natural reservoir(s) and the mode(s) of transmission to humans has been elusive. In most instances of human disease, the causative organisms have not been found in animals or the surrounding environment; and even when such organisms have been found in animals, milk, or water, the reservoir and the mode of transmission to humans have not been determined (1, 2, 13, 17, 30).

In our study, isolates of Y. enterocolitica serogroups 0:4,33, 0:5, 0:6,31, 0:7,8, and 0:8 with the same characteristics (biogroup, serogroup, and phage type) were found in both human or animal and environmental sources. This suggests the possibility of transmission from environment or animals to humans. Yersinia isolates with these serogroups have been implicated in human infections. In a study in Ontario, Canada (32), serogroups O:5,27 and O:6,30 were the second and third most frequent strains of Yersinia isolated from humans. In another report (T. F. Wetzler and D. McClellan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, c(H)69, p. 357), strains of serogroup O:4,32 were isolated from 14 of 21 patients with abdominal pain and diarrhea.

The pathogenic properties of Y. enterocolitica have been demonstrated in vitro by their ability to produce heat-stable enterotoxin, to invade HeLa cells, and to cause keratoconjunctivitis in guinea pig eyes (23). More recently, the ability of the WA strain of Y. enterocolitica O:8 to produce plague V and W antigens has been demonstrated (14). The relationship of these pathogenic properties to serogroups, biogroups, and phage types is unclear.

More in-depth study is needed to understand the pathogenicity of *Yersinia*. These studies should include a search for a suitable laboratory animal model, preparation of phages to distinguish strains that the phages now available cannot differentiate, and particularly investigation of possible adaptation of *Yersinia* from the environment to humans.

ACKNOWLEDGMENTS

We express our thanks to the laboratories submitting stool specimens for the gastroenteritis study; to the laboratories which sent isolates of organisms; to the New York State water laboratories at Albany and Syracuse and other officials for most of the water samples; and to C. W. Turner for her participation in part of this study. We also thank L. M. Parsons, P. S. Maupin, and the division's Media Department for their helpful assistance.

LITERATURE CITED

- Asakawa, Y., S. Akahane, K. Shiozawa, and T. Honma. 1979. Investigation of source and route of *Yersinia enterocolitica* infection, p. 115-121. In P. B. Carter, L. Lefleur, and S. Toma (ed.), Contributions to microbiology and immunology, vol. 5. S. Karger, Basel.
- Bercovier, H., J. Brault, N. Barre, M. Treignier, J. M. Alonso, and H. H. Mollaret. 1978. Biochemical, serological, and phage typing characteristics of 459 Yersinia strains isolated from a terrestrial ecosystem. Curr. Microbiol. 1:353-357.
- Bercovier, H., D. J. Brenner, J. Ursing, A. G. Steigerwalt, G. R. Fanning, J. M. Alonso, G. P. Carter, and H. H. Mollaret. 1980. Characterization of Yersinia enterocolitica sensu stricto. Curr. Microbiol. 4:201–206.
- Bercovier, H., J. Ursing, D. J. Brenner, A. G. Steigerwalt, G. R. Fanning, G. P. Carter, and H. H. Mollaret. 1980. Yersinia kristensenii: a new species of Enterobacteriaceae composed of sucrose-negative strains (formerly called atypical Yersinia enterocolitica or Yersinia enterocolitica-like). Curr. Microbiol. 4:219-224.
- Bissett, M. L. 1976. Yersinia enterocolitica isolated from humans in California 1968–1975. J. Clin. Microbiol. 4: 137-144.
- Black, R. E., R. J. Jackson, T. Tsai, M. Medvesky, M. Shayegani, J. C. Feeley, 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.
- Bottone, E. J. 1977. Yersinia enterocolitica: a panoramic view of a charismatic microorganism. Crit. Rev. Microbiol. 5:211-241.
- Bottone, E. J. 1978. Atypical Yersinia enterocolitica: clinical and epidemiological parameters. J. Clin. Microbiol. 7:562-567.
- Brenner, D. J. 1979. Speciation in Yersinia, p. 33-43. In P. B. Carter, L. Lafleur, and S. Toma (ed.), Contributions to microbiology and immunology, vol. 5. S. Karger, Basel.
- Brenner, D. J., H. Bercovier, J. Ursing, J. M. Alonso, A. G. Steigerwalt, G. R. Fanning, G. P. Carter, and H. H. Mollaret. 1980. Yersinia intermedia: a new species of Enterobacteriaceae composed of rhamnosepositive, melibiose-positive, raffinose-positive strains (formerly called Yersinia enterocolitica or Yersinia enterocolitica-like). Curr. Microbiol. 4:207-212.
- 11. Brenner, D. J., A. G. Steigerwalt, D. P. Falcao, R. E.

Weaver, and G. R. Fanning. 1976. Characterization of Yersinia enterocolitica and Yersinia pseudotuberculosis by deoxyribonucleic acid hybridization and by biochemical reactions. Int. J. Syst. Bacteriol. **26**:180-194.

