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Human carriage of *Yersinia* spp.

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Summary. Examination of faecal specimens for the presence of *Yersinia* spp. during a 1-year period yielded isolations from 3·5% of patients. *Salmonella* spp., *Campylobacter* spp. and *Shigella* spp. were isolated from 2·5%, 2·8% and 0·34% of patients respectively. Most isolates belonged to *Y. enterocolitica* biotype 1 (53%) and *Y. frederikseni* (39%). The most frequently encountered serotypable strains were serotypes O 5,27 and O 6,30. Serotype O 3, the commonly recognised pathogenic strain, was not isolated in this survey. A strong association between *Yersinia* excretion and the age group 1–14 years was demonstrated. Although biotype-1 strains and *Y. frederikseni* have not previously been thought to be pathogenic, clinical significance could be attributed to the presence of *Yersinia* spp. in almost 90% of patients aged 15 years or more, and in over 50% of patients in the younger age groups.

Introduction

During the last 20 years, *Yersinia enterocolitica* has become accepted as a causative agent of bacterial gastro-enteritis, terminal ileitis and mesenteric adenitis (Bottone, 1977; Lancet, 1984). Infection may also lead to several auto-immune conditions, in particular erythema nodosum and polyarthritis (Aho *et al.*, 1981; Winblad, 1981). The organisms may further be responsible for a wide variety of other clinical manifestations (Larson, 1979). Sporadic infection has been reported in many countries (Swaminathan *et al.*, 1982) and several outbreaks of infection due to *Y. enterocolitica* have also been recorded (Asakawa *et al.*, 1973; Black *et al.*, 1978; Ratnam *et al.*, 1982; Shayegani *et al.*, 1983; Tacket *et al.*, 1984). Despite the increasing number of reports of *Y. enterocolitica* isolations elsewhere, there is little published information concerning the incidence of this organism in Great Britain. Between 1980 and 1982, only 213 reports of patients with *Y. enterocolitica* infection were received by the Communicable Diseases Surveillance Centre (Public Health Laboratory Service, personal communication) of which 122 isolates were of faecal origin. Almost all of the strains which had been serotyped belonged to serotype O 3, which is the common pathogenic strain in this country.

To determine carriage rates in the local community, faecal samples submitted to the laboratory during a 1-year period were examined for the presence of *Yersinia* spp. The results obtained are presented.

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Materials and methods

Faecal specimens and rectal swabs submitted to the laboratory for conventional bacteriological examination from hospital and general practice patients were also cultured for the presence of *Y. enterocolitica* and related organisms. Specimens were applied directly to the surface of Cefsulodin-Irgasan-Novobiocin (CIN) agar plates (Yersinia Selective Agar, Oxoid CM653, with Oxoid antibiotic supplement SR109). Specimens were also inoculated into 10 ml of buffered peptone water (BPW; Oxoid CM509) containing peptone 1%; this was incubated at 4°C for 17–21 days before subculture to CIN agar. All CIN agar plates were incubated at 30°C for 20–24 h. Suspect colonies were identified by methods described elsewhere (Greenwood and Hooper, 1985). Briefly, isolates were screened with Triple Sugar Iron (TSI) agar (Oxoid) and urea broth. Those organisms that produced an acid butt in TSI without gas or H_2S production and were urease-positive were further tested for absence of motility at 37°C. Presumptive strains of *Yersinia* were sent to the reference facility at Leicester Public Health Laboratory for serotyping and biotyping according to the methods of Wauters (1970). Serum samples obtained from patients excreting *Yersinia* organisms were also sent to Leicester for antibody detection.

Results

During the 1-year period, 4585 faecal specimens or rectal swabs from 3784 patients were examined. A total of 135 strains of *Yersinia* spp. was isolated from 133 patients, representing a patient isolation rate of 3·5%. Corresponding isolation rates for

Salmonella spp., *Campylobacter* spp. and *Shigella* spp. were 2·5%, 2·8% and 0·34% respectively. Only five strains of *Yersinia* were isolated by direct culture; all other strains were obtained after cold enrichment. Species, biotypes and serotypes of the *Yersinia* strains are shown in tables I and II. Most isolates belonged to *Y. enterocolitica* biotype 1 or *Y. frederikseni*. Serotypes O 5,27 and O 6,30 occurred most frequently. Serotype O 3, the most commonly recognised pathogenic strain, was not isolated.

Table I. Species and biotypes of *Yersinia* isolated from human faeces

Species	Number of isolates
Y. enterocolitica biotype 1	72
Y. enterocolitica biotype 3	5
Y. frederikseni	51
Y. intermedia	4
Y. pseudotuberculosis	1
Not typed	2

Table II. Serotypes of *Yersinia* spp. isolated from human faeces

Species (biotype)	O serotype	Number of isolates
Y. enterocolitica (1)	5,27	23
Y. enterocolitica (3)	5,27	1
Y. enterocolitica (1)	6,30	13
Y. enterocolitica (1)	7	6
Y. enterocolitica (1)	15	2
Y. enterocolitica (1)	34	1
Y. enterocolitica (1)	NT	26
Y. enterocolitica (3)	NT	4
Y. frederikseni	16	9
Y. frederikseni	NT	43
Y. intermedia	NT	4
Y. pseudotuberculosis	IIa	1
Not typed	—	2

NT = not typable.

Analysis of patients by age and sex is shown in table III. Excretion was strongly associated with age ($x^2 = 42·8$; $p < 0·001$). Isolations in the 1–4 and 5–14 age groups were twice those expected. Although *Yersinia* excretion occurred more often in females aged 1–24 years, there was no overall association with sex ($x^2 = 6·32$; $p > 0·5$). Analysis of the occurrence of gastro-intestinal symptoms in relation to age is shown in table IV. Of patients from whom no other pathogen was isolated, 65% experienced symptoms of diarrhoea, vomiting, loose stools or abdominal pain. Most other isolates were made on routine testing of faeces from children admitted to hospital for other reasons (31), nursing staff (3) or people returning from foreign travel. Blood samples were obtained from 10 patients 3–10 weeks after the faecal samples were submitted, but no serum antibody response to *Yersinia* spp. could be demonstrated.

