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Two community outbreaks of human infection with *Yersinia enterocolitica*

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SUMMARY

Two outbreaks of human infection with *Yersinia enterocolitica* in Shizuoka, Japan are described. This is the first report of community outbreaks of infection with this organism in Japan, and possibly in the world. All the strains isolated in each outbreak belonged to O antigen group 3, biotype 4, of the species. Despite much effort, the source and mode of spread of the infection were not established.

INTRODUCTION

Although sporadic cases of human infection with *Yersinia enterocolitica* have been reported in western countries (Niléhn, Sjöström, Damgaard & Kindmark, 1968; Winblad, Niléhn & Sternby, 1966) and recently in Japan (Zen-Yoji & Maruyama, 1972), community outbreaks of infection have not previously been described.

Epidemics of *Y. enterocolitica* infection occurred among children in two separate communities in the Shizuoka Prefecture, Japan. These outbreaks were investigated by a team from the Shizuoka Department of Public Health and Public Health Laboratory; the organisms isolated were studied at the National Institute of Health, Tokyo. The results of these studies are presented in this paper.

MATERIAL AND METHODS

Stool specimens

Specimens were collected not only from patients but also from unaffected individuals in the same community or families as the patients. Additional stool specimens were obtained from staff working in a food catering institution which provided lunches for the community, as well as from individuals in other communities also supplied with lunch by the same caterer.

Stools were inoculated on Shigella-Salmonella (SS), MacConkey, thiosulphate citrate bile-salt sucrose (TCBS) and blood agar plates, and into selenite F and Rappaport broths. After incubation overnight, the selenite and Rappaport broths were subcultured on SS agar. In addition, enrichment culture in phosphate buffer

solution at pH 7·6 (Paterson & Cook, 1963) for 10 days or more at 8° C. was also used for most of the specimens.

At first, all the plates were incubated overnight at 37° C., but later, with the exception of blood and TCBS agar plates, they were all incubated for 48 hours at 30° C. and examined for the usual enteropathogens. Blood agar was incubated anaerobically at 37° C. for *Clostridium perfringens*.

Blood

Samples were collected from patients in the acute stage of illness and again after 14–20 days. Blood samples were also obtained from persons who were not ill. The sera were examined by an agglutinin titration procedure similar to that used with Widal tests.

Water

Samples of water used by the communities were investigated for total bacterial count, enumeration of coliform organisms and culture for enteropathogens several times during the period of the epidemic. For pathogens, 10 l. samples of water were filtered through Milipore membranes (0·45 μm) and the filters then placed in nutrient broth. After incubation for 48 hr. at 30° C., the broth was subcultured on SS and MacConkey agar.

Animals

Stool or cloacal swabs from various pet animals in the community, as well as autopsy material from rats caught in the vicinity were investigated for the presence of *Y. enterocolitica*.

Identification and characterization of the strains isolated were carried out with accepted media and methods used in enteric bacteriology (Edwards & Ewing, 1962).

RESULTS

The outbreaks

Outbreak A

Between 31 January and 26 February 1972, 182 children and one teacher among 390 persons at a primary school (A) and 6 of 51 children in the kindergarten associated with this school suffered from an illness resembling bacterial food poisoning. The majority of the 189 cases occurred during the first 10 days of the outbreak, but some continued to occur sporadically until 26 February (Table 1). With one possible exception, no secondary cases occurred in the families of the children affected. The exception was a junior high school boy whose brother was involved in the outbreak; they both suffered similar clinical symptoms at the same time.

The symptoms included abdominal pain (85·7%), fever (75·5%), diarrhoea (60·1%), nausea (23·5%), and vomiting (4·0%) as shown in Table 2. The body temperature ranged from 38° C. to 39·5° C. in most of the cases. Some patients, misdiagnosed as appendicitis, were treated by surgical operation. About half the cases with diarrhoea had only a few loose stools. There were no deaths and most of the patients recovered within one or two days.

Table 1. *Daily incidence of cases in two outbreaks of* Y. enterocolitica *infection*

Outbreak A		Outbreak B	
Date	Number of cases	Date	Number of cases
Jan. 31	4	July 11	79
Feb. 1	21	12	254
2	44	13	134
3	34	14	55
4	29	15	9
5	12	16	5
6	6	17	1
7	5	18	3
8	3	19	0
9	12	20	3
10	4	21	0
11	0	22	1
12	2		
13	0		
14	3		
15	1		
16	2		
17	1		
18	0		
19	0		
20	2		
21	1		
22	2		
23	0		
24	0		
25	0		
26	1		
Total no. of cases	189		544

Outbreak B

Between 11 and 22 July 1972, another explosive outbreak occurred among children in a primary school in another district, 15 km. from school A involved in the first epidemic. Of 993 children and 49 adults at risk, 544 (52·6%) pupils were ill. The majority of cases occurred within the first 4 days, but sporadic cases continued to occur until 22 July (Table 1). No secondary cases occurred in any family contacts.