- Brenner, D. J., J. Ursing, H. Bercovier, A. G. Steigerwalt, G. R. Fanning, J. M. Alonso, and H. H. Mollaret. 1980. Deoxyribonucleic acid relatedness in Yersinia enterocolitica-like organisms. Curr. Microbiol. 4:195-200.
- Caprioli, T., A. J. Drapeau, and S. Kasatiya. 1978. Yersinia enterocolitica: serotypes and biotypes isolated from humans and the environment in Quebec, Canada. J. Clin. Microbiol. 8:7-11.
- Carter, P. B., R. J. Zahorchak, and R. R. Brubaker. 1980. Plague virulence in antigens from Yersinia enterocolitica. J. Clin. Microbiol. 28:638-640.
- Cornelis, G., R. K. J. Luke, and M. H. Richmond. 1978. Fermentation of raffinose by lactose-fermenting strains of Yersinia enterocolitica and by sucrose-fermenting strains of *Escherichia coli*. J. Clin. Microbiol. 7:180-183.
- Dabernat, H. J., R. Bauriaud, J. Lemozy, J. C. Lefevre, and M. B. Lareng. 1979. Yersinia enterocolitica fermenting rhamnose. About 15 strains isolated in children, p. 270-276. In P. B. Carter, L. Lafleur, and S. Toma (ed.), Contributions to microbiology and immunology, vol. 5. S. Karger, Basel.
- Eden, K. V., M. L. Rosenberg, M. Stoopler, B. T. Wood, A. K. Highsmith, P. Skalig, J. G. Wells, and J. C. Feeley. 1977. Waterborne gastrointestinal illness at a ski resort. Isolation of *Yersinia enterocolitica* from drinking water. Public Health Rep. 92:245-250.
- Hanna, M. O., J. C. Stewart, Z. L. Carpenter, D. L. Zink, and C. Vanderzant. 1979. Isolation and characteristics of *Yersinia enterocolitica*-like bacteria from meats, p. 234-242. *In* P. B. Carter, L. Lafluer, and S. Toma (ed.), Contributions to microbiology and immunology, vol. 5. S. Karger, Basel.
- Hughes, D. 1979. Isolation of Yersinia enterocolitica from milk and a dairy farm in Australia. J. Appl. Bacteriol. 46:125-130.
- Knapp, W., and E. Thal. 1973. Differentiation of Yersinia enterocolitica by biochemical reactions, p. 10-16. In S. Winblad (ed.), Contributions to microbiology and immunology, vol. 2. S. Karger, Basel.
- Marks, M. I., C. H. Pai, L. Lafleur, L. Lackman, and O. Hammerberg. 1980. Yersinia enterocolitica gastroenteritis: a prospective study of clinical, bacteriologic, and epidemiologic features. J. Pediatr. 96:26-31.
- Mollaret, H. H., H. Bercovier, and J. M. Alonso. 1979. Summary of the data received at the WHO reference center for Yersinia enterocolitica, p. 174-184. In P. B. Carter, L. Lafleur, and S. Toma (ed.), Contributions to

microbiology and immunology, vol. 5. S. Karger, Basel. 23. Mors, V., and C. H. Pai. 1980. Pathogenic properties of

- Yersinia enterocolitica. Infect. Immun. 28:292-294.
 24. Niléhn, B. 1969. Studies on Yersinia enterocolitica with special reference to bacterial diagnosis and occurrence in human acute enteric disease. Acta Pathol. Microbiol. Scand. 206:1-48.
- Pedersen, K. B., and S. Winblad. 1979. Studies on Yersinia enterocolitica isolated from swine and dogs. Acta Pathol. Microbiol. Scand. Sect. B 87:137-140.
- Saari, T. N., and G. P. Jansen. 1979. Waterborne Yersinia enterocolitica in the midwest United States, p. 185-196. In P. B. Carter, L. Lafleur, and S. Toma (ed.), Contributions to microbiology and immunology, vol. 5. S. Karger, Basel.
- Schiemann, D. A. 1978. Association of Yersinia enterocolitica with the manufacture of cheese and occurrence in pasteurized milk. Appl. Environ. Microbiol. 36:274-277.
- Schiemann, D. A. 1978. Isolation of Yersinia enterocolitica from surface and well waters in Ontario. Can. J. Microbiol. 24:1048-1052.
- Schleifstein, J., and M. B. Coleman. 1939. An unidentified microorganism resembling *B. lignieri* and *Past. pseudotuberculosis*, and pathogenic for man. N.Y. State J. Med. 39:1749-1753.
- Shayegani, M., E. J. Menegio, D. M. McGlynn, and H. A. Gaafar. 1979. Yersinia enterocolitica in Oneida County, New York, p. 196-205. In P. B. Carter, L. Lafleur, and S. Toma (ed.), Contributions to microbiology and immunology, vol. 5. S. Karger, Basel.
- Thirumoorthi, M. C., and A. S. Dajani. 1978. Yersinia enterocolitica osteomyelitis in a child. Am. J. Dis. Child. 132:578-580.
- Toma, S., and L. Lafleur. 1974. Survey on the incidence of Yersinia enterocolitica infection in Canada. Appl. Microbiol. 28:469-473.
- 33. Toma, S., L. Lafleur, and V. R. Deidrick. 1979. Canadian experience with Yersinia enterocolitica (1966-1977), p. 144-149. In P. B. Carter, L. Lafleur, and S. Toma (ed.), Contributions to microbiology and immunology, vol. 5. S. Karger, Basel.
- 34. Ursing, J., D. J. Brenner, H. Bercovier, G. R. Fanning, A. G. Steigerwalt, J. Brault, and H. H. Mollaret. 1980. Yersinia frederiksenii: a new species of Enterobacteriaceae composed of rhamnose-positive strains (formerly called atypical Yersinia enterocolitica or Yersinia enterocolitica-like). Curr. Microbiol. 4:213-217.
- Weaver, R. E., and J. G. Jordan. 1973. Recent human isolates of Yersinia enterocolitica in the United States, p. 120-125. In S. Winblad (ed.), Contributions to microbiology and immunology, vol. 2. S. Karger, Basel.