An outbreak of gastroenteritis caused by both rotavirus and *Shigella sonnei* in a private school in Rio de Janeiro

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SUMMARY

In May 1980 an extensive outbreak of gastroenteritis occurred in a private school in the city of Rio de Janeiro. Examination of faeces and paired sera showed that this outbreak was caused by both rotavirus and a virulent strain of *Shigella sonnei*. In the first 19 stool samples collected seven (37 %) had rotavirus only, six (32 %) had *Sh. sonnei* only, while four (21 %) had both agents. Examination of the second and third stool collections revealed only the presence of *Sh. sonnei*. The 18 paired sera showed seroconversion for rotavirus in four cases (22 %) and in seven cases (39 %) for *Sh. sonnei*. The overall attack rate of the disease was approximately 75 %, the nursery and kindergarten having higher attack rates. Students in all grades became sick at the same time, and the unimodal curve of the onset dates of symptoms indicates a common source outbreak. Evidence suggested a contaminated water supply.

INTRODUCTION

Diarrhoeal disease has long been recognized as a major cause of mortality among young infants, especially in the underdeveloped and developing countries. In Brazil the occurrence of diarrhoeal disease is widespread, especially in the periphery of the large cities and rural areas where the water supply and sewage disposal systems are usually in a precarious condition. Epidemiological studies combined with a search for aetiologic agents have been infrequent.

This study describes a common-source outbreak in a private school where the aetiologic agents were both *Shigella sonnei* and rotavirus. In the literature which we have reviewed no previous mention has been made of an outbreak of diarrhoea caused by both agents.

MATERIAL AND METHODS

School and population description

The outbreak of gastroenteritis occurred in May 1980 in 1150 pupils of a day school aged between 4 and 18 years divided into nursery, kindergarten, primary

and secondary groups, which have limited contact with each other. The school is situated in a prosperous residential area of Rio de Janeiro located on a hillside and architecturally of high standard. It consists of a row of 10 buildings with all the necessary accommodation of a modern educational establishment.

The cafeteria serves food prepared on the premises to both the primary and secondary levels at different hours. The primary school children either have prepaid meals or bring food from home, while the secondary school children may buy food at school when they wish. The large majority of the secondary school students bring food from their homes.

A concession stand located in the school sells sandwiches, ice cream, milk, etc. to both primary and secondary pupils but is not permitted to sell to children in the kindergarten and nursery. The water supply comes from several wells on the school's property. The drinking water is obtained from some of these wells and is collected in a reservoir where it is chlorinated manually and pumped to another reservoir above the building complex. From there it is piped, under gravitational force, to the buildings. Sewage goes directly to the city's sewage system.

Specimen and data collection

Upon notification of a gastroenteritis outbreak on 16 May, a questionnaire was sent to all parents on 19 May, to obtain information about the extent of the outbreak, clinical symptoms, disease duration, hospitalization, and mode of spread.

Information regarding absenteeism, eating habits of the students and other data relating to possible mode of transmission were obtained from the school's director.

Three separate faecal collections were made: the first 19 stools were obtained by one of the authors from sick pupils on 17 and 18 May, 9 stools were obtained by a nurse on 20 May, and 25 stool samples collected from kitchen personnel on 22 May by the Public Health Department. Approximately one month later, 28 stools were collected from pupils at random and examined.

Eighteen paired blood samples were collected for serological tests. The first blood sample was obtained on 20 May and the second sample approximately six weeks later.