The main clinical symptoms were abdominal pain (63·9%), fever (49·6%), diarrhoea (32·4%), nausea (24·8%) and vomiting (10·6%). A temperature of 39° C. or higher occurred in 30% of 270 patients with fever. In the majority of cases with diarrhoea, watery stools were observed. Most patients recovered within 12–18 hr.; there were no deaths (Table 2).

Table 2. *Clinical features*

Main symptoms	Number of cases	
	Outbreak A*	Outbreak B†
Abdominal pain	84 (85·7 %)	348 (63·9 %)
Fever	74 (75·5 %)	270 (49·6 %)
Diarrhoea	59 (60·1 %)	176 (32·4 %)
Nausea	23 (23·5 %)	135 (24·8 %)
Vomiting	4 (4·0 %)	58 (10·6 %)

* Interview on 98 cases.
† Interview on 544 cases.

Table 3. *Isolation of* Yersinia enterocolitica *from stool specimens in outbreak A*

Source	Number of specimens tested	Number of specimens positive for *Y. enterocolitica*
School A		
Acute cases	113	48 (42·4 %)
Convalescent cases	175	36 (20·5 %)
Symptomless cases	234	9 (3·8 %)
Kindergarten	104	1*
Food catering institution	10	0
Family contacts of cases	122	0

* Symptomless case.

Bacteriology

Stool specimens

Outbreak A. When outbreak A was first investigated, no causative agent was isolated from stools because *Y. enterocolitica* was not considered and most plates were discarded after overnight incubation at 37° C. However, on examination of further stool cultures, *Shigella*-like colonies, later identified as *Y. enterocolitica*, were observed on SS agar plates kept for 24 hr. at room temperature after overnight incubation at 37° C. Accordingly, some of the earlier specimens, which had been kept at −20° C., were re-examined for this organism, and incubation for 48 hr. at 30° C. was adopted for subsequent bacteriological investigations.

In outbreak A, a total 113 stool specimens from acute cases were available for specific investigation and, as shown in Table 3, *Y. enterocolitica* was found in 42·4 % of these specimens. Other pathogens, including *Salmonella*, *Shigella*, enteropathogenic *Escherichia coli*, and *Clostridium perfringens*, were not found. On 11 March, 40 days after the outbreak started, stools were obtained from 175 persons who had been ill, and the same organism was isolated from 20·5 % of the specimens.

Of 234 specimens from symptomless persons in school A, 3·8 % were positive for the organism. In contrast, it was isolated from only one of 114 specimens from persons in institutions other than school A, including the catering establishment. The single positive specimen was from a symptomless child who had no connexion

Table 4. *Isolation of* Yersinia enterocolitica *from stool specimens in outbreak B*

Source	Number of specimens tested	Number of specimens positive for *Y. enterocolitica*
Acute cases	117	88 (75·1 %)
Convalescent cases	544	5 (0·9 %)
Food catering staff	5	0
Family contacts of cases	212	0

with school A. The organism was not isolated from 122 healthy individuals from the families of the cases.

Outbreak B. In outbreak B, a total of 117 stool specimens from cases were available on 15 July, and the same organism as that in outbreak A was isolated from 75 % of them (Table 4). On 5 September, 56 days after the first cases occurred, 544 specimens from convalescent patients were obtained and *Y. enterocolitica* was isolated from 0·9 % of them. It was not isolated from any specimens from the families of cases.

Isolation and identification. On SS agar, the organisms isolated in both outbreaks formed smooth colourless colonies resembling those of *Shigella* after 48 hr. incubation at 30° C.; they were only minute in size after overnight incubation at 37° C. Although the organism grew on ordinary and less inhibitory media, SS agar was the most convenient for isolation because it strongly inhibited other faecal organisms. Although selenite and Rappaport broths were both recommended for enrichment culture of *Y. enterocolitica* by Niléhn & Sjöström (1967*a*) and van Noyen & Vandepitte (1968), they were not as good as direct culture of stool specimens on SS agar in the present investigation. However, inoculation of the stools in phosphate buffer solution (pH 7·6) which was then kept for 10 days or more at 8° C. before subculture to SS agar, yielded more isolations than direct culture on SS agar.

The organisms isolated were Gram-negative, facultatively anaerobic, fermentative rods. All the strains were similar in morphology and gave the same cultural, biochemical, and serological results (Table 5) which confirmed their identity as *Y. enterocolitica* of O group 3 and biotype 4.

Water samples

Well-water, pumped up into large tanks and then chlorinated, was used in school A. Samples were tested repeatedly during the outbreak, but the concentration of residual chlorine in the water was satisfactory and no coliform organisms were detected. Indeed, only occasional viable organisms per ml. were found in the water samples examined.

In school B, chlorinated water from the city mains was supplied. This was also used in a swimming pool in the school grounds. The tap water and swimming pool water were both investigated early in the outbreak, but chlorine levels were again satisfactory and no coliform organisms or other Gram-negative rods were detected.

