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A large outbreak of gastroenteritis associated with a small round structured virus among schoolchildren and teachers in Japan

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SUMMARY

In March 1989 a large outbreak of acute gastroenteritis occurred simultaneously among schoolchildren and teachers at nine elementary schools in Toyota City, Japan. Illness was observed in 3236 (41·5%) of 7801 schoolchildren and 117 (39·4%) of 297 teachers. The main clinical symptoms were diarrhoea, vomiting, nausea and abdominal pain. Gastroenteritis was significantly associated with the consumption of school lunch served by one particular lunch preparation centre. One food handler at the centre suffered from gastroenteritis during the outbreak. Small round structured virus (SRSV) was detected in 4 of 8 stool specimens from sick persons. The school lunch contaminated by the infected food handler is the most probable source of this outbreak due to SRSV.

INTRODUCTION

Small round structured viruses (SRSVs) including Norwalk agent [1], Snow Mountain agent [2], Hawaii agent [3], Otofuke agent [4] have been identified as causes of acute gastroenteritis. Because almost all SRSVs are uncultivable, their properties have not been characterized in detail. SRSVs are morphologically indistinguishable from each other, but immunological relations among them are complex [5]. SRSVs are usually named after the location where the outbreaks occurred.

Outbreaks of acute nonbacterial gastroenteritis due to SRSVs have been reported in the USA [6, 7], Australia [8], United Kingdom [9] and Japan [4]. These outbreaks have commonly been associated with contaminated water (drinking and swimming), and food (shellfish and salad items).

In the present paper we describe a large outbreak of acute gastroenteritis associated with SRSV among schoolchildren and their teachers in Toyota City, Japan.

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MATERIALS AND METHODS

Microbiological investigations

Microbiological examinations were performed on stool specimens which were collected as close to the onset of illness as possible. Stool specimens and throat swabs were obtained from 2 schoolchildren aged 10 and 11 years old, and 6 teachers aged 25–49 years at one school 3 or 4 days after onset of acute gastroenteritis.

Stools specimens were examined for *Salmonella* spp., enterotoxigenic *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio parahaemolyticus*, *V. cholerae* non-O1, *V. mimicus*, *V. fluvialis*, *Clostridium pefringens*, *Bacillus cereus*, *Aeromonas hydrophila*, *A. sobria*, *Plesiomonas shigelloides*, *Shigella* spp., *Campylobacter jejuni/coli* and *Staphylococcus aureus*. In view of the epidemiological background, parasitic examinations including those for cryptosporidium and giardia were not performed. Stool specimens were inoculated to BGMK, HeLa, HEL, RD-18S cells (cloned cells from RD cells) and analysed for the presence of enteroviruses. Throat swabs were inoculated into MDCK cells for the isolation of influenza viruses, because diarrhoea and vomiting may be caused by respiratory infections due to influenza virus. Enzyme-linked immunosorbent assay (ELISA) was used to detect adenoviral antigens and group A rotaviral antigens in stool specimens [10].

Electron microscopy (EM) examination

Stool specimens were prepared for EM investigation according to the method of Flewett and colleagues [11]. Briefly, stool specimens were prepared as 10% suspensions in phosphate-buffered saline (pH 7·2). The suspensions were centrifuged at 7000 rev./min for 30 min, and then the supernatants were centrifuged at 35000 rev./min for 150 min in a Hitachi SCP85H2 centrifuge with RP-40 rotor. The pellets were resuspended in a few drops of distilled water. The specimens were negatively stained with 3% phosphotungstic acid (pH 7·0) and examined with a JEOL JEM-100CX electron microscope.

Statistical analysis

Univariate χ^2 and Fisher's exact test were used for data analysis.

RESULTS

Epidemiological features

An outbreak of acute gastroenteritis occurred simultaneously among schoolchildren and teachers at nine elementary schools in Toyota city between 4 and 6 March 1989. Gastrointestinal illness was identified by self-administered questionnaires in 3236 (41·5%) of 7801 schoolchildren (ages from 6–12 years) and 117 (39·4%) of 297 teachers (Table 1). The first person became ill at noon on 4 March and the number of patients peaked that night. There was no significant difference in attack rates for males (42·5%) and females (40·3%). No systematic effort was made to determine the frequency of secondary spread, but the available data suggested that it was uncommon. All cases were restricted to the persons at nine schools and no other outbreaks of gastroenteritis in the area were reported to the regional health centre during this period.

