J. Hyg., Camb. (1986), 97, 281–287
Printed in Great Britain

Faecal carriage rate of *Yersinia* species

By ANN M. LEWIS and B. CHATTOPADHYAY
Public Health Laboratory and Department of Microbiology, Whipps Cross Hospital, London E11 1NR

(*Received 17 January 1986; accepted 14 May 1986*)

SUMMARY

A total of 1203 unselected routine faecal samples from 1006 patients were cultured for *Yersinia* species by a cold enrichment technique. Seventy-five specimens (6·1 %) from 63 patients were culture-positive for *Yersinia* spp. Fifty-two were *Yersinia enterocolitica*, 22 *Yersinia frederiksenii* and 1 *Yersinia intermedia*. The predominant *Y. enterocolitica* isolates belonged to biotype 1 – serotype 0:6, 30 or serotype 0:5, 27. *Y. frederiksenii* strains were non-typable. Forty isolates were recovered from 33 patients with gastroenteritis. During the study period 83 *Salmonella* spp. from 33 patients, 17 *Shigella sonnei* from 13 patients and 13 *Campylobacter jejuni* from 12 patients were cultured. *Yersinia* spp. was isolated in association with salmonella on three occasions, twice with rotavirus and once each with *Shigella sonnei*, *Campylobacter jejuni* and *Trichuris trichiura*.

INTRODUCTION

Yersinia enterocolitica is capable of producing a spectrum of disease. The most common clinical manifestation in man is abdominal pain with or without bloody diarrhoea. Fever may be present. Other forms of presentation include mesenteric lymphadenitis, terminal ileitis, pseudo-appendicitis, septicaemia, meningitis and urinary tract infection (Bottone, 1977; Larsen, 1979; Weissfeld & Sonnenwirth, 1980). Sequelae include arthritis, Reiter's syndrome and erythema nodosum (Wimblad, 1973). Infants are most susceptible to *Y. enterocolitica* infections during the first year of life (Vandepitte & Wauters, 1979).

The organism is ubiquitous and has not only been isolated from faeces but also from food and water. Several community and hospital-acquired outbreaks from outside the United Kingdom reported over the last few years have implicated water, milk and food as the vehicles of transmission and person-to-person spread has also been recorded (Highsmith, Feeley & Morris, 1977).

Paucity of information concerning this organism in the UK prompted this present study in order to assess the carriage rate of *Yersinia* spp. in stool, to note the prevalent serotypes and their association or otherwise with gastrointestinal disorders.

MATERIALS AND METHODS

Source of samples

A total of 1203 unselected stool samples from 1006 patients (524 males and 482 females) were examined over a period of 5 months.

Isolation methods

All specimens were examined for *Salmonella*, *Shigella* and *Campylobacter* species by standard methods and, where the history indicated, for other enteric pathogens including rotavirus and parasites.

Approximately 1 g of faeces was emulsified in 10 ml of 1% buffered peptone water (BPS-Oxoid) of pH 7·2 and incubated at +4 °C for 3 weeks. The broths were then subcultured to Yersinia Selective Medium – Cefsulodin–Irgasan–Novobiocin agar (CIN medium – Oxoid) and incubated overnight at 30 °C. *Y. enterocolitica* strains. NCTC 10938 and 10463 were used as controls. The plates were examined for mannitol fermenting colonies (pink or red). Colonies were tested for motility at 37 °C. Those which were non-motile at 37 °C, and were urease-positive using Christensen's urea slopes (Central Public Health Laboratory, Colindale), were considered provisionally to be *Yersinia* spp. The identity of isolates was confirmed using API 20E. Strains were biotyped and serotyped at Leicester Public Health Laboratory by the method of Wauters (Wauters, 1970).

Sensitivity testing was by the disc diffusion technique using the Stokes method (Stokes & Waterworth, 1972) with *Escherichia coli* NCTC 10418 as the control organism.

