

An outbreak of food poisoning in Tamil Nadu associated with *Yersinia enterocolitica*

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An outbreak of food poisoning in a Tamil Nadu village, affecting 25 of 48 individuals who participated in a feast, was investigated. The risk of developing illness was associated with consumption of buttermilk (relative risk 3.8). None of the food items consumed during the feast was available for analysis. Toxin-producing *Y. enterocolitica* (serotype 3, biotype 4) was grown from 1 of 11 stool samples from affected individuals, as well as from a water sample from the source used to dilute the buttermilk. High titres of antibody of *Yersinia* were detected in 2 of 12 patients but in neither of the two groups of controls. Toxin production was noted in buttermilk incubated for 6 h with *Y. enterocolitica*. This is the first report from India of a food poisoning outbreak associated with this organism.

Key words Food poisoning - India - outbreak - *Yersinia enterocolitica*

Food hygiene and safety receive scant attention in India despite the fact that several million people, including children, are fed every day under State-sponsored supplementary feeding programmes such as the Tamil Nadu Integrated Nutritional Project¹. Food poisoning outbreaks are not uncommon, but are seldom investigated. The present report describes the investigation of an outbreak of food poisoning which, though delayed, provided evidence for contamination of water and buttermilk with a toxin-producing bacterium as the probable cause.

Material & Methods

Outbreak investigation : The outbreak began on 29th September 1996 in a village in North Arcot District of Tamil Nadu, India. Villagers (48) partook of a feast comprising a standard vegetarian south Indian meal. At

varying times subsequently 25 (52 %) developed symptoms requiring medical attention, and seven had to be hospitalized.

The outbreak came to the attention of the investigation team the next day, by which time all remnants of the meal and water had been discarded. The investigation was begun on 2nd October 1996, by interviewing 47 of the 48 attendees at the feast (response rate 98 %). Details of the symptoms and food consumed during the feast were obtained. Any person who gave a history of fever and loose stools with or without vomiting or abdominal cramps was considered a case. Stool samples were collected the same day from 11 cases. No blood samples were collected at this time. Five litre samples of water were collected for analysis from the borewell which supplied water for the feast (including buttermilk preparation), as well as from another well

located in a field. Two samples of milk were obtained for analysis, one from the vendor who supplied milk for the feast, and the other from another source.

Blood samples were collected for serology nearly a month after the epidemic (October 28th), because by this time *Yersinia enterocolitica* was grown from the stool of one of the affected individuals, as also from the water. Samples were obtained from 12 feast participants who fell ill, from 8 who did not develop illness (controls) and from 15 residents of the village who did not participate in the feast (general population). Paired blood samples were again obtained on 23rd December. Two further samples of bore well water were obtained in November and December.

Laboratory methods : Stool was transported to the laboratory on ice, and samples were plated on MacConkey agar, xylose lysine desoxycholate agar, blood agar and thiosulphate citrate bile salt sucrose agar and inoculated into selenite F broth which were incubated overnight at 37°C. Samples were also plated onto Butzler's medium and incubated at 42°C under microaerophilic conditions. Selenite F broth was subcultured onto MacConkey agar after 16 h. All plates were examined at 24 and 48 h after incubation and appropriate biochemical tests were done. Cold enrichment was done by inoculating 2 g of the original stool sample with one tube each of phosphate buffered saline (pH 7.2) and selenite F broth and incubated for 6 wk at 4°C². Weekly subcultures onto MacConkey agar were made. All media were from Difco Labs and chemicals from Sigma Chemical Co.

Standard techniques² were used to analyze water and milk samples, and all the above culture media were utilized.

Biotyping of *Yersinia* was done by fermentation of sucrose, raffinose, rhamnose, melibiose and cellobiose³. Motility of suspected *Yersinia* colonies was examined at 25 and 37°C by hanging drop and Craigie's tube method². Isolates biochemically identified as *Y. enterocolitica* were tested for fermentation of salicin and esculin and for production of pyrazinamidase³. They were also plated on Congo Red magnesium

oxalate agar⁴ and tested for production of enterotoxin in the suckling mouse assay⁵. Appropriate positive and negative controls were used. Serotyping and biotyping of the two *Y. enterocolitica* isolates was done by kind courtesy of Dr Bernard Rowe (PHLS, Colindale, UK).

Y. enterocolitica antigen was prepared⁶ from a known pathogenic strain of *Y. enterocolitica* (Statens Serum Institut, Copenhagen, Denmark, serotype 03) and used for agglutination assay. The assay was done by standard method and the reaction read after overnight incubation at 37°C.

The two toxin-producing *Y. enterocolitica* isolates were grown in Luria broth (prepared from materials from Sigma and Difco) overnight and 10⁴ bacteria were inoculated into 10 ml samples of buttermilk which were held at 4°C and at room temperature for 6 h. The buttermilk was centrifuged and the supernatant tested for toxin production in the suckling mouse assay⁵.