Table 1. *Frequency of acute gastroenteritis at nine elementary schools*

School	Schoolchildren			Teachers		
	No. at risk*	No. sick	Attack rate (%)	No. at risk*	No. sick	Attack rate (%)
A	1177	409	38·0	40	13	32·5
B	1142	540	47·3	41	20	48·8
C	1411	713	50·5	49	25	51·0
D	940	558	59·4	32	21	65·6
E	364	247	67·9	18	11	61·1
F	654	202	21·2	36	4	11·1
G	545	182	33·4	25	11	44·0
H	956	136	14·2	36	4	11·1
I	412	249	60·4	20	8	40·0
Total	7801	3236	41·5	297	117	39·4

* Numbers eating the school lunch.

Table 2. *Clinical symptoms in schoolchildren and teachers with gastroenteritis*

Symptom	Schoolchildren (n = 3236)		Teachers (n = 117)	
	No.	%	No.	%
Diarrhoea	982	30·4	71	60·7
Vomiting	1745	53·9	43	36·8
Nausea	1679	51·9	57	48·7
Abdominal pain	1794	55·4	51	43·6
Fever	789	24·4	26	22·2
Headache	1139	35·2	23	19·7
Malaise	441	13·6	18	15·4
Chills	489	15·1	28	23·9

The prevalence of clinical symptoms among sick schoolchildren and teachers is shown in Table 2. Abdominal pain and vomiting were predominant symptoms among schoolchildren, whereas diarrhoea and nausea were common among teachers. Clinical symptoms were of similar prevalence among nine schools. None of those affected was hospitalized and all recovered within a few days.

No special school events took place prior to the outbreak, and the children and teachers from the nine schools had no chance to meet together in the days preceding the outbreak. Drinking water or food was suspected as a common source of this outbreak. The drinking water for nine schools was supplied by the municipal water system and the residual chlorine levels between 1 and 10 March ranged from 0·1–0·7 p.p.m. The infection was unlikely to have been transmitted by water because no problems were reported with the water supply system and no outbreaks occurred in the community which shared the same water supply.

All children and teachers at nine schools ate the common school lunch served by one lunch preparation centre. Food history analysis indicated that the illness was significantly associated with consumption of school lunch on 3 March ($P < 0·01$). Food items consumed at the lunch included rice, boiled vegetables with peanut butter, fried fish, pudding and milk. Boiled vegetables with peanut butter was mostly suspected as a causative food of this outbreak from statistical analysis (P