Table 5. *Characteristics of strains isolated*

Test	Reaction	Test	Reaction
Gram stain	−	Acid only from:	
Oxidase	−	Arabinose	+
Motility, 37° C	−	Celliobiose	+
Motility, 25° C	+	Lactose	−
Nitrate reduction	+	Maltose	+
Indole	−	Mannose	+
Voges–Proskauer, 37° C	−	Melezitose	−
Voges–Proskauer, 25° C	+	Melibiose	−
Ammonium glucose	+	Raffinose	−
Ammonium citrate	−	Rhamnose	−
Ammonium acetate	+	Sorbose	+
Malonate utilization	−	Sucrose	+
Urease	+	Trehalose	+
Hydrogen sulphide (TSI)	−	Xylose	+
Lysine decarboxylase	−	Adonitol	−
Arginine dihydrolase	−	Dulcitol	−
Ornithine decarboxylase	+	Erythritol	−
Phenylalanine deaminase	−	Glycerol	+
Gelatinase	−	Mannitol	+
Haemolysis	−	Sorbitol	+
Lipase, corn-oil	−	Salicin	−
Kauffmann–Petersen, Citrate	−	Aesculin	−
Kauffmann–Petersen, D-Tartrate	−	Inositol	+
Kauffmann–Petersen, Mucate	−	α-Methylglucoside	+
O–F medium, glucose (sealed)	+	β-Methylglucoside	+
Gas from glucose	−	β-Galactosidase	+

Animals

In outbreak A, faecal specimens from pet animals in the school including three chickens, four pheasants, three pigeons and one rabbit, as well as autopsy material from 10 rats caught near the school were examined for *Y. enterocolitica* with negative results.

Serological survey

Sera from patients and from unaffected persons associated with both outbreaks were examined. Agglutinin titres ranged from 1/160 to 1/1280 after 3 weeks in the majority of convalescent cases, compared with 1/20 or less in the acute stage of illness. Agglutinin titres rarely exceeded 1/20 in sera from persons who were not ill.

DISCUSSION

As it was isolated from the faeces from most of the cases and since no other enteropathogenic organisms were recognized, *Y. enterocolitica* was almost certainly the causative organism in the two outbreaks described. Significantly raised agglutinin titres against this organism in convalescent sera further confirmed this view. Although sporadic cases of human infection with *Y. enterocolitica* have been reported (Carlsson, Ryd & Sternby, 1964; Winblad, Niléhn & Sternby, 1966; Winblad, Niléhn & Jonsson, 1966; Niléhn & Sjöström, 1967*a*, *b*; Winblad, 1968; Niléhn *et al.* 1968; Ahvonen, Sievers & Aho, 1969; Braunstein, Tucker & Gibson, 1971), community outbreaks have not previously been described.

The isolation of *Y. enterocolitica* from numerous animal sources including hares, chinchillas, pigs, dogs, cattle and the bush-baby (Dickinson & Mocquot, 1961; Becht, 1962; Akkermans & Terpstra, 1963; Daniëls & Goudzwaard, 1963; Daniëls, 1963; Knapp & Thal, 1963; Struve, 1963; Mollaret, Chevalier & Deplanche, 1964; Mollaret & Lucas, 1965; Siegmann, 1965; Mair, White, Schubert & Harbourne, 1970) suggests that human infection with *Y. enterocolitica* may be food-borne. Rabson & Koornhof (1972), Esseveld & Goudzwaard (1972) and Rakovský, Pauckova & Aldova (1972) considered that pigs were the main source of human infection. On the other hand, Szita, Káli & Rédey (1972) thought that spread of infection from man to man was more likely because there was no indication of transmission from animal sources in their study. It is difficult, however, to reconcile this view with their statement that an oral dose of $3{\cdot}5 \times 10^9$ organisms was needed to cause illness in human volunteers, as it seems unlikely that such large numbers of *Y. enterocolitica* would be transmitted in this way.

In the present incidents, cases continued to occur for about one month in outbreak A and for 11 days in outbreak B. This picture is more like that seen in outbreaks of *Shigella* infection than of food poisoning, and does suggest that infection from man to man can occur. However, all cases in the present outbreaks were restricted to pupils and one teacher, and spread of infection to family contacts was not observed. Although no conclusions can be drawn, these findings suggest that the outbreaks described were probably food- or water-borne.

More recently, in a further outbreak of *Y. enterocolitica* infection, 198 cases occurred in a junior high school in a different locality 200 km. from Shizuoka (Zen-Yoji *et al.* 1973). In addition, an outbreak of infection at a primary school 400 km. from Shizuoka was thought to be due to *Y. enterocolitica* (Sakazaki *et al.* unpublished). In this instance, high agglutinin titres to O antigen 3 were demonstrated in convalescent sera taken 2 months after the outbreak, although bacteriological examination for *Y. enterocolitica* was not performed during this outbreak. In both these incidents, the source and mode of infection were again unknown.

The outbreaks described in the present paper were first thought to be food-poisoning and *Y. enterocolitica* was not even considered. It is thus possible that other outbreaks of 'food-poisoning' of unknown aetiology may be caused by this organism. More attention should therefore be paid to *Y. enterocolitica* infection, not only in sporadic cases but also in community outbreaks of infection.

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