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Yersinia enterocolitica: Serotypes and Biotypes Isolated from Humans and the Environment in Quebec, Canada

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Thirty-one strains of *Yersinia enterocolitica* were isolated from food and surface water. During the period of January 1975 to June 1977, 157 strains from 143 human cases were also isolated. Among the different serotypes from nonhuman sources, serotypes 6,30 and 4,32 were the most common. Serotype 4,32 was present only in food and not in water. Serotype 3 was isolated only from humans. Among the different serotypes from human cases, 73.9% belonged to serotype 3. Only serotype 3 was isolated from children under 4 years old. The presence of other serotypes increased and that of serotype 3 decreased in frequency as the age progressed. No serotype 3 was isolated from human cases aged 50 years and more.

Human infections, either in sporadic or epidemic form, due to *Yersinia enterocolitica* have been reported from various countries (1, 2, 6, 8, 12, 16-19; A. C. Sonnenwirth, Bacteriol. Proc., p. 87, 1969). This organism has been isolated from the environment, particularly from water (10, 15, 21, 24). It is frequently found in the intestinal tract of wild animals (12, 14, 20). Despite its isolation from various sources, no definite correlation regarding its mode of transmission has been established so far. Some authors (11, 15, 22) think that water might be a reservoir of infection and that the disease might be transmitted by consumption of contaminated water. On the other hand, many authors found no correlation between the serotypes isolated from humans and the environment and suspect that the transmission of this microorganism might be through personal contact (18) or food (27).

The present study was undertaken to establish a correlation between the serotypes isolated from humans and the environment in the province of Quebec.

MATERIALS AND METHODS

The study was undertaken during the period from January 1975 to June 1977. Water samples were collected from a local river during the months of July, August, and September 1976.

Strains. Cultures of *Y. enterocolitica* were either isolated from specimens sent to the State Laboratory by hospitals, local health units, clinical laboratories, private physicians, and state environmental and agricultural laboratories, or received directly from state bacteriologists on semisolid agar or agar slants for identification and/or confirmation purposes. Strains were mostly isolated from water, food, and human stools.

Isolation of *Y. enterocolitica* strains. (i) Water. One liter of water was filtered through a 0.45-μm nitrocellulose filter (Millipore Corp., Bedford, Mass.). The filter was dropped into 10 ml of modified Rappaport medium (23) and incubated overnight at 29°C. The next day, MacConkey and salmonella-shigella (SS) agar plates were streaked and incubated at 29°C for another 18 h. Typical lactose-negative colonies were picked and maintained on triple sugar iron agar slants for further identification.

(ii) Food. Ten grams of food was added to 90 ml of sterile phosphate buffer solution or physiological saline (0.85% NaCl) and homogenized in a sterile blender or mixer. A 0.1-ml sample of this mixture was directly inoculated onto SS and MacConkey agar plates. For enrichment purposes, 1 ml of the homogenate was inoculated into 25 ml of selenite enrichment broth (7) and incubated at 35°C for 18 h. The next day, MacConkey and SS agar plates were streaked. All the SS and MacConkey agar plates were incubated at 29°C for 18 h. Lactose-negative colonies picked up from the plates were maintained on triple sugar iron agar slants.

(iii) Human specimens. A small quantity of the human specimen sample was directly inoculated onto SS and MacConkey agar plates and incubated overnight at 29°C. For the enrichment technique, the sample was inoculated in selenite broth. After overnight incubation at 29°C, the broth culture was streaked on an SS agar plate and incubated for 18 h at 29°C. Typical lactose-negative colonies were picked up from MacConkey and SS agar plates and maintained on triple sugar iron agar slants.

