

## Isolation of *Yersinia enterocolitica* from Swine

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Data pertaining to 20 strains of *Yersinia enterocolitica* isolated from the cecal content of swine slaughtered at Toronto Abattoirs are presented. Fifteen of 20 cultures belonged to the two predominant human serotypes in Ontario (i.e., serotype O:3 and O:5,27). Seven cultures of *Y. enterocolitica* O:3 belonged to the "Canadian" human phage type 9b. These findings are further proof that swine are reservoirs for *Y. enterocolitica* human infection. Seventeen *Y. enterocolitica* cultures were isolated by the cold enrichment method only. Difficulties encountered in isolating *Y. enterocolitica* from feces specimens are discussed. It would appear that the reported rate of *Y. enterocolitica* isolation from feces specimens is far below the actual incidence. Fourteen isolates of *Y. pseudotuberculosis* serotype III were isolated during the same survey.

The number of *Yersinia enterocolitica* species diagnosed in humans has increased considerably during the last decade. There has been a steady increase in the recorded incidence of infection and the number of countries which report *Y. enterocolitica* infections. Clinical aspects of the disease have widened, and the various animal species or environmental specimens in which this bacterium is found have increased.

There are many unknown aspects to human yersiniosis; one is the lack of knowledge as to the natural reservoir of human infections. Although there have been more than 5,000 recorded cases of human yersiniosis and five outbreaks produced by *Y. enterocolitica* (2, 6, 9, 15), the human carrier rate is very low. *Yersinia* has never been isolated from food involved in outbreaks, and strains isolated from epizootics in chinchillas, from hares, rodents, and water specimens, as well as other environmental sources, proved to be bioserotypes, which are seldom involved in human infection.

In Europe and Japan *Y. enterocolitica* serotype O:3, phage type 8, the most predominant human serophage type in these areas, was isolated from swine, the only animal species which consistently harbors this human serophage type (1, 4, 12, 14, 16). These isolations suggested the possibility of a relationship between some human infections and swine. The European specialists have long expected that Canada would confirm this epidemiological link because *Y. enterocolitica* serotype O:3 is the most predominant serotype in Canada and belongs to a specific phage type, 9b, referred to as the "Canadian" phage type. Phage type 9b has never been

isolated in Europe, Africa, Asia, or elsewhere (7). The purpose of this study was to search for the presence of *Y. enterocolitica* in swine and to determine their phage types.

### MATERIALS AND METHODS

From September 1974 to June 1975, we examined 544 specimens of cecal content collected from swine slaughtered for human consumption at an abattoir in Toronto. About 1.0 g of cecal content was taken immediately after slaughter and immersed in 10 ml of 0.067 M phosphate-buffered saline, pH 7.6 (8.5 g of NaCl, 120 ml of 0.067 M  $\text{KH}_2\text{PO}_4$ , and 880 ml of 0.067 M  $\text{Na}_2\text{HPO}_4$ ), in a screw-cap bottle (8, 12). Prior to this survey, four different fluid media (Selenite-F Enrichment, Banxang and Elliott, cooked meat broth, and 0.067 M phosphate buffer solution, pH 7.6) were tried in parallel to study their "cold enrichment" capacity for the isolation of *Y. enterocolitica* from feces specimens. These four fluid media were inoculated with the same amount of feces suspension into which one of seven *Y. enterocolitica* strains was added (*Y. enterocolitica* serotypes O:3 [three different strains]; 5, 27; 6, 30; 8; and 9). Each of these four fluid media was plated on MacConkey and Salmonella-Shigella (SS) plates after being stored at +4 C for 1 day and 1, 2, 3, and 4 weeks, as well as for 2 and 3 months. The phosphate-buffered saline, fluid medium yielded the highest number of *Y. enterocolitica* isolates and was therefore used for the collection and enrichment of specimens from swine. There were more *Yersinia* isolates after 3 weeks of storage at +4 C than at any other period.

The phosphate-buffered saline specimens were plated on SS and MacConkey media (pH 7.2 to 7.4) after 1 day and again after 3 weeks at +4 C. The last batch of 198 specimens was inoculated on a plate medium called Y-L in addition to MacConkey and SS plates. This medium was successfully used by T. Saari for the isolation of *Yersinia* from water speci-

mens in Denver, Colo. (personal communication; in press). The plates were examined after overnight incubation at 30 C and again after another 24 h at room temperature. A plate microscope with direct or oblique illumination was used to examine the plates (10). Small lactose-negative colonies were picked with a straight platinum wire and inoculated onto a triple sugar iron agar and a semisolid *o*-nitrophenyl- $\beta$ -D-galactopyranoside-PA-M medium (5). Biochemical identification of cultures was carried out by standard methods used for identification of *Enterobacteriaceae* (3). Some of the tests were modified according to Wauters (13). Serological typing was done by slide agglutination using cultures grown on Trypticase soy slants or blood agar plates incubated for 24 to 48 h at 33 C. We used 34 "O" *Y. enterocolitica* and 8 *Y. pseudotuberculosis* absorbed and unabsorbed antisera prepared in rabbits by our laboratory. The phage typing was performed at the *Yersinia* Center, Institut Pasteur, Paris, by H. H. Mollaret.