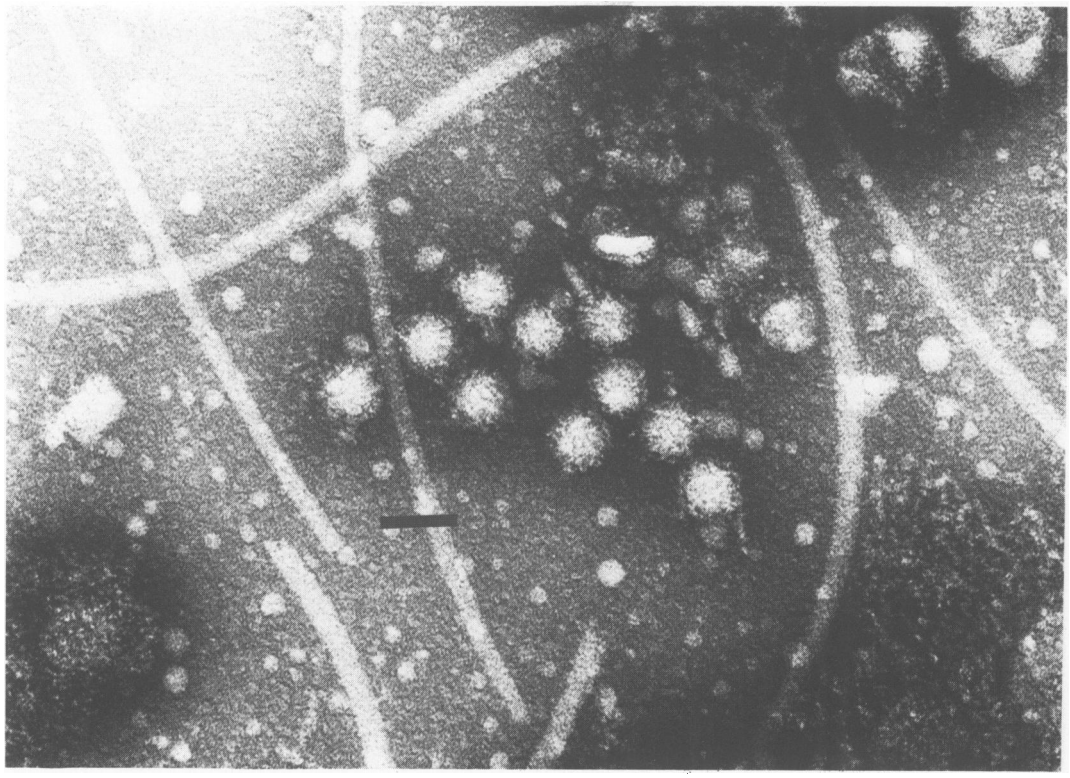

Fig. 1. Small round structured virus from patient with gastroenteritis. Bar represents 50 nm.

$< 0{\cdot}01$). One of 48 food handlers who prepared the school lunch reported gastrointestinal illness on 3 March. She had three episodes of vomiting, two episodes of diarrhoea and fever (38 °C) on that day.

Microbiological investigations

Stool specimens from eight patients were all negative for pathogenic bacteria and enteroviruses. Adenovirus and group A rotavirus antigens were not detected in stool specimens. Influenza viruses were not isolated from throat swabs.

SRSV was detected in stool specimens obtained from 1 of 2 schoolchildren and 3 of 6 teachers by electron microscopy (Fig. 1). The virions were 30–35 nm in diameter and possessed a ragged edge. Unfortunately we failed to obtain stool specimens from food handlers at the lunch preparation centre.

DISCUSSION

The epidemiological results of this outbreak, including incubation period (35 h, range 12–72 h), attack rate, duration of illness and the explosive spread of infection, were similar to those of other outbreaks due to SRSV [1, 7, 12–14]. The different frequency of vomiting and diarrhoea between schoolchildren and teachers was also consistent with previous clinical observations that vomiting is more common than diarrhoea among children and diarrhoea occurs more

frequently among adults with SRSV infection [7, 13, 15]. We received no reports of outbreaks of gastroenteritis among inhabitants near the schools before and during this outbreak from the regional health centre. The detection of SRSV and absence of other enteropathogens in the stool specimens of sick patients supported the clinical and epidemiological findings that this outbreak was caused by SRSV. The SRSVs detected in sick patients were morphologically similar to Norwalk virus, the prototype SRSV. Because the standard sera to SRSVs are not commercially available, immunological properties of the detected virions are uncertain.

The acute onset (88% of sick persons became ill within 48 h) and high incidence at nine schools indicated a common source. Water, food, aerosol and person-to-person contact have been suggested as a route of transmission of SRSV [13]. Epidemiological evidence from this outbreak indicated that it was foodborne with a common school lunch as the most probable means of transmission.

It has been reported that persons infected with SRSV excrete virus in faeces and vomitus, and can be infectious for as long as 2 days after resolution of symptoms [14, 15]. Such results suggest that food handlers were the probable source of infection in this outbreak; it seems likely that some foods were contaminated by faeces and/or vomitus via one infected food handler during the preparation of the school lunch.

Among the large foodborne outbreaks due to SRSV, shellfish such as oysters and clams, and raw foods such as salad, bakery products and ham were the commonly identified source [6, 8, 9, 18–20]. The suspected school lunch included none of such food items. There is little information on the stability of SRSV. Dolin and colleagues [21] reported that Norwalk virus is acid-stable (pH 2·7 for 3 h at room temperature), relatively heat-stable (60 °C for 30 min), and ether-stable (20% ether for 25 h at 4 °C). Thus although viruses do not multiply in foods, SRSVs may survive in inadequately heated foods. However, the currently available methods can not identify SRSVs in suspected foods.

We believe that the source of infection is probably the school lunch contaminated by one infected food handler. The present outbreak is particularly large among the reported foodborne outbreaks due to SRSV [18–20].

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