Identification of *Y. enterocolitica* strains. Biochemical studies (at 29°C) (Table 1) were done according to Wauters (23) and Brenner et al. (5). The strains were classified (Table 2) according to Wauters (23). For serotyping, 31 somatic antisera were prepared

TABLE 1. *Biochemical reactions of 188 strains of Y. enterocolitica*[a]

Determination	Wauters biotypes[b]																															
	I																					II			III	IV						
Triple sugar iron agar	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	K/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	K/A	A/A	A/A	A/A	A/A	A/A	A/A	K/A	A/A	A/A
Voges-Proskauer (22°C)	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+
Voges-Proskauer (37°C)	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Simmons citrate	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Urease	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mobility at 22°C	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	(+)	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	)	+	+	+	+	+	+	+	+	+	+	+	+	(+)	−	(+)	(+)	(+)	(+)	(+)	(+)
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	−	−	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+	−	−	−	−	−	−	−	−
Salicin	+	+	+	+	+	+	+	+	+	+	+	−	−	(+)	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Raffinose	−	−	−	+	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Rhamnose	−	+	−	+	+	−	−	+	+	+	(+)	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
α-methyl-D-Glycoside	−	+	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Melibiose	−	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lecithinase (22°C)	+	+	+	+	+	+	+	(+)	+	−*	+	+	+	−*	+	+	+	−*	+	+	−	+*	+*	−	−	−	−	−	−	−	−	−
Indole	+	+	+	+	+	(+)	+	+	+	+	+	+	(+)	+	−*	+	+	+	+	+	(+)	(+)	(+)	−	−	−	−	−	−	−	−	−
Lactose (oxidation)	+	+	+	+	+	+	+	−*	+	+	−*	−*	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−*	+	+	+	+	+	−	−	−	−	−	−	−	−
Nitrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−*	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ornithine decarboxylase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−*
β-Galactosidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−*	−*	+	+	+	+	+	+	+	+	+	+	+	+	−*	+	
Total strains	39	1	1	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	2	1	3	1	6	1	92	12	4	2	1	2	2	1

[a] *Y. enterocolitica* strains were all positive by the following tests: motility at 22°C, methyl red, dextrose, arabinose, and sorbitol; the strains were all negative by these tests: H_2S, dulcitol, lysine, arginine, malonate, adonitol, and phenylalanine.

[b] Symbols: ( ), Slow (2 days or more); A, acid; K, alkaline; +, positive reaction; −, negative reaction; *, atypical reactions with reference to Wauters classification (23).

in rabbits according to the method of Wauters and coworkers (25, 26) by inoculating with the stock strains furnished by S. Toma of the Ontario Public Health Laboratory, Toronto. Slide and tube agglutinations, whenever necessary, were done according to the method of Edwards and Ewing (7).

RESULTS

Table 3 represents the distribution of the serotypes and biotypes of *Y. enterocolitica* strains isolated from nonhuman sources. In total, 25 strains representing seven different serotypes were isolated, of which 6,30 and 4,32 were the most common. Six strains which could not be serotyped are being studied further. Six of the seven typable serotypes were found in water. Serotype 4,32, found in food, was also isolated from water.

TABLE 2. *Y. enterocolitica biotypes according to Wauters (23)*[a]

Determination	*Y. enterocolitica* biotypes[a]				
	1	2	3	4	5
Lecithinase activity	+	−	−	−	−
Indole production	+	(+)	−	−	−
Lactose oxidation	+	+	+	−	−
Xylose fermentation	+	+	+	−	−
Nitrate reduction	+	+	+	+	−
Trehalose fermentation	+	+	+	+	−
Ornithine decarboxylation	+	+	+	+	−
β-Galactosidase activity	+	+	+	+	−

[a] Symbols: ( ), Slow (2 days or more); +, positive reaction; −, negative reaction.

Table 4 represents the types of *Y. enterocolitica* isolated from human sources. One hundred fifty-seven isolates from 143 cases, belonging to 13 different serotypes, were isolated. Of the 140 strains, 82.9% belonged to 3, a common serotype in Canada. Of the 157 strains, 17 could not be typed by the existing sera.

Of the 143 human cases positive for *Y. enterocolitica,* 59.4% were males; 72.0% of the 143 cases harbored serotype 3 (Table 5). Of the 81 known cases harboring serotype 3, 65.4% were aged 4 years or below (Table 6). In this age group, no serotype other than 3 was isolated. The percentage of cases with serotype 3 and other serotypes in the age group below 10 years, among the 111 cases for which age was known, was 55.0 and 0.9, respectively, whereas it was 18.0 and 26.1%, respectively, in cases aged 10 years and above.