RESULTS

A total of 20 *Y. enterocolitica* and 14 *Y. pseudotuberculosis* strains were isolated (Table 1). Fifteen of 20 *Y. enterocolitica* cultures belonged to the two predominant human serotypes in Ontario (10), 10 isolates of serotypes O:3 and five isolates of serotype O:5, 27 (Table 2). Seven *Y. enterocolitica* serotype O:3 strains have already been phage typed, and all belong to the same Canadian human phage type 9b.

All *Y. pseudotuberculosis* isolates belonged to serotype III. This is perhaps the only serotype present in swine, or it is possible that only this particular serotype grows on the conventional enteric bile media which we used. It is known

that *Y. pseudotuberculosis* does not grow well on enteric bile plates and is very seldom, if ever, isolated from feces specimens or intestinal content.

The rate of isolation was higher during the cold months, November to March (6 to 12%), than during the warmer months of September/October and May/June (1 to 5%).

Seventeen *Y. enterocolitica* cultures were isolated by the cold enrichment method, and only two cultures were isolated by direct plating (Table 3). The number of *Y. pseudotuberculosis* strains isolated was approximately the same using either method. The last 198 specimens yielded six *Yersinia* (two *Y. enterocolitica* and four *Y. pseudotuberculosis*). Five out of these six cultures were isolated from the Y-L medium only, either by direct plating (two cultures) or after cold enrichment (three cultures).

DISCUSSION

The epidemiology of *Y. enterocolitica* infections is still obscure. It is probable that human

TABLE 3. Isolation of *Yersinia* from swine (cecal content)

Culture	No. of cultures isolated	Method of isolation		
		Direct plating	Indirect plating (cold enrichment)	Both methods
<i>Y. enterocolitica</i>	20	2	17	1
<i>Y. pseudotuberculosis</i>	14	7	6	1

TABLE 1. Isolation of *Yersinia* from swine (cecal content)

Batch no	Date of collection	No. of specimens	Isolates			
			<i>Y. enterocolitica</i>		<i>Y. pseudotuberculosis</i>	
			No.	%	No.	%
1	Sept–Oct 1974	102	3	(3)	2	(2)
2	Nov–Dec 1974	138	9	(6.5)	7	(5)
3	Feb–Mar 1975	106	6	(6)	1	(0.9)
4	May–June 1975	198	2	(1)	4	(2)

TABLE 2. Isolation of *Yersinia* from swine (cecal content)

Batch no.	Cultures isolated	<i>Y. enterocolitica</i>							<i>Y. pseudotuberculosis</i>	
		0:3 <sup>a</sup>	5, 27	5	6, 30	7, 13	8	14	Cultures isolated	Serotype III
1	3	—	3	—	—	—	—	—	2	2
2	9	7	1	—	1	—	—	—	7	7
3	6	2	1	—	—	1	1	1	1	1
4	2	1	—	1	—	—	—	—	4	4

<sup>a</sup> Serotypes.

infection occurs through the oral route, but the possibility of airborne transmission has been considered, especially during outbreaks in groups of infants. Healthy carriers are known to occur, as shown in contacts with *Yersinia*-infected patients or by different epidemiological surveys. Certainly carriers represent an important factor in the spread of this infection, but the ultimate reservoir and the main sources of human contamination have not yet been discovered.

At the present time the only help the laboratory can give to trace the epidemiology of *Yersinia* is to compare the main biochemical, serological, and phage type features of human cultures with those cultures isolated from animals or environmental sources. There are bioserotypes common to particular animal species: e.g., biotype 5, serotype O:2, isolated mainly from hares, and biotype 3, serotype O:1, responsible for most epizootics or isolated infections in chinchillas. Neither of these two types of *Y. enterocolitica* has been isolated from humans. The predominant human serotype O:3 has been only rarely isolated from dogs, cats, or cattle, but has been isolated quite regularly from a single animal reservoir, that is, swine (1, 4, 12, 14, 16).

Our findings are further proof that the swine population is a reservoir of human infections for the following reasons. (i) Fifteen of the 20 *Y. enterocolitica* isolates from swine belonged to the two predominant human serotypes in Ontario, serotypes O:3 and O:5, 27; and (ii) the serotype O:3 isolates from both humans and swine belonged to the same phage type, the Canadian phage type 9b.