Virological studies

Twenty per cent faecal suspensions were tested for rotavirus particles by direct and immunoelectron microscopy, counter immunoelectro-osmophoresis (CIE) and enzyme-linked immunosorbent assay, (ELISA), and antibody assays were carried out by complement fixation, ELISA and CIE. Direct electron microscopy (DEM) and immunoelectron microscopy (IEM) were done by standard methods described by Flewett *et al.* (1974*a*, *b*) and Kapikian, Dienstag & Purcell (1976), respectively. CIE was done according to the method described by Middleton *et al.* (1976). Faecal suspensions were tested against rabbit antiserum to newborn calf diarrhoea virus. Enzyme-linked immunosorbent assay (ELISA) for antigen was performed by the double antibody sandwich method described by Voller, Bidwell & Barlett (1976) as applied to rotaviruses by Yolken *et al.* (1977*a*). For the titration of antibodies

we used the techniques described by Yolken *et al.* (1977*b*). Reagents used in these tests included an antigen prepared from MA 104 cells infected with bovine rotavirus (Compton strain), a goat anti-human rotavirus (supplied by Dr R. M. Chanock, NIH, Bethesda, Md) used as capture antibody, guinea-pig IgG anti-simian rotavirus (SA II) coupled with alkaline phosphatase as described by Avrameas (1969) and goat anti-human IgG coupled with the same enzyme (Miles Yeda Ltd, Kiryat Weizmann, Rehovot, Israel). Complement fixation (CF) was done according to the LBCF/CDC standard method, using an antigen supplied by Behring (Marburg, W. Germany).

Bacteriological studies

The isolation of *Sh. sonnei* was done on Hektoen and EMB agar media. The identification was done by biochemical analysis described by Edwards & Ewing (1972) and the serological characterization was done according to Kauffmann (1966). Biotyping these samples was done in three substrates (betagalactosidase, xylose, rhamnose) according to methods standardized by Rubinstein (1964). Each *Sh. sonnei* positive sample was tested in vivo by the Serény virulence test (Serény, 1955; Mackel, Langley & Vanice, 1961). The antibiogram spectrum was determined by the diffusion of antibiotics in a disk system. The method of inoculation, incubation time and antibiograms were according to the criteria described by Bauer *et al.* (1966).

Serological studies were carried out by Mata & Carreras' (1970) modification of Neter & Walder's (1954) passive haemagglutination method.

RESULTS

Descriptive epidemiology

The notification of the gastroenteritis outbreak was made on 16 May 1980 by a parent who contacted the authors. Information on the number of cases and the date of onset of symptoms, obtained from the questionnaire sent to all parents, showed a unimodal curve with a peak incidence on 15 May and a spread of five days (Fig. 1). Absenteeism rates and questionnaire data showed an overall attack rate of 70 to 80 per cent. Table 1 summarizes these data. All grades became sick at the same time with a higher absenteeism among the younger children. The rates decreased the week following the beginning of the outbreak, with the exception of the kindergarten and nursery which returned to normal levels the week after.

Interviews with patients revealed a large variation in symptomatology and severity. Also opinions of physicians attending individual cases varied largely as to the possible aetiology.