DISCUSSION

Despite its isolation from food, water, humans, and animals reported from various countries, no

TABLE 3. *Strains isolated from nonhuman sources*

O serotype	Biotype	Origin	No. of specimens	No. of strains
4,32	2 atypical (lecithinase positive)	Pancake	1	
		Cheese	1	
		Ham	1	
		Sausage	2	
		Raw beef	1	6
5	1	Water	1	1
6,30	1	Water	9	
		Milk	2	11
	1 atypical (indole negative)	Water	1	1
6,31	1	Water	1	
		Sausage	1	2
7,8	1	Water	1	1
11,24	2 atypical (lecithinase positive)	Water	1	1
34	1	Water	2	2
Nontypable	1 atypical (lecithinase negative)	Water	3	
		Poultry	2	
	1 atypical (lactose oxidation negative)	Raw beef	1	6
Total			31	31

TABLE 4. *Strains isolated from human sources*

O serotype	Biotype	Origin	No. of cases	No. of strains
3	4	Abscess	1	
		Feces	74	113
		Pus	1	
		Unknown	37	
	4 atypical (β-galactosidase negative; ornithine negative)	Feces	3	3
4,32	1	Feces	1	1
4,33	1	Expectoration	1	1
5	1	Feces	2	
		Nose swab	1	3
5,27	2	Feces	3	3
6,30	1	Feces	6	6
6,31	1	Feces	3	3
7,8	1	Urine	1	1
8	1 atypical (β-galactosidase negative)	Feces	1	1
8,19	1	Feces	1	1
13,7	1	Feces	1	1
	1 atypical (β-galactosidase negative)	Feces	1	1
16	1 atypical (lecithinase negative)	Feces	1	1
10[a]	1	Feces	1	1
Nontypable	1 atypical: Xylose negative (2)[b] Nitrate negative (1) Lactose fermentation negative (3) Lecithinase negative (1) Indole slow (1)	Feces	16	
		Nose swab	1	17
Total			143	157

[a] Also gives a cross-reaction with antiserum O34.
[b] ( ), Number of strains.

TABLE 5. *Distribution of strains from human sources according to sex*

Sex	Serotype 3 (no. of cases)	Other serotypes (no. of cases)	All serotypes (no. of cases)
Male	65	20	85
Female	38	20	58

definite mode of transmission of *Y. enterocolitica* has been established so far. Our data confirm some of the results published elsewhere. Serotype 3, normally present in humans and pigs (25), and frequently isolated in Canada (21) but rare in United States (3), was isolated neither from water nor from food. The situation was the same for other serotypes less frequent in humans, e.g., 4,33; 5,27; 8; 8,19; 13,7; 16; and 10, which were not isolated from water or food. Similarly, serotypes 11,24 and 34, present in water, were absent in humans. These data may indicate that: (i) the number of samples from the environment is too small to come to any valuable conclusion; (ii) there is difficulty in isolation of *Yersinia* strains, especially from the environment; (iii) the serotypes normally pres-

TABLE 6. *Distribution of strains from human sources according to age*

Age (years)	Serotype 3 (no. of cases)	Other serotypes (no. of cases)	All serotypes (no. of cases)
<1	18	0	18
1-4	35	0	35
5-9	8	1	9
10-19	10	9	19
20-29	7	10	17
30-39	2	2	4
40-49	1	2	3
>50	0	6	6
Unknown	22	10	32

ent in humans and the environment are different, as reported by Wauters et al. (25). These authors presume that serotype 1 is found almost exclusively in chinchilla, serotype 2 in hares, serotypes 3 and 9 in humans, and serotype 3 in pigs.

It is interesting that children under 4 years of age harbored almost exclusively serotype 3, which tends to decrease in frequency while the presence of other serotypes increases as the age progresses. In patients aged 50 years or more, only serotypes other than 3 were isolated.

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