Escherichia coli isolated from the stool of all individuals were tested for enteropathogenic serotypes, for production of heat-labile and heat-stable toxins, Hep-2 cell adherence and for production of shiga-like toxins I and II by enzyme immunoassay⁷.

Statistical analysis : Epi-Info software (Version 5, CDC and WHO) was used for data analysis. Relative risks and 95 per cent confidence intervals were used to measure the strength of association between development of symptoms and consumption of a particular food item.

Results

All cases occurred between 29 September and 1 October. The time from consumption of the food to development of illness ranged from 7 to 49 h (median 16 h). The overall attack rate was 52 per cent, and was similar for either sex. The affected individuals ranged in age from 3 to 80 yr, 11 per cent being children. The commonest symptoms were fever (96 %), diarrhoea (92 %), abdominal cramps (92 %), headache (75 %), rigors (71 %) and vomiting (67 %). Bloody stools were not reported nor did any of the affected individuals have an appendicitis-like illness. There were no deaths.

The mean stool frequency was 9 per day, and the mean duration of diarrhoea was 66 h.

The Table shows the magnitude of association (measured as relative risk) between consumption of various food items and illness. The only food item significantly associated with risk of developing illness was buttermilk. All those who had consumed buttermilk fell ill.

Microbiological analysis revealed *Y. enterocolitica* in 1 of 11 stool samples of the patients. *Y. enterocolitica* was also isolated from the borewell water sample taken initially. Both isolates exhibited all biochemical attributes of pathogenic *Y. enterocolitica* (including Congo Red binding) and both were positive for toxin production in the suckling mouse assay. Both isolates were of serotype 3 and biotype 4. No other enteric pathogens (including enteropathogenic, enterotoxigenic, enteroadherent or enterohaemorrhagic *E. coli*) were isolated from stool, water or milk samples. Cold enrichment of the samples yielded *Y. enterocolitica* only in the two instances where the organism had already been detected on direct plating, but not in any of the other samples. All water samples had coliform counts greater than 180/ml. *Y. enterocolitica* was not isolated from the second and third samples from the borewell, or from the other well sampled.

Serology of the first paired samples revealed high anti-*Y. enterocolitica* antibody titres (1 in 640 and 1 in 5120 dilution) in 2 of 12 patients and borderline titres (1 in 160) in 1. No significant antibody titres were detected in any of the 8 controls or 15 general population samples. The second paired samples were all negative for antibody to this organism.

Toxin was detected in inoculated buttermilk kept for 6 h, both at 4°C and at room temperature.

Discussion

Y. enterocolitica infection may cause intestinal, extraintestinal and reactive (postinfectious) syndromes⁸. Infection with this organism has been reported sporadically from India⁹⁻¹¹, primarily in patients with diarrhoea. The organism usually causes illness by invasion of the intestine. However, it also produces a heat-stable enterotoxin resembling the heat-stable enterotoxins

Table. Magnitude of association between consumption of food items and illness

Food item	Relative risk (RR)	95% confidence intervals
Rice	2.14	0.38 - 11.95
Rasam	1.03	0.54 - 1.97
Sambar	2.26	0.66 - 7.73
Vadai	1.02	0.44 - 2.41
Payasam	0.93	0.51 - 1.70
Curry (plantain)	1.41	0.70 - 2.81
Buttermilk	3.88*	2.13 - 7.04
* Drinking water	1.03	0.55 - 1.91

*Statistically significant ($P < 0.001$)

(ST) of *E. coli* and non-01 *Vibrio cholerae*. The organism is a known cause of food poisoning,¹² and several outbreaks have been reported. The contaminated source responsible for such epidemics has usually been some form of milk,¹³⁻¹⁵ bean sprouts⁸ or soya tofu¹⁶. In the latter two, spring water or well water were also implicated. In a study from Ludhiana, the organism was detected in milk and fruit juice during testing of a number of food samples for pathogens¹⁷. The organism has also been isolated from many environmental water sources¹⁸ where the non-pathogenic variety is more commonly found⁸. However, there is a report of an outbreak due to ingestion of unchlorinated well water¹⁹. There is no report, to date, from India of an outbreak of food poisoning due to this organism.