To control the most probable routes of transmission immediate general hygiene measures were instituted, hygiene education stressed, food was temporarily obtained from a catering service and only mineral water and bottled soft drinks were available for drinking.
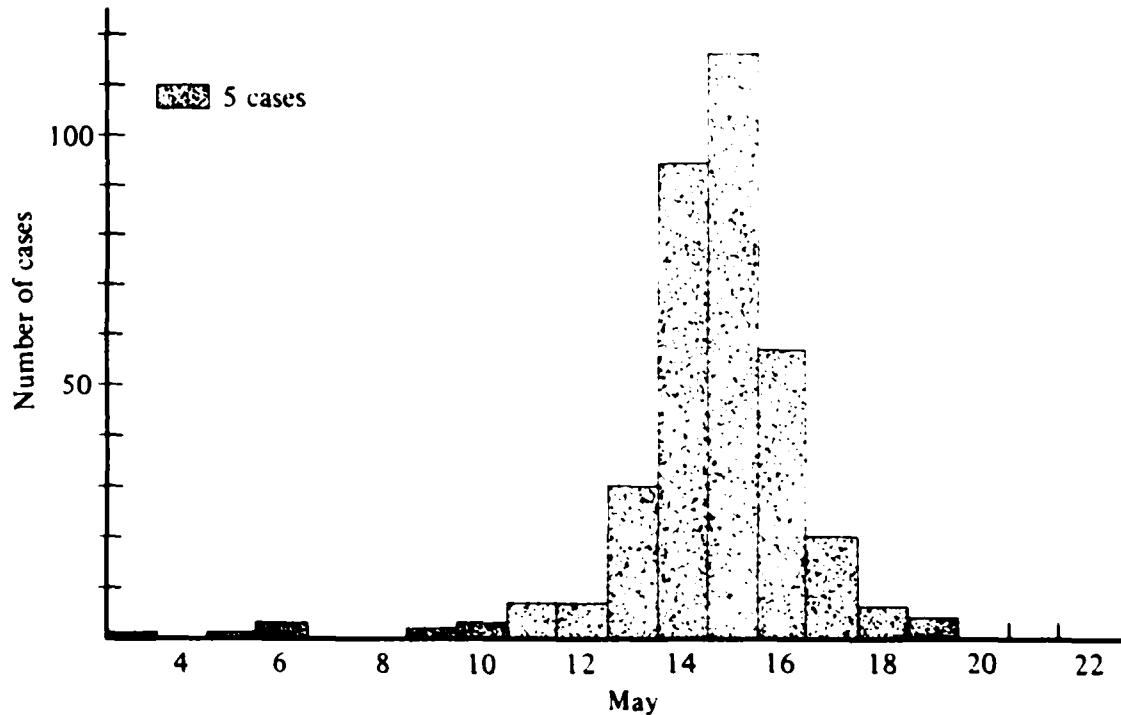
Fig. 1. Number of cases versus onset dates of gastroenteritis symptoms in a school in Rio de Janeiro, 1980.

Table 1. *Student absenteeism (A) and disease attack rate (B) per grade*

Age group	A*		B†	
	Total	Absent (%)	Total	Sick (%)
3–5	85	81 (95·2)	35	31 (88·6)
6–8	220	166 (75·4)	130	102 (78·5)
9–11	229	227 (75·9)	197	158 (80·2)
12–13	169	127 (75·1)	49	43 (87·7)
14–18	377	211 (55·0)	76	52 (68·4)
Total	1150	812 (70·6)	487	386 (79·3)

* Based on school records.   † Based on returned questionnaires.

Laboratory findings

Table 2 shows the results of bacteriological and virological examination of the first, second and third collections of faeces. In the first collection seven (37 %) had rotavirus particles, six (32 %) had *Sh. sonnei*, while four (21 %) had both agents in their faeces. In the second collection, only *Sh. sonnei* was isolated in five out of nine stools. In the faeces obtained from the 25 adults two were positive for *Sh. sonnei*. Faecal material obtained a month after the outbreak from 28 persons were negative for both rotavirus and *Sh. sonnei*.

Comparing the virological techniques employed for the detection of rotavirus particles, IEM and ELISA techniques were more sensitive than CIE and DEM. While the IEM and ELISA identified the same eleven positive samples, the CIE identified only eight and DEM only six of these.

Table 2. *Rotavirus detection and* Sh. sonnei *isolation in the three specimen collections*

Specimen collection		Number positive for		
Date	Number	*Sh. sonnei*	Rotavirus	Both agents
17 & 18 May	19	6	7	4
20 May	9	5	0	–
22 May	25	2	0	–

All *Sh. sonnei* isolates were further tested in vivo by inoculation on the rabbit conjuctiva (Serény test). Sixteen of these were extremely virulent, provoking kerato-conjunctivitis between 24 and 48 h.

The antibiogram spectra determined for each *Sh. sonnei* strain isolated were identical, showing them to be sensitive to chloramphenicol, colistin, gentamicin, phosphomycin, nalidixic acid and nitrofurantoin, and resistant to ampicillin, streptomycin, trimethoprim + sulphamethoxazole, tetracycline and erythromycin.