RESULTS

Yersinia spp. was isolated from 75 (6·1%) of the 1203 specimens analysed. The isolates came from 63 patients, 24 of whom were male (30 strains) and 39 female (45 strains). Fifty-two strains were *Y. enterocolitica*, 22 *Y. frederiksenii* and 1 *Y. intermedia*. Biochemically *Y. frederiksenii* differs from *Y. enterocolitica* by being rhamnose-positive, whereas *Y. intermedia* is melibiose-positive. Other types of intestinal pathogens recovered are shown in Table 1. *Yersinia* spp. was isolated in association with *Salmonella* sp. on three occasions, twice with rotavirus (in the same patient) and once each with *Campylobacter jejuni*, *Shigella sonnei* and *Trichuris trichiura*. Forty of the isolates (53%) were recovered from 33 patients with diarrhoea or other symptoms of gastroenteritis. In five of these patients, other intestinal pathogens were also present.

Table 2 records the isolations by age group and provides details of bio- and serotypes of the organism recovered. The proportion of patients with symptoms is also presented. Patients aged 1–5 years yielded the highest proportion of positive cultures, which accords with findings of other workers in Europe (Vandepitte & Wauters, 1979).

In Table 3 the biotypes and serotypes of *Yersinia* spp. isolated from symptomatic patients are compared with those from patients without symptoms. There was no preponderance of any particular type or types in the different age groups amongst the symptomatic patients. In five patients with symptoms other intestinal pathogens were present.

In 10 patients more than one specimen was received. Table 4 presents the findings. In 5 cases second specimens were received within 48 h of the first and in 3 of these the serotypes of the isolates differed. Where the interval was longer the serotypes had changed in 1 case and the biotypes as well in 2 others. In each of

Table 1. *Numbers and percentages of various types of isolate*

	No. of isolations	%	No. of patients
Yersinia spp.	75	6·1	63
Salmonella spp.*	83	6.7	33
Shigella spp.	17	1.4	13
Campylobacter jejuni	13	1.1	12
Clostridium perfringens	11	0.9	11
Staphylococcus aureus	9	0.7	9
Giardia lamblia	8	0.6	8
Ascaris	4	0.3	3
Ankylostoma/Necator	3	0.2	2
Trichuris	5	0.4	4
Rotavirus	3	0.2	3

A total of 1203 specimens of faeces from 1006 patients were studied. Though expressed as a percentage of the total, pathogens below the dotted line were only looked for when clinically indicated.

* Also includes enteric fever group.

Table 2. *Distribution of* Yersinia *spp. according to age groups along with the bio-serotypes of 75 strains*

	Age (years)								
	< 1	1–5	6–10	11–18	19–35	36–60	60+	Not stated	Total
Y. enterocolitica									
Biotype 1									
Non-typable	1	6	—	—	4	—	2	1	14
0:4	1	—	—	—	—	—	—	—	1
0:5, 27	1	4	—	—	1	—	2	3	11
0:6, 30	1	4	—	2	2	2	1	3	15
0:7	1	—	—	1	2	—	1	—	5
0:34	—	—	2	—	—	—	—	—	2
Biotype 3									
Non-typable	—	1	—	—	—	1	—	—	2
0:5, 27	—	—	—	—	—	—	—	1	1
Biotype 4									
0:6, 30	1	—	—	—	—	—	—	—	1
Y. frederiksenii									
Non-typable	—	6	1	2	1	2	3	3	18
0:2	—	1	—	—	2	—	—	—	3
0:16	—	—	—	—	—	—	—	1	1
Y. intermedia	—	—	—	—	1	—	—	—	1
Total	6	22	3	5	13	5	9	12	75
Percentage positive of total examined in age group	6	15	5	10	8	3	5	3	6.1
Percentage symptomatic of positives in age group	17	50	67	60	69	60	78	33	53

The total number of cases in various age groups along with percentages of positive and symptomatic patients have been shown.

https://doi.org/10.1017/S0022172400065372 Published online by Cambridge University Press

Table 3. *Isolations from symptomatic and asymptomatic patients by biotype and serotype*

Isolate	Biotype	Serotype	No. from* symptomatic	No. from† asymptomatic
Y. enterocolitica	1	0:5, 27	10	1
	1	NT	8	6
	1	0:6, 30	5	10
	1	0:7	2	3
	1	0:34	1	1
	1	0:4	0	1
	3	NT	2	0
	3	0:5, 27	0	1
	4	0:6, 30	0	1
Y. frederiksenii	—	NT	9	9
	—	0:2	2	1
	—	0:16	0	1
Y. intermedia	—	NT	1	0

NT, not typable.
* 40 isolations from 33 patients.
† 35 isolations from 30 patients.

