Epidemiological and laboratory investigations of outbreaks of diarrhoea in rural South India: implications for control of disease

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SUMMARY

Two epidemics of acute, watery diarrhoea in villages in North Arcot district, India, were investigated. The attack rates were 10·03 and 15·53 per 100 population, the median duration was 5 days and enteric pathogens were present in 56·8 % and 60·3 % of specimens from the two villages, but no predominant pathogen was identified. Examination of stools from a 20 % age-stratified random sample of the population of one of the villages after the epidemic found 22·9 % of asymptomatic subjects excreted bacterial enteric pathogens. Despite the high background of enteric pathogen carriage, the isolation rates for shigellae, enteropathogenic *Escherichia coli* and Shiga-toxin producing *E. coli* were significantly higher ($P < 0.001$, $P < 0.02$, $P < 0.05$) during the epidemic. The epidemics may have been caused by faecal contamination of well water following rain. Point-of-use techniques for water disinfection may be most effective for preventing such outbreaks, but further research into the development of appropriate technology is required.

INTRODUCTION

Epidemics of acute diarrhoea occur frequently in tropical developing countries and diarrhoeal epidemics have been reported earlier from the rural areas of North Arcot District in Tamil Nadu [1–6]. We have maintained a surveillance programme in North Arcot district, investigating reported outbreaks of diarrhoea in order to identify causative organisms and to appropriately advise local health authorities about control measures to be instituted in order to control the outbreak. While identification of a single pre-dominant enteric pathogen in the laboratory supported by epidemiological studies can assign a cause to an epidemic and help in formulating control measures [7], in some epidemics, no single predominant organism is found. In an environment where asymptomatic carriage and excretion of enteric pathogens is known to occur in children and adults [8, 9], it can be difficult to identify the cause of the epidemic or to devise appropriate control measures. It is therefore important to obtain epidemiological data about diarrhoeal outbreaks in order to formulate control and prevention policies, which may need to be designed to suit area and population specific needs.

Here we report the laboratory and epidemiological investigation of two epidemics of diarrhoea that were unusual in that they were closely related in time and although a number of enteric pathogens were identified, no single organism predominated. We also present a later follow-up study and discuss potential modes of occurrence and implications for control.

MATERIALS AND METHODS

Epidemiological investigation

After receipt of information about outbreaks of diarrhoea by the surveillance team in June and July 1991, the affected villages were visited. A house-to-

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house survey was carried out where each house in the village was visited and at least one member of each family was questioned. Information was obtained about family structure and socio-economic status. For each member of the household, a questionnaire was used to collect information on previous and present acute and chronic illness. For all those reporting diarrhoea, information was obtained about date of onset, number, colour and consistency of stool, presence of blood and/or mucus, other associated symptoms such as fever, abdominal pain, nausea and vomiting. Follow-up visits were made twice weekly for the first 4 weeks and then once a week for the following 4 weeks.

Collection of specimens from patients during the epidemics

During the epidemics, stool specimens were requested from all patients complaining of diarrhoea over a period of 3 days in the two villages, this time point coincided with the peak of the epidemics. For children, diarrhoea was defined as a change in consistency or number of stool perceived as diarrhoea by the mother.

Collection of specimens from asymptomatic subjects during an inter-epidemic period

In a follow-up study carried out 2 years later in the same months as the epidemics, an age-stratified random sample of 20% of the population of one of the villages were asked to provide stool specimens.

Transport and processing of specimens

Specimens (approximately 5 g) were collected in sterile screw-capped containers and transported on ice to the laboratory and processed within 3 h of collection.

Identification of enteric pathogens

Laboratory procedures for the identification of bacterial, viral and parasitic enteric pathogens, including adherence assays and toxin testing for *Escherichia coli* were as described earlier [9–11]. Briefly, culture, biochemical and serotyping techniques were used to identify *Salmonella*, *Shigella*, *Vibrio*, *Yersinia*, *Campylobacter*, *Plesiomonas* and *Aeromonas* spp. Diarrhoeagenic *Escherichia coli* were identified by toxin testing and adherence assays. Agents of viral
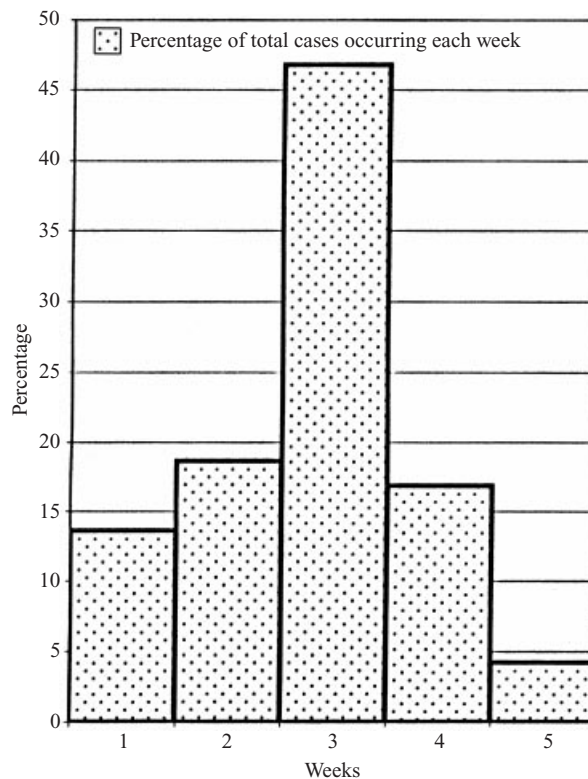

