Excretion of *Yersinia* spp. associated with consumption of pasteurized milk

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SUMMARY

Yersinia enterocolitica biotype 1, serotype O.10K was isolated from 19 patients in the paediatric wards of a district general hospital over a period of 3 months. Fifteen cases were patients on the medical ward. Shortly afterwards, *Y. enterocolitica* biotype 1, serotype O.6,30 was isolated from a further 17 patients on this ward in 1 month. The same serotypes of *Y. enterocolitica* were isolated from the pasteurized milk supplied to the ward. Epidemiological evidence indicated that contaminated pasteurized milk was the source of the yersinia organisms excreted by the patients.

INTRODUCTION

A number of outbreaks of infection due to the consumption of food contaminated with *Yersinia enterocolitica* have now been reported in other countries. In particular, an outbreak associated with the consumption of contaminated pasteurized chocolate milk occurred in the USA in 1976 [1], and a large multi-state outbreak of infection in the USA in 1982 was traced to pasteurized milk [2].

Infection caused by *Y. enterocolitica* does not appear to be common in the UK, although the Communicable Disease Reports of the Public Health Laboratory Service indicate that the isolation of *Y. enterocolitica* has increased over recent years. In this report two episodes of yersinia infection are described which occurred on a single ward of a district general hospital, and which were traced to the consumption of pasteurized milk.

METHODS

Stool samples from all patients admitted to the paediatric wards of the hospital were routinely submitted to the laboratory for pathogen screening. In addition specimens were submitted if a patient exhibited gastrointestinal symptoms or if a patient was in contact with another shown to be excreting an intestinal pathogen. All stool samples were examined for the presence of salmonella, shigella, campylobacter, rotavirus, and enteropathogenic *Escherichia coli* using standard techniques. Samples were examined for the presence of *Yersinia* spp. by cold enrichment at 4 °C in buffered peptone water pH 7.2 (BPW; Oxoid), followed by subculture after 2.5-3 weeks to Cefsulodin-Irgasan-Novobiocin agar (CIN;

Fig. 1. Isolation of *Y. enterocolitica* O.10K and *Y. frederiksenii* NT from patients on the paediatric wards by date of specimen. ○, Patient excreting *Y. enterocolitica* O.10K; ●, patient excreting *Y. frederiksenii* NT; ◐, patient excreting *Y. enterocolitica* O.10K and *Y. frederiksenii* NT.

Gibco) [3]. Samples were also enriched in tris-buffered peptone water pH 8.0 (TPW) [4], incubated at 14 °C and subcultured to CIN agar after 1–3 and 4–6 days as part of a comparative survey. Suspect colonies were examined for their reaction in triple sugar iron agar (TSI; Oxoid), presence of urease activity, and absence of motility at 37 °C [5]. Presumptive strains of yersinia were sent to the reference facility at the Public Health laboratory, Leicester, for confirmation, biotyping according to Bercovier and Mollaret [6] and serotyping by the methods of Wauters [7, 8].

Samples of milk were examined on 28 January, 29 April, and 3 June for the presence of yersinia by enriching 25 ml aliquots in 225 ml BPW incubated at 4 °C and TPW incubated at 14 °C, with weekly subculture to CIN agar for up to 3 weeks.

RESULTS

On 15 January 1985, a one-year old female was admitted to hospital suffering from diarrhoea, thought to be caused by lactose intolerance. Faecal samples were submitted to the laboratory for analysis on 16 and 18 January. a urease-negative strain of *Yersinia enterocolitica*, biotype 1, serotype O.10K was isolated from both specimens. No other recognized pathogen was isolated. This strain of *Y. enterocolitica* was subsequently isolated from seven more children during January.

Because pasteurized milk had frequently been found to be contaminated with *Yersinia* spp. a sample of milk supplied to the ward was examined for the presence of yersinia on 28 January. *Y. frederiksenii* (non-typable) was isolated.

Between 1 February and 1 April isolations of *Y. enterocolitica* O.10K were made from a further 11 children. Of the 19 paediatric patients with *Y. enterocolitica* O.10K, 15 were from the medical ward for children over the age of one year. Five of these patients were also excreting a serologically non-typable strain of *Y. frederiksenii*, and between 16 January and 1 April four additional patients were found to be excreting *Y. frederiksenii* only. During this time other yersinia strains were isolated from three patients. The incidence of excretion of *Y. enterocolitica* O.10K and *Y. frederiksenii* by date of specimen is shown in Fig. 1.

Isolation of *Y. enterocolitica* O.10K from patients ceased after 1 April, and there was one further isolation of *Y. frederiksenii* from a patient on this ward on 18 April. Isolation of *Yersinia* spp. from patients on this ward began again on 25 April, and in the next 30 days 15 patients were found to be excreting *Y. enterocolitica* biotype 1, serotype O.6,30, 7 patients excreting non-typable *Y. frederiksenii*, and 2 patients excreting both strains. Specimen dates are shown in

Fig. 2. Isolation of *Y. enterocolitica* O.6,30 and *Y. frederiksenii* NT from patients on the medical paediatric wards by date of faecal specimen. ○, Patient excreting *Y. enterocolitica* O.6,30; ●, patient excreting *Y. frederiksenii* NT; ◐, patient excreting *Y. enterocolitica* O.6,30 and *Y. frederiksenii* NT.

Fig. 3. Cases of yersinia infection on the paediatric medical ward from August 1984 until December 1985.

Fig. 2. No other strains of yersinia were isolated from patients on the ward during this time.

Pasteurized milk obtained from the ward on 29 April was found to contain *Y. enterocolitica* O.6,30 and *Y. frederiksenii* O.16. A further sample of milk tested on 3 June was found to contain *Y. enterocolitica* O.10K and serologically non-typable *Y. frederiksenii*.