Table 4. *Analysis of isolates where more than one specimen was taken*

(i) Interval between specimens < 48 h

Case no.	Age (years)	Sex	Isolate
1	3	M	*Y. entero* bio 1, serotype NT
			Y. frederiksenii serotype NT
2	35	F	*Y. entero* bio 1, serotype 0:7
			Y. frederiksenii serotype 0:2
3	4	F	*Y. entero* bio 1, serotype NT
			Y. entero bio 1, serotype 0:6, 30
4	13 months	M	*Y. entero* bio 1, serotype 0:5, 27
			Y. entero bio 1, serotype 0:5, 27
5	16	M	*Y. frederiksenii* serotype NT
			Y. frederiksenii serotype NT

(ii) Interval between specimens < 48 h

Case np.	Age (years)	Sex	Interval (days)	Isolate
6	2	F		*Y. entero* bio 1, serotype NT
			8	*Y. entero* bio 1, serotype 0:5, 27
7	72	M		*Y. entero* bio 1, serotype 0:6, 30
			13	*Y. frederiksenii* serotype NT
8	3	M		*Y. entero* bio 1, serotype 0:6, 30
			18	*Y. frederiksenii* serotype NT
			4	*Y. entero* bio 1, serotype NT
9	1	F		*Y. entero* bio 1, serotype 0:4
			6	*Y. entero* bio 1, serotype NT
			15	*Y. entero* bio 1, serotype 0:7
10	84	F		*Y. entero* bio 1, serotype NT
			7	*Y. entero* bio 1, serotype NT

NT, not typable.

https://doi.org/10.1017/S0022172400065372 Published online by Cambridge University Press

the 2 cases in which 3 specimens were examined from the same patient, all the strains differed from each other.

DISCUSSION

While routine culture methods may fail to detect *Yersinia* spp. the value of the cold enrichment technique has been clearly demonstrated (Bottone, 1977; Highsmith, Feeley & Morris, 1977) and in a recent survey the rate of isolation of *Y. enterocolitica* was as high as that of *Salmonella* spp. (Weissfeld & Sonnenwirth, 1980). We, therefore, did not attempt direct faecal culture.

There are 23 serogroups based on their 0 (somatic) and H (flagellar) antigens (Wauters, 1981). Cross-reactions with Gram-negative bacteria have been noted (Swaminathan, Harmon & Mehlman, 1982). In Europe and Canada serogroup 0 : 3 is most prevalent followed by 0 : 9 (Toma & Lafleur, 1974; Vandepitte & Wauters, 1979). Recently in a survey in the Netherlands, while 0 : 3 and 0 : 9 were found most frequently in enteric disease, 0 : 8 was equally common in extra-mesenteric infections (Van Noyen *et al.* 1981). In the United States groups 0 : 5 and 0 : 8 are the most common (Weissfeld & Sonnenwirth, 1980). Though none of these serotypes was isolated in this study, 53 % of the patients presented with symptoms of gastroenteritis, and other recognized pathogens were isolated as well in only six.

The majority of the strains in our study either belonged to *Y. enterocolitica* biotype 1, serotype 0 : 6, 30, 0 : 5, 27 or non-typable or *Y. frederiksenii* serotype non-typable (Table 2), which are normally regarded as non-pathogenic types (WHO, 1983). However, 0 : 6, 30 was isolated from the faeces of an immunosuppressed patient with diarrhoea in the United States (Greenwood *et al.* 1975). In the United Kingdom too an unusual outbreak of *Y. enterocolitica* infection has been documented where the offending pathogen was serotype 0 : 6, 30 (CDSC, unpublished).

Some 30 % of isolates were either *Y. frederiksenii* or *Y. intermedia*, neither of which has been recognized as a human pathogen. As such strains are culturally similar or identical to *Y. enterocolitica*, the need for precise identification is emphasized. These strains, which were once considered to be atypical *Y. enterocolitica*, are now classified separately and are biochemically distinct.