Fig. 1. Epidemic curve for village On, 15 June–21 July, 1991.

gastroenteritis were looked for by electron microscopy. For parasites, saline and iodine preparations were examined directly and after formol-ether concentration. A modified Ziehl–Neelsen stain was used to identify *Cryptosporidium* species.

Examination of water sources

In the inter-epidemic period, when stool samples were collected from a stratified sample of the residents of village Ka, water samples were also collected for analysis by the most probable number technique to identify faecal contamination [12].

RESULTS

The two villages, Ka and On, are within 15 km of each other in the North Arcot district of Tamil Nadu, about 35 km south of Vellore. They are situated in agricultural land between 80 and 120 m above sea level. The annual rainfall in this area is 75–150 cm and occurs mainly in September to November. The average daytime temperature is 30–38 °C, which may rise to 45 °C in May and fall to 25 °C in December. The majority of the adult population in On are weavers and in Ka, they are agricultural labourers.

Table 1. *Attack rates per 100 population in the two epidemics in On and Ka*

Age group (years)	On			Ka		
	Popln	Cases	Attack rate	Popln	Cases	Attack rate
< 5	168	43	25·4	166	47	35·0
5–12	396	34	8·6	199	31	15·7
12–18	331	26	8·2	141	21	16·1
> 18	1625	147	9·2	819	91	11·4
Total	2520	280	11·1	1325	190	15·5

Drinking water is obtained from semi-protected open public wells in On and Ka and is never boiled. On village also had a 'protected' drinking water supply through an overhead tank in the village centre, but this supply system had broken down in April 1991, due to problems with the pump. During June, in both villages, the surrounding areas were waterlogged because of rain.

There were nine reported cases of diarrhoea in On during the 3 months preceding June 1991. A total of 280 cases of acute, watery diarrhoea were reported during epidemic, over the next 5 weeks. The epidemic curve is shown in Figure 1. The overall attack rate was 10·03 per 100 population. Males and females were equally affected. Attack rates were significantly higher ($P < 0.001$) in preschool children than in other age groups (Table 1). The duration of illness ranged from 1–45 days with a median of 5 days. The illness lasted longer than 2 weeks in 7·2%. Nausea, vomiting, blood and mucus were reported by less than 5% of those affected and associated abdominal pain was reported by approximately 10%. There was no mortality.

In Ka, there had been eight reported cases of diarrhoea during the 3 months preceding the epidemic which began in the first week of July 1991. During the next 5 weeks, 189 cases were reported and the epidemic curve is shown in Figure 2. The overall attack rate was 15·54 per 100 population. Males and females were equally affected. Preschool children (35%) were affected significantly more frequently ($P < 0.001$) than other age groups (Table 1). The attack rates were similar in both sexes. The duration of illness ranged from 1–48 days with a median of 5 days. The illness lasted longer than 2 weeks in 9·4%. Nausea and vomiting were not reported. Blood and mucus in the stool were reported by approximately 10% and abdominal pain by 16% of those affected.

During the epidemics, stool samples were obtained from 44 cases in On and 58 cases in Ka. Recognized bacterial pathogens were present in 25 of 44 (56·8%)
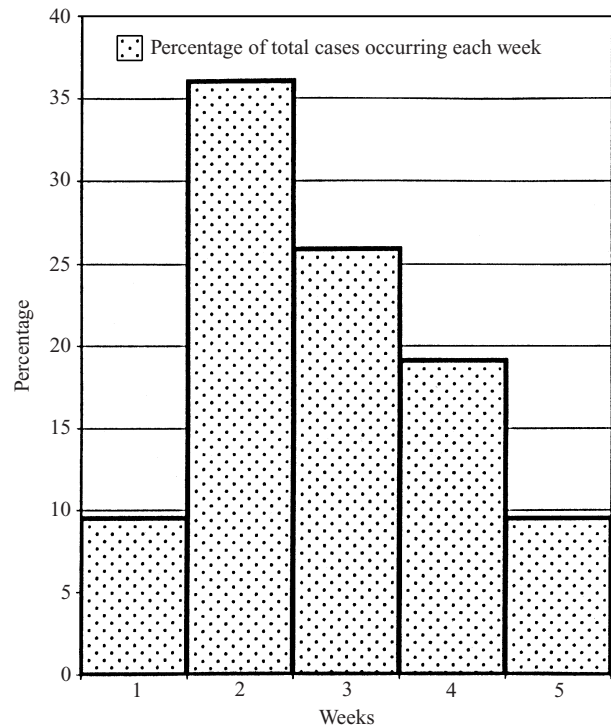

