The prevalence of bacterial intestinal pathogens in a healthy rural population in southern India

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Summary. In a one-year prospective survey bacterial intestinal pathogens unassociated with diarrhoeal episodes were isolated from 20.5% of stool samples from 48.5% of a stratified random sample of the population of a village in southern India. *Campylobacter jejuni* was the pathogen most frequently isolated, followed by enteropathogenic serotypes of *Escherichia coli*. The incidence of diarrhoea in the study population was lower than the frequency of isolation of bacterial intestinal pathogens. It is necessary to understand the prevalence of intestinal pathogens in this ecosystem to know the dynamics of intestinal infection and the pathogenesis of diarrhoea.

Introduction

Bacterial intestinal pathogens and rotaviruses can be isolated from or demonstrated in most children and adults with acute diarrhoea coming to hospital in tropical developing countries (Maiya *et al.*, 1977; Pickering *et al.*, 1978; Hieber *et al.*, 1978; Black *et al.*, 1980; Mata *et al.*, 1983; Sen *et al.*, 1983). The human habitat in such countries is insanitary; water supplies are unprotected, waste disposal is inadequate, and there is close association between man and domestic animals. Furthermore, bacterial pathogens are present in the faeces of apparently healthy people (Beck *et al.*, 1957; Mata *et al.*, 1965; Feldman *et al.*, 1970; Baker and Mathan, 1972; Rajan and Mathan, 1982). Understanding the pattern of prevalence of pathogens in the community is necessary to plan adequate measures for the control and prevention of intestinal infections and diarrhoea. This paper reports the results of a systematic prospective study of the faecal excretion of bacterial intestinal pathogens, including *Campylobacter jejuni*, by individuals from a stratified random sample of the families in an agricultural village near Vellore, in southern India.

Subjects studied and methods

The population of a village 20 km south of Vellore was stratified on the basis of family income and location in the village and from the 379 families in the village, 63, consisting of 311 individuals, were randomly selected. Approximately 25 stool samples were collected each week, all members of a family providing specimens on the same day, at least 4 weeks after the last known episode of

diarrhoea in the family. The average sampling interval for a family was 3.3 months. From November 1980 to December 1981, stool cultures to identify bacterial intestinal pathogens including *C. jejuni* were done (1188 samples). Each family was sampled on four separate occasions during this initial period. Subsequently, cultures for *C. jejuni* only were done from January 1982 to August 1982 (853 samples). Episodes of diarrhoea in the study population were recorded by a public-health nurse once a week. Diarrhoea was recorded when individuals gave a history of more than three loose stools in a day, a single large watery stool, or the presence of mucus and blood in the stool. Because the median duration of episodes of diarrhoea was 2 days and families were visited only once a week, it was not possible to obtain stool samples during diarrhoeal episodes.

Laboratory methods

Stool samples were passed into sterile plastic containers early in the morning and transported on ice to the laboratory, where they were processed, usually within 1 h of receipt. Samples were inoculated on to McConkey Agar, Desoxycholate Citrate Agar, Xylose-Lysine-Desoxycholate Agar and into Selenite Broth which, after overnight incubation, was subcultured on Salmonella-Shigella Agar. Special cultures for *Yersinia enterocolitica* were not done because preliminary surveys had shown that this organism was extremely infrequent in this warm climate. All pathogens detected were identified by standard biochemical methods and by serology (Edwards and Ewing, 1972). Approximately five lactose-fermenting colonies of each colony morphology from McConkey-agar plates were screened for enteropathogenic serotypes of *Escherichia coli* (EPEC) by slide agglutination. Sera for the following serotypes were available: O55 B5; O86 B7; O26 B6; O111 B4; O127 B8; O119 B14; O124 B17; O125 B15; O126 B16; O128 B12. Stool samples were also plated directly on to Butzler's Medium and isolates of *C. jejuni*

were characterised by standard techniques as described by Rajan and Mathan (1982). Significant differences in rates of isolation were tested by the χ^2 test.