Table 1. *Gastrointestinal symptoms associated with excretion of Yersinia spp.*

Symptoms	<i>Y. ent.</i> 10K	<i>Y. fred.</i>	<i>Y. ent.</i> 6.30
Diarrhoea	3	—	1
Vomiting	3	—	—
Colic, abdominal pain	1	2	—
Constipation	3	1	—
Loose/offensive stools*	2	—	2
Semi-formed stools†	14	10	6
Other pathogen	1	2	1
Total no. of cases	19	16	17

* Loose/offensive stools – clinical observation.

† Semi-formed stools – laboratory observation.

Following this episode, isolations of yersinia were made from only six patients on this ward in the subsequent 3 months, and remained at a low level throughout the rest of the year. The monthly number of ward patients excreting *Yersinia* spp. from August 1984 until December 1985 is shown in Fig. 3.

The isolation rates from patient samples examined from the ward in the first episode were 10% for *Y. enterocolitica* O.10K and 5% for *Y. frederiksenii*. In the second episode the rates were 25% for *Y. enterocolitica* O.6,30 and 13% for *Y. frederiksenii*. Gastrointestinal symptoms associated with *Yersinia* excretion are shown in Table 1. *Y. enterocolitica* was not isolated from repeat faecal specimens from the index case 6 days after the first isolation, or from two patients 7 and 17 days after primary isolation. It was however isolated again from one patient 7 days after initial isolation.

DISCUSSION

In August 1984, the contract for supplying the hospital with pasteurized milk and other dairy products was awarded to a different dairy. Milk products were transported from this dairy in an insulated van and refrigerated on arrival at the hospital. Most wards were supplied with three-gallon cartons of pasteurized, homogenized milk, but due to complaints about the taste of cartoned milk, the paediatric wards were supplied with one-pint bottles of pasteurized milk. There are three paediatric wards at this hospital. The ward in question is a medical ward which takes children aged 1–15 years. The second ward takes all children under the age of one year, most of whom drink formula milk. The third ward takes surgical cases, whose diet is often restricted on admission due to the imminence of surgery. It is the policy of the hospital that faecal samples are examined from all paediatric cases as soon after admission as possible.

The index case of *Y. enterocolitica* O.10K infection was admitted to hospital on 15 January. This organism was isolated from faecal specimens submitted on 16 and 18 January, and was unusual in its inability to hydrolyse urea (urease activity is a property usually used to identify yersinia organisms). This strain had not been encountered in the 3 years that the laboratory had been seeking the presence of *Yersinia* spp. In the subsequent fortnight six more cases were identified, and by 1 April this organism had been isolated from a total of 19 patients, mostly from the medical ward.

Person-to-person transmission was at first considered to be a strong possibility, as there was a coincidental outbreak of *Shigella sonnei*/campylobacter infection in the ward which had commenced with the admission of a patient on 18 January. Because of this outbreak, strict precautions to prevent cross-infection were instigated, which resulted in cessation of further cases within 10 days. Although the index case could have transmitted *Y. enterocolitica* O.10K to other children on the ward, during this period three isolates of O.10K were made from children on other wards with whom there was no physical contact. In addition the lengthy period of time over which isolations of O.10K were made and the 3-week interval between isolations in March indicated that another cause was more probable.

The isolation of such a rare strain of *Yersinia* spp. from so many patients on the same ward suggested a common source. Although *Y. enterocolitica* O.10K was not isolated from the pasteurized milk supplied to the ward at this time, non-typable *Y. frederiksenii* was obtained. This strain was also isolated from a total of nine patients on the ward, five of whom were also excreting *Y. enterocolitica* O.10K. The practice of selecting a single colony of each morphological type of suspect colony on the isolation medium may have prevented detection of multiple strains in this sample of milk.

A sample of milk examined in early June yielded a growth of both *Y. enterocolitica* O.10K and non-typable *Y. frederiksenii*. The milk sample examined during the second episode of *Yersinia* infection was shown to contain *Y. enterocolitica* O.6,30, the same strain as that isolated from the patients in the ward, together with *Y. frederiksenii* O.16. Investigations were instigated at the dairy supplying the hospital with pasteurized milk, and several strains of yersinia, including O.10K, O.6,30 and *Y. frederiksenii* were subsequently isolated from the pasteurized milk [9]. The absence of yersinia excretion by patients in the remainder of the hospital and the different type of milk supplied to the paediatric wards help to support the theory that contaminated bottled pasteurized milk was the source of yersinia infection.

In a 1-year survey of presence of *Yersinia* spp. in faecal samples [3], it was found that although almost 90% of adults excreting yersinia were suffering from gastrointestinal symptoms, this proportion was considerably lower in the under 15-year age group. Available clinical information in the episodes described here was unfortunately mainly limited to details recorded on the laboratory request forms. The clinical significance of strains belonging to *Y. enterocolitica* biotype 1 and *Y. frederiksenii* is unclear, but it is thought that these strains might be capable of causing a self-limiting enteritis [10]. These so-called 'environmental' strains are able to cross the intestinal barrier, reach the mesenteric lymph nodes and elicit a weak humoral response, which is independent of the presence of virulence plasmids [11]. Results shown in Table 1 indicate that several of the children were suffering gastrointestinal symptoms in the absence of any other known pathogen.

Previous results obtained in the laboratory have shown that contamination of pasteurized milk with yersinia organisms is not uncommon [5]. The frequency with which pasteurized milk is found to be contaminated with *Yersinia* spp., the unusual ability of these organisms to grow at refrigeration temperatures, and the possibility that hospitalized children may be more susceptible to infection than their healthy counterparts suggest that screening of hospital milk supplies for the presence of *Y. enterocolitica* and related species is a worthwhile procedure.

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