Fig. 2. Epidemic curve for village Ka, 1 July–8 August, 1991.

specimens from On and in 35 of 58 (60·3%) specimens from Ka (Table 2), but no single predominant pathogen was identified. Six patients from Ka but no patients from On had more than one bacterial pathogen identified from their stool. Different serotypes of enteropathogenic *E. coli* (EPEC) were the most commonly isolated bacterial pathogens (10/44) in On with *Shigella* spp. being isolated from 3 of the specimens. The EPEC were of various serotypes with a maximum of 3 isolates for any one serotype. In Ka, *Shigella* spp. were identified in 12 of 58 specimens, with a total of 7 serotypes belonging to 3 species. *Shigella flexneri* serotype 3, the commonest isolate, was found in 6 patients. EPEC were isolated from 8 patients and *Campylobacter* spp. from 3 patients from Ka. Production of heat-labile toxin (LT) was demon-

Table 2. *Pathogen isolation from patients with diarrhoea during the two epidemics in On and Ka and from asymptomatic controls from Ka*

Pathogen*	On ($n-44$)	Ka ($n-58$)	Controls ($n-261$)
Bacteria			
Shigellae	3	12	1
EPEC	10	8	2
Plesiomonas	1	1	—
Campylobacter	—	3	9
Aeromonas	—	1	1
ETEC	5	1	11
STEC	—	7	12
LAEC	7	5	15
EAggEC	3	3	17
Parasites			
Hookworm	8	2	5
Giardia	2	1	9
Cryptosporidium	1	6	16
Hymenolepis nana	1	—	6
Viruses			
Rotavirus	2	2	ND†
Adenovirus	1	1	ND
Calicivirus	2	1	ND

* EPEC, enteropathogenic *Escherichia coli*; ETEC-enterotoxigenic *Escherichia coli*; STEC, Shiga-toxin producing *Escherichia coli*; LAEC, locally adherent *Escherichia coli*; EAggEC, enteroaggregative *Escherichia coli*.
† ND, not done

strated in *E. coli* isolates from 4 subjects in On, but in none of the samples from Ka (Table 2). Heat-stable toxin (ST) was produced by 1 isolate from each village. Shiga-like toxin (Stx) producing *E. coli* were not found in any specimens from On, but were demonstrated in 7 samples from Ka.

On doing the HEp-2 cell adherence assay, diffusely adherent *E. coli* (DAEC) were identified in 18/41 *E. coli* isolates from On and 23/53 *E. coli* isolates from Ka. Enteroaggregative *E. coli* (EAggEC) were found in 3 samples from each village. *E. coli* producing localized adherence (LAEC) were seen in 7 (15·8%) samples from On and 5 (8·6%) from Ka (Table 2).

Parasites were demonstrated in 12 of 44 samples from On and 9 of 58 samples from Ka. Hookworm, *Giardia*, *Cryptosporidium* and *Hymenolepis nana* were the commonest parasites, with On village accounting for 8 of 10 hookworm ova seen (Table 2).

Rotaviruses were identified in 2 children below the age of 5 years in both villages. Adenovirus was found in 1 adult in each village, and calicivirus in 2 children in On and an adult in Ka (Table 2).

Identification of enteric pathogens in asymptomatic subjects during an inter-epidemic period was also done to study background asymptomatic excretion. A total of 261 residents of village Ka, a 20% stratified random sample of the population, gave single stool samples for examination during the year 1993. None of the subjects had diarrhoea for the month preceding collection of the sample. Parasites were detected in stools from 36 subjects, the commonest being *Cryptosporidium* and *Giardia*. Other than *E. coli*, the bacterial pathogens encountered were *Aeromonas* and *Campylobacter* spp. Only 8 subjects had more than one enteric bacterial pathogen in their stool, but 23 excreted both parasites and bacteria.

E. coli were obtained from 248 subjects. EPEC were identified by serogrouping from the original plates in only 2 subjects. Toxin testing identified a total of 11 enterotoxigenic *E. coli* (ETEC), of which 5 produced only ST, 3 produced only LT and 3 produced both LT and ST. A total of 12 isolates were found to produce Shiga-like toxins, 9 produced both Stx 1 and Stx 2, 1 produced only Stx 1 and 2 produced only Stx 2. Slide agglutination with O157 antiserum was positive for 4 isolates that produced both Stx 1 and 2.