Results

From November 1980 to December 1981 the pathogen isolated with highest frequency (table I) was *C. jejuni* (10.9%) and the rate of isolation in preschool children, below 5 years of age (21.3%) was significantly higher than that in older age groups (8.6%) ($p < 0.005$). *C. jejuni* was isolated in association with another pathogen in 19 instances, cultivated from two separate samples from the same individual in 21 instances, from three samples in nine instances and from five separate but consecutive samples in one individual. The frequency of such multiple isolations was also higher in pre-school children. The rate of isolation of *C. jejuni* from January to August 1981 (72 isolates from 693 stools; 10.3%) was significantly higher than in the

Figure. The percentage of stool samples from which *C. jejuni* (O) and other pathogens (●) were isolated each month during the study. No samples were collected during Mar. 1981.

same period in 1982 (41 isolates from 853 stools; 4.8%) ($p < 0.005$). Isolations were lowest in March–May in both 1981 and 1982 (figure). The high rates of isolations during November–December 1980, January 1981 and August 1981 were not found in the subsequent year.

The rates of isolation of other pathogens in children below 5 years and in older age groups was not significantly different (table I). Shigellae and EPEC were found with similar frequency in all age groups; salmonellae were less frequent in preschool children.

The total of 284 bacterial intestinal pathogens isolated between November 1980 and December 1981 was from 255 stool samples provided by 151 (48.5%) of the 311 individuals in the study. As shown in table II, the rate of isolation of pathogens, the frequency of more than one pathogen in the same sample, and the frequency of pathogens in more than one sample from an individual were highest in pre-school children. In 53 instances (157 isolates), pathogens were isolated at the same time from more than one individual in a family. The

Table I. Rates of isolation of bacterial intestinal pathogens from stool samples from the study population, Nov. 1980–Dec. 1981

Pathogenic species	Percentage of samples with pathogens in subjects		
	< 5 years old (n = 221)	> 5 years old (n = 967)	Total (n = 1188)
<i>Salmonella</i> spp.*	0.5	1.8	1.5
<i>Shigella</i> spp.†	2.3	1.8	1.9
EPEC‡	7.2	6.0	6.2
<i>Plesiomonas shigelloides</i>	0.5	1.7	1.4
<i>Aeromonas hydrophila</i>	0.5	1.5	1.3
<i>Alkalescens dispar</i>	0.5	0.7	0.6
<i>C. jejuni</i>	21.3	8.6	10.9

* Salmonellae grouped only by polyvalent antisera A–G.

† *Sh. dysenteriae* 4; *Sh. flexneri* 7; *Sh. boydi* 3.

‡ EPEC serotypes O125, B15 24; O119, B14 17; O124, B17 1; O126, B16 6; O128, B12 5; O86, B7 10; O26, B6 8; O127, B8 3.

Table II. The isolation of bacterial intestinal pathogens from different age groups and the incidence of diarrhoea

Age groups (years)	Total number of individuals sampled	Percentage from whom pathogens were isolated	Percentage with > 1 pathogen in same sample	Percentage with pathogens in > 1 sample	Number of cases of diarrhoea
0–4.9	62	62.9	14.5	22.5	99
5–11.9	67	56.7	10.4	10.4	34
12–17.9	36	38.8	5.5	2.7	10
18–and over	146	41.1	5.4	2.7	44
Total	311	48.5	8.3	8.3	187

same pathogen was present in 25 of these instances but different pathogens were isolated from individuals of the same family in 28 instances. In only six of the 63 families were no pathogens isolated during the 22 months of the study.

Between November 1980 and December 1981, there were 187 episodes of acute diarrhoea affecting 132 individuals (table II). There was no significant difference in the incidence of diarrhoea in individuals from whom pathogens were isolated during the year and in those without. Of these episodes, 14 occurred during the 2 weeks following stool sampling, only four in individuals from whom bacterial pathogens were isolated. No diarrhoea occurred in three children below 2 years old from whose stool samples bacterial pathogens were isolated on more than one occasion.