In Europe, there is a clear seasonal variation in the occurrence of *Y. enterocolitica* infections, the incidence reaching its peak in late autumn and winter months (Vandepitee, Wauters & Isebaert, 1983) which may be associated with the ability of the organism to survive and grow at lower temperatures. Our survey which was conducted during winter months gave an isolation rate of 6·1 %, which compares with 0·39 % and 10·4 % in two surveys in Japan (Asakowa *et al.* 1979; Kanazawa & Ikemura, 1979), and 1·8 % in Belgium (Vandepitte & Wauters, 1979). Other surveys in Belgium showed isolation rates of 5·9 % from patients and 4 % from controls (Van Noyen *et al.* 1981). In Netherlands the overall recovery rate was 2·9 %, of which 1·6 % was from patients (Hoogkamp-Korstanje, De Koning & Samson, 1986). Other countries have reported the numbers of isolations generally in association with symptoms, and carriage rates cannot be assessed. A variety of isolation techniques were used which makes direct comparisons difficult and probably unrewarding.

The frequent association of *Y. enterocolitica* with *Salmonella* spp. (8·5%) in the stool of patients with enteritis has been noted. In 25–50% of the slaughtered pigs *Y. enterocolitica* serotype 3 and *Salmonella* spp. have been demonstrated (Vandepitte & Wauters, 1979). Consumption of pork meat may lead to intestinal infection by these two enteric pathogens simultaneously. In our study *Y. enterocolitica* was isolated in association with salmonella on three occasions.

Although in previous antibotic susceptibility studies aminoglycosides and co-trimoxazole were shown to be the most active agents, now new-generation cephalosporins like ceftizoxime and ceftriaxone (Scribner, 1982), and in our hands cefotaxime, appear to be equally effective.

As this work was not designed to study pathogenicity we cannot comment in any detail on the significance of the isolation only of *Yersinia* spp. in some symptomatic patients. Neither is it possible to say whether carriage is intermittent or may follow convalescence from symptomatic illness.

The findings on the patients from whom more than one specimen was examined (Table 4), are interesting and important. In only 2 out of 10 circumstances was the same biotype/serotype isolated subsequently from the same patient and on both occasions when 3 samples were taken, all isolates were different. The explanation for this might be that the serotypes come from the environment and replace each other rapidly in their colonization of the gut or that many types are usually present together. Clearly there is a need to pick several colonies for identification in such studies on carriage. Even this creates a problem as far as sampling from the colonies is concerned as they may appear the same irrespective of their fine characteristics. In our study two colonies from each apparently pure culture were chosen for further tests. It is likely that in the presence of infection one strain only dominates. However, in pathogenicity studies these findings should be borne in mind.

In conclusion our study has demonstrated that 6·1% of an unselected population, but particularly children, may be carrying *Yersinia* spp. at any one time. Studies in North America have shown that infections due to *Y. enterocolitica* including gastroenteritis are more common in children (Kohl, Jacobson & Nahmias, 1976; Marks *et al.* 1980). Although the majority of the strains belonging to biotype 1 are considered to be non-pathogenic, the capacity of *Y. enterocolitica* to invade tissue (Leading Article, 1984) and four recently documented cases of septicaemia due to *Y. enterocolitica* biotype 1 (Seto & Lau, 1984) should be noted with concern. Clearly further studies are needed to evaluate the clinical significance of the carriage of *Yersinia* spp. in man.

We are grateful to Leicester Public Health Laboratory for bio/serotyping the strains. We thank Mr J. C. Teli for technical help in the beginning of the study, and Mrs M. M. White for the typescript.

REFERENCES

ASAKOWA, Y., AKAHANE, S., SHIOZAWA, K. & HONMA, T. (1979). Investigations of source and route of *Yersinia enterocolitica* infection. *Contributions to Microbiology and Immunology* **5**, 115–121.

BOTTONE, E. J. (1977). *Yersinia entercolitica:* a panoramic view of a charismatic microorganism. *Critical Reviews of Microbiology* **5**, 211–241.

GREENWOOD, J. R., FLANIGAN, S. M., PICKETT, J. J. & MARTIN, W. J. (1975). Clinical isolation of *Yersinia enterocolitica:* cold temperature enrichment. *Journal of Clinical Microbiology* **2**, 559–560.