Paired sera from eighteen subjects, five of whom claimed not to have had clinical symptoms, were tested for antibodies to rotavirus and *Sh. sonnei* (Table 3). Four had significant rises of rotavirus antibody by ELISA, indicating a recent infection and two of these also had significant rises by CF. One of the latter and another patient seroconverted by CIE. Two others had high initial ELISA titres which subsequently decreased, suggesting a recent infection. In seven cases there was a significant rise in antibody to *Sh. sonnei* and 5 of these seroconverted. In the acute phase only 3 sera had titres of 40, while a month and a half later 12 patients had titres of 40.

Source of infection

Efforts to identify the mode of transmission in this outbreak were made. Attention was given to the mechanisms usually involved in gastroenteric disease. Person-to-person transmission may have occurred among a few students and this mode was observed among family contacts but its frequency was not determined. Food-borne transmission was excluded because no statistical difference existed in the absenteeism rates among the students who ate lunch at school and those who did not. Also food obtained from the concession stand could be excluded as the nursery and kindergarten are not allowed to purchase food there. With regard to the water supply system, information obtained from the school personnel revealed that the school was accidentally pumping water from a shallow well which was poorly protected and known to contain high coliform counts, and the chlorination was done manually. On 16 May the State Foundation for Environmental Protection (FEEMA) collected five water samples (from the deep and shallow wells, elevated reservoir, kitchen sink and a bathroom) for residual chlorine determination and for total and faecal coliform counts. The residual chlorine was zero except in the elevated reservoir and the kitchen sink where the value was only 0·1 p.p.m. In the shallow well the total coliform count was 33 and at the kitchen sink 8 per decilitre,

Table 3. *Results of serological tests for antibody to rotavirus and* Sh. sonnei *on paired sera from* 18 *patients*

Patient no.	Rotavirus						Sh. sonnei	
	CF titre		ELISA titre		CIE		HA titre	
	Acute	Conv.	Acute	Conv.	Acute	Conv.	Acute	Conv.
56	Ac	8	.	.	—	—	80	80
57	16	32	2100	2700	+	+	20	80
59	8	<8	160	100	—	—	20	40
60	32	32	33100	5700	+	+	0	40
61	16	16	1290	2570	+	+	40	160
62	8	≥128	440	17000	—	+	0	0
63	16	16	1230	1580	+	+	0	320
64	<8	32	930	10500	+	+	20	0
65	16	32	<100	3100	—	+	0	0
66	≥128	64	22000	6800	+	+	40	40
67	≥128	64	13180	4270	+	+	20	40
69	16	32	3300	5250	±	+	0	80
70	32	32	3240	3710	+	+	0	40
71	Ac	8	<100	<100	—	—	0	0
73	16	32	1800	1900	+	+	0	0
76	8	16	70	1700	—	—	20	40
77	16	8	180	<100	+	—	20	0
78	8	8	<100	<100	—	—	0	40

Ac = anti-complementary sera.

the other samples contained no coliforms. After a thorough cleaning of the reservoir and the disconnection of the shallow well from the water system, further water analyses were done by the F.E.E.M.A. and private companies. The water samples were tested for both total and faecal coliforms and its physico-chemical properties. Data from the F.E.E.M.A. identified faecal coliforms but the follow-up studies were not consistent as to the point of contamination and were difficult to interpret. Efforts to prove possible cross-connections with tracers (NaCl, rotamine, methylene blue) failed to reveal this fact. Water analysis by three private companies showed that the water was of potable quality but their criteria and techniques were not available to us.

DISCUSSION

Diarrhoeal disease has long been recognized as a major cause of mortality and morbidity in developing and underdeveloped countries. By one estimate there were 3–5 billion cases of diarrheoa occurring annually in children under the age of 5 in Asia, Africa, and Latin America resulting in at least 5–10 million deaths. (Walsh & Warren, 1979). Besides death it often leads to malnutrition which in turn makes children more susceptible to other infections by impaired body defences. From the official data available in the state of Rio de Janeiro from 1968 to 1972, it can be

shown that diarrhoeal disease represents 15–20 % of the infant mortality, while the percentage for the city of Rio de Janeiro is a little over 10 %. These data should be considered as a low estimate since this information contains a group of about 30 % of 'poorly defined' diseases.