Table III. Analysis of patients excreting *Yersinia* spp. by age and sex

Age (years)	Number of patients				Percentage of total patients
	male	female	not stated	total	
<1	5	2	1	8	6·0
1–4	15	27	—	42	31·6
5–14	7	10	—	17	12·8
15–24	7	12	—	19	14·3
25–34	6	3	—	9	6·8
35–44	5	2	—	7	5·3
45–54	3	3	—	6	4·5
55–64	6	1	—	7	5·3
>65	5	6	—	11	8·3
Not stated	4	3	—	7	5·3
Total	63	69	1	133	100

Table IV. Occurrence of gastro-intestinal symptoms in relation to age

Age	Symptoms*		Not known	Other pathogens
	+(%)	−(%)		
<1	4 (50)	4 (50)	0	0
1–4	23 (59)	16 (31)	2	3
5–14	6 (46)	7 (54)	1	1
>15	52 (88)	7 (12)	0	6
Not known	1	0	0	0
Total	85 (71)	34 (29)	3	10

* Symptoms: + = diarrhoea, vomiting, loose stools or abdominal pain; − = no gastro-intestinal symptoms.

Discussion

The incidence of yersinia infection rivals that of salmonella infection in some surveys (Marks *et al.*, 1980; Weissfeld and Sonnenwirth, 1980), but appears to be low in other reports (Dajani and Maurer, 1980; Yamauchi *et al.*, 1981). The excretion of *Yersinia* revealed in this survey exceeds that of *Salmonella*, *Campylobacter* and *Shigella* spp. Before the introduction of CIN agar the isolation of *Y.*

enterocolitica from faecal material relied heavily on the use of media designed for the detection of other enteric pathogens. The differences in isolation rate obtained by other workers are probably due to differing isolation techniques rather than to different geographical location.

Y. enterocolitica biotype 1 and *Y. frederikseni* were the predominant groups of *Yersinia* spp. isolated. The isolation of *Y. frederikseni* from clinical sources has not been reported before in this country (Public Health Laboratory Service, personal communication), possibly as a result of the use of unsuitable isolation media, but perhaps also because this organism has only recently been identified amongst the so-called "*Y. enterocolitica*-like" organisms (Bercovier *et al.*, 1980; Brenner *et al.*, 1980, Ursing *et al.*, 1980). This investigation has shown that serotype O 3 is not the most common type of *Yersinia* spp. to be found in human faecal specimens in the UK. Serotype O 3 was not isolated during this 1-year study, although subsequent studies in which the methodology described here was used have resulted in several isolations of this serotype.

The pathogenicity of serotypes O 3, O 9 and O 8 is well established, but opinion varies concerning the pathogenicity of other serotypes and of strains such as *Y. frederikseni*. Van Noyen *et al.* (1981) found that strains of *Yersinia* other than serotypes O 3 and O 9 were recovered only by use of cold enrichment techniques. These workers did not consider such strains to be significant as they were twice as prevalent in controls as in patients. Weissfeld and Sonnenwirth (1980) found only biotype-1 strains in a survey of patients in hospital, and felt that all their isolates were of clinical significance. They found an incidence rate of *Y. enterocolitica* equal to that of *Salmonella* and emphasised the need for cold enrichment. Martin *et al.* (1982) also stressed the need for cold enrichment methods, and showed that a biotype-1 strain of serotype 21 was pathogenic. Pai *et al.* (1979) found that although recovery of serotype O 3 from symptomatic patients was usually obtained by direct culture, recovery of other serotypes was very poor without cold enrichment. These workers also found that recovery of serotype O 3 from the stools of asymptomatic carriers and convalescent patients was considerably enhanced by the use of cold enrichment. Results obtained in our study confirm the need for cold enrichment; less than 5% of isolations were made by direct culture.

Results shown in tables III and IV indicate that yersinia carriage is strongly associated with the 1–14 year age group. However, this group also contains the largest proportion of apparently symptomless excreters. It has been shown (Greenwood and Hooper, 1985) that pasteurised milk often contains *Yersinia* organisms. The greatest consumption of milk occurs in this age group and may account partly for this frequency of isolation. If results obtained from patients less than 15 years of age are excluded, it can be seen that almost 90% of excreters exhibit gastro-intestinal symptoms. It seems unlikely that the finding of *Yersinia* serotypes other than those of the recognised pathogenic strains in such a large proportion of symptomatic patients is merely fortuitous. In a review of *Y. frederikseni* diarrhoea (Scholey and Freeman, 1984), the authors speculated that the organism might be a cause of antibiotic-associated diarrhoea. Other clinical manifestations of yersiniosis may also include pharyngitis (Tacket *et al.*, 1983) fever, arthritis and erythema nodosum (Bottone, 1977) which may occur in the absence of, or following, gastro-intestinal symptoms. The availability of clinical information in this survey was limited to the details given on the request forms, and follow-up of positive cases was hampered by the time lapse involved in cold enrichment methods.

The regimen combining enrichment in BPW incubated at 4°C for 17–21 days with subculture to CIN agar has allowed a more complete picture of the incidence of *Yersinia* spp. in man to emerge. Studies are in progress in an effort to reduce the length of time required to isolate *Yersinia* spp. from enrichment media. This will allow better follow-up of clinical cases and help to clarify the role of all strains of *Yersinia* in enteric disease.

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