In the adherence assay, 84 of the 248 subjects were found to excrete adherent *E. coli*. Of these, 52 isolates were DAEC, 15 LAEC (6·0%) and 17 EAggEC (6·9%) in the asymptomatic population studied here.

Water samples from 14 water sources in village Ka, including open wells, protected wells and hand pumps were analysed in the inter-epidemic period. All sources showed evidence of gross contamination, with coliform counts of $> 180/ml$, although *E. coli* were identified in six open wells. The other sources had *Klebsiella*, *Enterobacter* and *Aeromonas* spp., and non-agglutinating vibrios.

DISCUSSION

Two epidemics of acute, watery diarrhoea have been described, they were of short duration, reached a peak within 2 weeks from the beginning and rapidly subsided. The patterns of the epidemics described here in On and Ka are similar to those reported from this area earlier [1, 2, 4, 6, 13].

The age-specific attack rates showed that pre-school children were affected significantly more frequently than adults ($P < 0.05$) in both epidemics, a finding that indicates infection of the community with a pathogen to which adults are resistant due to an immune response acquired after prior infection. Although no single pathogenic agent could be associated with the epidemics from Ka and On, a significantly higher rate of isolation of a variety of pathogens was obtained. The high rate of identification of potentially pathogenic organisms could not be checked against controls without diarrhoea from the same village at the same time. However, when these data from the epidemics were compared with pathogen identification in asymptomatic individuals in Ka village 2 years later the isolation rates for shigellae, EPEC and Stx producing *E. coli* were significantly higher ($P < 0.001$, $P < 0.02$, $P < 0.05$) in Ka patients with diarrhoea (Table 2). The isolation rates for ETEC, LAEC and EAggEC were not significantly different for diarrhoeal patients affected by the outbreak in Ka and controls. However, when the data from the On outbreak is compared with the control data from Ka, EPEC and LAEC were found significantly more frequently in patients with diarrhoea ($P < 0.001$, $P < 0.02$, Table 2).

The data in the Ka controls are similar to earlier reports in the rural population around Vellore [10], where it has been shown that asymptomatic adults have a high rate of carriage of enteric pathogens. All pathogens isolated during the two epidemics were also isolated from controls in the follow-up study done 2 years later when there was no increased incidence of diarrhoea in the community. In such a setting, it is essential to have background epidemiological data on enteric pathogen excretion in order to assess the significance of isolation of a single or multiple pathogens in the causation of diarrhoea.

The patterns of adherence of *E. coli* isolated from the two villages shows that adherent *E. coli* could not have been the primary organisms in these two outbreaks. EAggEC were found in three samples each in the two villages, not significantly higher prevalence than that seen in asymptomatic Ka controls. Among the toxin producing *E. coli*, LT producing organisms were not identified in Ka, however, all seven Stx producing *E. coli* were isolated from this village. Ka is a predominantly agricultural village and close contact with livestock may be the reason for a high incidence of Stx producing organisms. Contamination of water with cattle dung may also be responsible for trans-mission of these organisms to humans. In Ka, during the epidemic, drinking water was obtained from wells located in the fields. The higher incidence of *Crypto-sporidium* in Ka ($> 10\%$) than in On ($< 5\%$) could also be due to these factors, since *Cryptosporidium* can be a waterborne pathogen for which cattle can act as a reservoir [14].

Although, no single predominant enteric pathogen was isolated during either of the two epidemics, it is possible that these outbreaks were caused by organisms that were not identified by the diagnostic techniques used. Viral agents of gastroenteritis can be excreted in low numbers, and electron microscopy may not be a sufficiently sensitive detection technique. However, the higher isolation rates for shigellae, EPEC and Stx producing *E. coli* in Ka and EPEC and LAEC in On indicate that a mixture of organisms may have been the cause of disease, especially in young children who may not have been previously exposed to these pathogens in numbers exceeding the infective dose. With the breakdown of the water pump and rain causing waterlogging in fields where wells were located, runoff from the fields which are used for defaecation is likely to have caused heavy contami-nation of the well water. In the water sources sampled in the inter-epidemic period, faecal contamination was evident in at least six wells, with high bacterial counts in the other sources.

In developing countries, waterborne diarrhoeal illness continues to be a leading cause of morbidity and mortality. The optimal approach to prevention of disease is the construction of water disinfection and delivery systems and sewage disposal and treatment facilities. This is expensive, time-consuming, and

breakdowns in the system, as occurred in one of the outbreaks reported here can result in outbreaks of disease. In recent years, point of use disinfection by a range of techniques including chlorination [15], the use of oxidized coal [16] and solar disinfection [17] has been suggested as a method of ensuring a safe, reliable year-round supply of drinking water. In India, we need research into and development of inexpensive, rapidly implementable techniques for water quality improvement using appropriate, locally available technology.

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