There are many difficulties involved in the isolation of *Y. enterocolitica* from feces specimens, some of which may be overcome by the use of fresh, quality-controlled plate media, a plate microscope having oblique illumination (10), and experience in the recognition of *Y. enterocolitica* colonies on isolation plates and screening media. Xylose lysine deoxycholate agar and Hektoen enteric agar media have been proven unsuitable for the isolation of *Y. enterocolitica* because they contain sucrose, which is fermented by this organism. Xylose in xylose lysine deoxycholate medium and salicin in Hektoen enteric agar medium are also fermented by some *Y. enterocolitica*. The Y-L medium, introduced in the latter part of our survey, proved to be highly suitable. Five of the six *Y. enterocolitica* cultures were isolated from this medium only and not from MacConkey and SS plates. This strongly indicates that some *Yersinia* isolates have been previously missed in our laboratory, either in feces specimens or during the present swine survey.

Difficulties encountered in isolating *Y. enterocolitica* are further suggested by following example. Two laboratories in Europe processed same specimens from animals in order to isolate *Y. enterocolitica* (14). As a result of this joint project a total of nine *Y. enterocolitica* strains were isolated. Only three cultures were isolated simultaneously by both laboratories, whereas the other six *Y. enterocolitica* were isolated by one participating laboratory only.

The above-mentioned factors indicate that the reported rate of *Y. enterocolitica* isolation from feces specimens is far below the actual incidence.

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#### LITERATURE CITED

- Ahvenon, P., E. Thal, and H. Vasenius. 1973. Occurrence of *Yersinia enterocolitica* in animals in Finland and Sweden, p. 135-136. In Contributions to microbiology and immunology, vol. 2. Karger, Basel.
- Asakawa, Y., S. Akahane, N. Kagata, M. Masateru, R. Sakazaki, and K. Tamura. 1973. Two community outbreaks of human infection with *Yersinia enterocolitica*. *J. Hyg.* 71:715-723.
- Edwards, P. R., and W. H. Ewing. 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
- Esseveld, H., and C. Goudzwaard. 1973. On the epidemiology of *Y. enterocolitica* infections: pigs as the source of infections in man, p. 99-101. In Contributions to microbiology and immunology, vol. 2. Karger, Basel.
- Giammanco, G., and A. Falci. 1971. The detection of *Salmonella* and *Shigella* with the aid of a new medium for the simultaneous assay of beta-galactosidase, phenylalanine-deaminase and motility. *Ann. Inst. Pasteur Paris* 120:525.
- Gutman, L. T., E. A. Ottesen, T. J. Quan, P. S. Noce, and S. L. Katz. 1972. An inter-familial outbreak of *Yersinia enterocolitica* enteritis. *N. Engl. J. Med.* 288:1372-1377.
- Nicolle, P., H. Mollaret, and J. Brault. 1973. Recherches sur la lysogénie, la lysosensibilité, la lysotypie et la sérologie de *Yersinia enterocolitica*, p. 54-58. In Contributions to microbiology and immunology, vol. 2. Karger, Basel.
- Paterson, J. S., E. A. Cook. 1963. A method for the recovery of *Pasteurella pseudotuberculosis* from faeces. *J. Pathol. Bacteriol.* 85:241-242.
- Toivanen, P., A. Toivanen, L. Olkkonen, and S. Aantaa. 1973. Hospital outbreak of *Yersinia enterocolitica* infection. *Lancet* 1:801-803.
- Toma, S. 1973. Survey on the incidence of *Yersinia enterocolitica* in the Province of Ontario. *Can. J. Public Health* 64:477-487.
- Toma, S., and L. Laffleur. 1974. Survey on the incidence of *Yersinia enterocolitica* infection in Canada. *Appl. Microbiol.* 28:469-473.
- Tsubokura, M., K. Otsuki, and K. Itagaki. 1973. Isolation of *Y. enterocolitica* from swine. *Jpn. J. Vet. Sci.* 35:419-424.
- Wauters, G. 1970. Contribution à l'étude de *Yersinia enterocolitica*. Vander, Louvain.
- Wauters, G., J. Vandepitte, and R. VanNoyen. 1971.

- Yersinia enterocolitica* chez les grandes espèces domestiques. *Med. Mal. Infect.* 1:226-228.
15. Zen-Yoji, H., T. Maruyama, S. Sakai, S. Kimura, T. Mizuno, and T. Momose. 1973. An outbreak of enteritis due to *Yersinia enterocolitica* occurring at a junior high school. *Jpn. J. Microbiol.* 17:220-222.
16. Zen-Yoji, H., S. Sakai, T. Maruyama, and Y. Yanagawa. 1974. Isolation of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from swine, cattle and rats at an abattoir. *Jpn. J. Microbiol.* 18:103-105.