Despite several limitations in the investigation of the outbreak, the circumstantial evidence outlined below leads us to believe that the outbreak was due to contamination of buttermilk by *Y. enterocolitica*. *Y. enterocolitica* of similar characteristics was isolated from the stool of a symptomatic individual, as well as from the water used to dilute the buttermilk. No other enteric pathogen (including pathogenic *E. coli*) was isolated from the stool of symptomatic individuals. The demonstrable fall in antibody titre in paired samples from 3 of 12 affected individuals suggests that they had been exposed to the organism recently. Delay in obtaining the first blood sample may explain why more of the affected individuals did not have an antibody response to the organism. It is likely that the *Y. enterocolitica*

present in the water used to dilute the buttermilk proliferated *in vitro* producing toxin, and that ingestion of this buttermilk caused the symptoms of food poisoning. The short incubation period (median incubation period 16 h) and the associated fever and vomiting is consistent with symptomatology due to ingestion of a pre-formed toxin. However, it is possible that symptoms in those affected individuals with longer incubation periods can be attributed to invasion of bowel mucosa by the organism. The reason for the well water contamination was not investigated. However, it is likely that there was a transient contamination, possibly from pigs or cattle which are both known to be reservoirs of the organism⁸.

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References

1. Shekar M, Latham MC. Growth monitoring can and does work! An example from the Tamil Nadu Integrated Nutrition Project in Rural South India. *Indian J Pediatr* 1992; 59: 5-15.
2. Collee JG, Duguid JP, Fraser AG, Marmion BP, McCartney's *Practical microbiology*. 13th edn. Edinburgh: Churchill Livingstone: 1989.
3. Farmer JJ, Kelly MT. Enterobacteriaceae. In: Balows A, Hausler WJ Jr, Isenberg HD, Shadomy HJ editors *Manual of clinical microbiology*. 5th edition. Washington DC: American Society for Microbiology, 1991; 360-83.
4. Riley G, Toma S. Detection of pathogenic *Yersinia enterocolitica* by using Congo Red-magnesium oxalate agar medium. *J Clin Microbiol* 1989; 27: 213-4.
5. Dean AG, Ching YC, Williams RG, Harden LB. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhoea in children in Honolulu. *J Infect Dis* 1972; 125: 407-11.
6. Fowler JM, Brubaker RR. Immunology of *Yersiniae*, Rose NR, Friedman A, Fahey JL, editors. In: *Manual of clinical and laboratory immunology*. 3rd edition. Washington DC: American Society for Microbiology, 1989; 371-4.
7. Law D, Hamour AA, Acheson DW, Panigrahi H, Ganguli LA, Denning DW. Diagnosis of infections with Shiga-like toxin-producing *Escherichia coli* by use of enzyme-linked immunosorbent assay for Shiga-like toxins on cultured stool samples. *J Med Microbiol* 1994; 40: 241-5.
8. Cover TL. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL, editors. *Infections of the gastrointestinal tract*. New York: Raven Press, 1995: 811-23.
9. Singh G, Arora NK, Bhan MK, Ghai OP, Dhar S, Shrinivas DC. Role of *Yersinia enterocolitica* in childhood diarrhoea. *Indian J Pediatr* 1983; 50: 39-42.
10. Varghese A, Ramachandran VG, Agarwal DS. Isolation and characterization of *Yersinia enterocolitica* from patients of gastroenteritis. *Indian J Med Res* 1984; 79: 35-40.
11. Ram S, Khurana S, Singh R, Sharma S, Vadehra DV. *Yersinia enterocolitica* diarrhoea in north India. *Indian J Med Res* 1987; 86: 9-13.
12. Bishai WR, Sears CL. Food poisoning syndromes. *Gastroenterol Clin North Am* 1993; 22: 579-608.
13. Black RE, Jackson RJ, Tsai T, Medvesky M, Shayegani M, Feeley JC et al. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. *N Engl J Med* 1978; 298: 76-9.
14. Shayegani M, Morse D, DeForge I, Root T, Parsons LM, Maupin PS. Microbiology of a major foodborne outbreak of gastroenteritis caused by *Yersinia enterocolitica* serogroup 0: 8. *J Clin Microbiol* 1983; 17: 35-40.
15. Tacket CO, Narain JP, Sattin R, Lofgren JP, Konigsberg C, Jr Rendtorff RC et al. A multistate outbreak of infections caused by *Yersinia enterocolitica* transmitted by pasteurized milk. *JAMA* 1984; 251: 483-6.
16. Tacket CO, Ballard J, Harris N, Allard J, Nolan C, Quan T et al. An outbreak of *Yersinia enterocolitica* infections caused by contaminated tofu (soybean curd). *Am J Epidemiol* 1985; 121: 705-11.
17. Ram S, Khurana S, Khurana SB, Vadehra DV, Sharma S, Chhina RS. Microbiological quality and incidence of organisms of public health importance in food and water in Ludhiana. *Indian J Med Res* 1996; 103: 253-8.
18. Javier-Castillo F, Larraz V, Asuncion-Lafarga M, Navarro M, Gomez-Luz R. Phenotypic characterization and distribution of *Yersinia* in human and environmental samples. *Microbiologia* 1994; 10: 83-92.
19. Thompson JS, Gravel MJ. Family outbreak of gastroenteritis due to *Yersinia enterocolitica* serotype 0: 3 from well water. *Can J Microbiol* 1983; 32: 700-1.