For many years attempts to identify the aetiological agent involved in diarrhoeal disease were frustrated by a low isolation rate of bacteria known to cause disease, such as salmonella, shigella and enteropathogenic *Escherichia coli*. After the publication of Bishop *et al.* (1973), showing the presence of rotavirus-like particles in stools of infants with acute gastroenteritis, many publications have shown considerable evidence that this virus is the aetiologic agent involved in a large proportion of the diarrhoea of infants and children (Albrey & Murphy, 1976; Hieber *et al.* 1978; Pickering *et al.* 1978). Rotavirus has also been shown to occur in adults (WHO, 1981; Bonsdorff *et al.* 1978) and has been detected in many developed and underdeveloped countries.

An interesting feature of this study was the demonstration of two agents (*Sh. sonnei* and rotavirus) as the aetiologic cause of a common source epidemic. Although we did not find a similar situation in the literature the existence of multiple agents should be considered since in sporadic cases the detection of multiple agents has been achieved (Madeley *et al.* 1977).

Epidemics by one or other of these two agents have been described. Black, Graun & Blake (1980) studied 110 common-source outbreaks caused by shigella in the United States and Gangarose (1971) has described a large outbreak in Central America. Rotavirus was the agent that caused a large gastroenteritis outbreak in an isolated Pacific island group causing more than 3000 cases (Foster *et al.* 1980) and Linhares *et al.* (1979) described an outbreak among Indians living in Pará State (Brazil). Lycke *et al.* (1978) described an epidemic in a small Swedish town where 3172 cases were registered, indicating that at least 30 per cent of the population had been afflicted. All age groups were infected but the most pronounced effect was in the school age groups. Observation suggested that the water supply was contaminated with sewage effluent.

From the preliminary interviews, the symptomatology and its severity showed considerable variation. Many patients presented upper gastrointestinal symptoms (nausea, vomiting, abdominal pains) while others had dysentery-like disease (mucus and blood in their stools) and again others only produced frequent watery diarrhoea. The clinical opinions of the physicians attending the students also varied. This wide spectrum of symptoms can be explained, since shigella causes a self-limiting disease with a sudden onset of abdominal pain, cramps, diarrhoea and fever. The stools are liquid and can contain mucus and blood. Rotavirus infection is also a self-limiting disease with abdominal cramps, but vomiting and nausea are more predominant and the diarrhoea is copious and watery.

The incubation periods for both rotavirus (Middleton, 1978) and shigella infection is approximately 48 h (variation 1–3 days) and explains why the distribution of onset of symptoms occurred in a unimodal fashion (fig. 1).

The overall attack rate of 70–80 % of the students was high but again may be explained by the multiple agents in this study. Reviewing the waterborne outbreak

of shigella in the United States, Black, Graum & Blake (1980) found an average attack rate of 65 per cent in semi-public water systems. The attack rate by rotavirus alone in common-source outbreaks has not been described.

In view of the explosive nature of the outbreak special attention was given to the possibility of a common source such as food or water. Food served could be eliminated by statistical analysis, while the concession stand could also be excluded because the nursery and kindergarten were also sick but they were not allowed to buy food at this stand. The possibility that the stand, outside the school, was a cause is unlikely because only a small minority bought food there.

Although the information on the water supply system after the epidemic was confusing, the fact remains that this school was accidentally pumping water known to contain coliforms into the system and that the chlorination was inadequate. For this reason the water is the prime suspect as the vehicle of transmission in this epidemic. In the majority of waterborne shigella outbreaks reviewed by Black, Graun & Blake (1980) it was shown that untreated or inadequately treated water was incriminated, due to deficient or interrupted chlorination, and that most outbreaks involved semi-public water systems such as the one described in this study.

The diagnostic methods utilized for the detection of rotavirus antigen in this study showed that the ELISA and IEM are sensitive methods in agreement with observations made by other works such as Ellens *et al.* (1978).

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