Discussion

The isolation of bacterial intestinal pathogens from 20.5% of stool specimens, 48.5% of individuals and 90.5% of the families from a stratified random sample of the healthy rural population of southern India in a year confirms earlier reports from tropical developing regions (Beck *et al.*, 1957; Feldman *et al.*, 1970; Baker and Mathan, 1972). The rate of isolation of pathogens in the present study was higher than in the earlier studies because techniques were employed to isolate *C. jejuni* and may have been even higher if toxigenic coliforms had also been identified. Studies such as this can only provide an estimate of the prevalence of pathogens; stool samples would have to be cultured daily from each individual to determine the true incidence and duration of excretion of bacterial intestinal pathogens by healthy populations. Extrapolation of the present data suggests that in this population, bacterial intestinal pathogens can be found in stools on about 75 days each year in apparently healthy people, emphasising the magnitude of the problem.

The detection of the same pathogen in more than one individual of a family at the same time in 25 instances suggests that environmental factors may be important. In this village, as in most others in southern India, there is no protected water supply and no sanitary facilities. The streets, back yards and surrounding fields are used for defaecation and waste disposal. Domestic animals, which are the most valuable possessions of the family, are kept in close proximity to houses or often share living quarters. Under these circumstances, does the isolation of a bacterial intestinal pathogen from the stool of an asymptomatic individual indicate merely a transient "passenger" from the environment, "col-

onisation" or "infection"? The higher frequency of isolation of single and multiple pathogens in pre-school children than in older age groups suggests that protective immunity may develop with age. Whether this is due to asymptomatic excretion or to the approximately two episodes of diarrhoea per year in the pre-school children is difficult to determine.

Several reports are now available of the isolation of *C. jejuni* from apparently healthy individuals (Bokkenheuser *et al.*, 1979; Blaser *et al.*, 1980; Macaden *et al.*, 1984) and from children with diarrhoea in tropical countries (DeMol and Bosmans, 1978; Blaser *et al.*, 1980; Ayyagiri *et al.*, 1982). Unlike the situation in temperate climates, where the isolation of *C. jejuni* is almost exclusively from patients, in tropical countries the rate of isolation appears to be as high in controls as in patients with diarrhoea. Although the lower rate of isolation in older age groups suggests acquired resistance to intestinal colonisation, it is not yet clear whether the first exposure of children to *C. jejuni* causes enteritis in southern India. If this exposure occurs early in the first year of life, antibodies acquired from the mother may modify the clinical response (Gothefors *et al.*, 1976).

The incidence of acute diarrhoea was similar in individuals from whom pathogens were cultured during the year and in those without. There were also children from whose stools pathogens were isolated on more than one occasion who did not develop diarrhoea during the study. In a community-based study in El Salvador, enterotoxigenic and enteropathogenic strains of *E. coli* were detected with equal frequency in patients with diarrhoea and in matched controls (Spencer *et al.*, 1980). In Bangladesh, 7–19% of asymptomatic controls in communities from which cases of diarrhoea were hospitalised were found to have shigellae in their stool (Boyce *et al.*, 1982). The rate of isolation of bacterial pathogens (66%) in hospitalised children at Vellore (Maiya *et al.*, 1977) was not significantly different from that in the same age group in the present study. The taxonomic identification of an organism as an "intestinal pathogen" is only a preliminary stage to understanding the complex interaction that produces diarrhoea. Virulence of the organism, microbial competition, host response and gastro-intestinal epithelio-luminal interface defence mechanisms are poorly understood factors in the pathogenesis of diarrhoea. There may also be other factors in the ecosystem, as suggested by the wide fluctuations in the month-to-month rates of isolation of pathogens (fig.) and the significant differences in the rates of isolations of

Campylobacters during identical periods in succeeding years, which may have an influence on the dynamics of enteric bacterial infection in the community. The high prevalence of bacterial intestinal pathogens in asymptomatic individuals in several areas in tropical developing countries suggests that the question "why are enteric pathogens not more strongly associated with diarrhoea in indigenous populations?" is important. Since this high prevalence rate of intestinal bacterial pathogens is a

reflection of environmental factors which are unlikely to change radically in the foreseeable future, a full understanding of the microbial ecosystem in this habitat is necessary to plan effective interventions to prevent the morbidity and mortality of diarrhoea.

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