HIGHSMITH, A. K., FEELEY, J. C. & MORRIS, G. K. (1977). *Yersinia enterocolitica:* a review of the bacterium and recommended laboratory methodology. *Health Laboratory Sciences* **14**, 253–260.

HOOGKAMP-KORSTANJE, J. A. A., DE KONING, J. & SAMSON, J. P. (1986). Incidence of human infection with *Yersinia enterocolitica* serotypes 03, 08 and 09 and the use of indirect immunofluorescence in diagnosis. *Journal of Infectious Diseases* **153**, 138–141.

KANAZAWA, Y. & IKEMURA, K. (1979). Isolation of *Yersinia enterocoliticia* and *Yersinia pseudotuberculosis* from human specimens and their drug resistence in the Niigata district of Japan. *Contribution to Microbiology Immunology* **5**, 106–114.

KOHL, S., JACOBSON, J. A. & NAHMIAS, A. (1976). *Yersinia enterocolotica* infections in children. *Journal of Pediatrics* **89**, 77–79.

LARSEN, J. H. (1979). The spectrum of clinical manifestations of infections with *Yersinia enterocolitica* and their pathogenesis. *Contribution to Microbiology Immunology* **5**, 257–269.

LEADING ARTICLE (1984). Yersiniosis today. *Lancet* i, 84–85.

MARKS, M. I., PAI, C. H., LAFLEUR, L., LACKMAN, L. & HAMMERBERG, O. (1980). *Yersinia enterocolitica* gastroenteritis: a prospective study of clinical, bacteriologic and epidemiologic features. *Journal of Pediatrics* **96**, 26–31.

SCRIBNER, R. K., MARKS, M. I., WEBER, A. & PAI, C. H. (1982). *Yersinia enterocolitica:* comparative in vitro activities of seven new β-lactam antibiotics. *Antimicrobial Agents and Chemotherapy* **22**, 140–141.

SETO, W. H. & LAU, T. K. (1984). Septicaemia due to *Yersinia enterocolitica* biotype 1 in Hong Kong. *Journal of Infection* **8**, 28–33.

STOKES, E. J. & WATERWORTH, P. M. (1972). *Antibiotic Sensitivity Tests.* Association of Clinical Pathologists Broadsheet No. 55 (revised).

SWAMINATHAN, B., HARMON, M. C. & MEHLMAN, I. J. (1982). A review – *Yersinia enterocolitica. Journal of Applied Bacteriology* **52**, 151–183.

TOMA, S. & LAFLEUR, L. (1974). Survey on the incidence of *Yersinia enterocolitica* infection in Canada. *Applied Microbiology* **28**, 469–473.

VANDEPITTE, J. & WAUTERS, G. (1979). Epidemiological and clinical aspects of human *Yersinia enterocolitica* infections in Belgium. *Contribution to Microbiology Immunology* **5**, 150–158.

VANDEPITTE, J., WAUTERS, G. & ISEBAERT, A. (1973). Epidemiology of *Yersinia enterocolitica* infections in Belgium. *Contribution to Microbiology Immunology* **2**, 111–119.

VAN NOYEN, R., VANDEPITTE, J., WAUTERS, G. & SEEDERSLAGHS, R. (1981). *Yersinia enterocolitica:* its isolation by cold enrichment from patients and healthy subjects. *Journal of Clinical Pathology* **34**, 1052–1056.

WAUTERS, G. (1970). Contribution à l'étude de *Yersinia enterocolitica.* Thèse d'agrégation (Vander, Leuven).

WAUTERS, G. (1981). In *Yertsinia enterocolitica* (ed. E. G. Bottone), pp. 41–53. Boca Raton, Fla.: C.R.C. Press.

WEISSFELD, A. S. & SONNENWIRTH, A. C. (1980). *Yersinia enterocolitica* in adults with gastrointestinal disturbances: need for cold enrichment. *Journal of Clinical Microbiology* **11**, 196–197.

WIMBLAD, S. (1973). The clinical panorama of human *Yersinia enterocolitica. Contribution to Microbiology Immunology* **2**, 129–132.

WORKING GROUP ON YERSINIOSIS. (1983). Yersiniosis Euro. Rep. Studies 60. Copenhagen: World Health Organization.