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CALICIVIRUS DETECTED IN OUTBREAKS OF ACUTE GASTROENTERITIS IN SCHOOL CHILDREN

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SUMMARY Outbreaks of acute gastroenteritis occurred in AK and N Primary Schools of Osaka Prefecture early in March and early in November, respectively, 1977.

Epidemiological surveys were carried out in both schools with the following results. The morbidity rates were 21.8% (AK) and 8.5% (N) among all pupils, and 41.7% (AK) and 36.8% (N) in special age groups. The main clinical symptoms were abdominal pain (77.2-91.3%), diarrhea (38.4-41.3%) and vomiting (6.5-41.6%). Electron microscopic observations of negatively stained specimens showed that five of 15 fecal extracts obtained from patients in the two schools contained virus particles of 35-40 nm diameter with some black hollows on the surface of complete particles and ten projection-like structures at the edge of empty shells. These morphological characteristics resemble those of calicivirus particles. Immune electron microscopic examination suggested that outbreak in AK School was associated with this virus. Attempts to cultivate the viruses in various cultured cells and in suckling mice were unsuccessful.

INTRODUCTION

Caliciviruses have been isolated from porcine, pinniped and feline species, as reviewed by Studdert (1978), and this virus group has been proposed as a new family of vertebrate viruses, "Caliciviridae" (Matthews, 1980). Calicivirus has also been detected in human feces by electron microscopic (EM) examination and it has been reported that this virus may be associated with acute gastroenteritis of infants and young children (Flewett and Davies, 1976; Madeley and Cosgrove, 1976; Kjeldsberg, 1977; Spratt et al., 1978; McSwiggan et al.,

1978; Cubitt et al., 1979; Chiba et al., 1979; Suzuki et al., 1979). In addition, it is suggested that calicivirus is an etiological agent of acute gastroenteritis on the basis of immune electron microscopic (IEM) examinations (McSwiggan et al., 1978; Cubitt et al., 1979; Chiba et al., 1979; Suzuki et al., 1979). Calicivirus has also been investigated in relation to outbreaks of gastroenteritis in an infant home (Chiba et al., 1979) and of winter vomiting disease in an infant and junior school (McSwiggan et al., 1978; Cubitt et al., 1979), but there

are fewer reports about these cases than about cases of rotavirus infection.

This paper reports that calicivirus was detected in outbreaks of acute gastroenteritis in two primary schools in Osaka and suggests that outbreaks were associated with this virus.

MATERIALS AND METHODS

1. Subjects of epidemiological survey

In 1977 there were outbreaks of acute gastroenteritis in AK Primary School (AK School) in Higashiosaka City early in March and in N Primary School (N School) in Matsubara City early in November. Clinical symptoms were surveyed by questionnaires to the parents of cases in both schools.

2. EM observation

Purification and EM observation on fecal extracts obtained from patients in both schools were carried out by the methods described previously (Oishi et al., 1979).

3. Serological studies

Eight paired sera obtained from cases in AK School were tested for antibodies against various antigens as follows. The presence of antibodies to human adenovirus type 4 and calf rotavirus (NCDV) were tested by the complement fixation (CF) test, those to influenza viruses (A/Yamanashi/20/76 (H3N2) and B/Gifu/2/73) by the hemagglutination-inhibition (HI) test, and those to feline calicivirus, which was kindly supplied by Dr. M. Hara, Azabu Veterinary College, Kanagawa, Japan, by the neutralization (NT) test.

4. Immune electron microscopic (IEM) examinations

IEM examinations were performed on 1:5 diluted samples of two paired sera and a partially purified virus suspension of a mixture of fecal extracts from cases in the two schools. (Tests were not made on individual fecal specimens.) The amounts of antibody in the sera were assessed by the grades of aggregation and coating with antibody, as described by Kapikian et al. (1972).

5. Virus cultivation

Fecal extracts known to contain virus particles were inoculated onto the following cultured cells: primary green monkey kidney, human embryonic lung (HEL) and kidney (HEK), primary feline kidney, HEp-2, FL, GMK (a cell line of green monkey kidney), Vero, BHK-21, and Pky cells (a pig kidney cell line, which was supplied by Dr. N. Ueba of this laboratory). The cells were examined daily for cytopathic changes. Some samples were also inoculated into one-day-old suckling mice by the intracerebral or intraperitoneal route.

RESULTS

1. Outbreaks of acute gastroenteritis

Data on the outbreaks of acute gastroenteritis, which occurred in AK School early in March and N School early in November, 1977, are shown in Table 1. These outbreaks each lasted only two days, but there were a few cases before and after the outbreaks. The numbers of cases, scored from data on the onset of the disease in AK School, are shown

TABLE 1. Outbreaks of acute gastroenteritis in two primary schools in Osaka

Primary school	Outbreak	No. of cases	Morbidity rate	
			Whole school	Special age group
AK	Early March 1977	259		6-10 years
			21.8% (259/1189) ^a	41.7% (250/600)
N	Early November 1977	91		10-11 years
			8.5% (91/1068)	36.8% (14/38) ^b

^a No. of cases/No. of pupils.

^b Data on one class.

in Fig. 1. In AK School the morbidity rate was 21.8% among all pupils, and 41.7% among children aged 6–10 years old, while in N School it was 8.5% among all pupils and 36.8% among children of 10–11 years old in one class. One teacher in N School also suffered from the same illness. Secondary infections

among 250 members of the patients' families in AK School, including 23 cases in siblings and 7 cases in mothers, mainly occurred two or three days after the onset of the disease in the school.

The clinical symptoms summarized in Table 2 show that the frequency of abdominal pain (77.2–91.3%) is rather higher than those of diarrhea (38.4–41.3%) and vomiting (6.5–41.6 %).

2. Results of EM observations

On EM observations of 15 fecal extracts from cases in the two schools (13 from School AK and 2 from School N), small spherical particles of 35–40 nm diameter were detected in five samples (4/13 from AK and 1/2 from N). Negative staining particles showed that these were of various shapes. A fringe-like structure and some black hollows were seen on complete particles (Fig. 2) and some projection-like structures were seen on empty shells (Fig. 2A, C). The rotation enhancement technique, established by Markham et al. (1963), showed that complete virions had a central hollow surrounded by six similar hollows (Fig. 3A, B) and that empty shells had ten surface projections (Fig. 3C, D) in $n=3$ -fold and $n=5$ -fold rotation, respectively. The shapes of these virions seem to resemble those of calicivirus described by other workers (Almeida et al., 1968; Studdert, 1978; Burroughs et al., 1978; Madeley, 1979).

3. Results of serological and IEM tests

None of the 8 paired sera showed any specific antibody responses against the various virus antigens tested.

IEM examinations showed that 2 convalescent phase sera from cases in AK School contained specific antibody against the virus obtained from these cases, giving a rating of 2–3+ as shown in Fig. 4A and B, while the acute phase sera contained no detectable antibody. These results suggest that, at least in AK School, the acute gastroenteritis was associated with this virus.

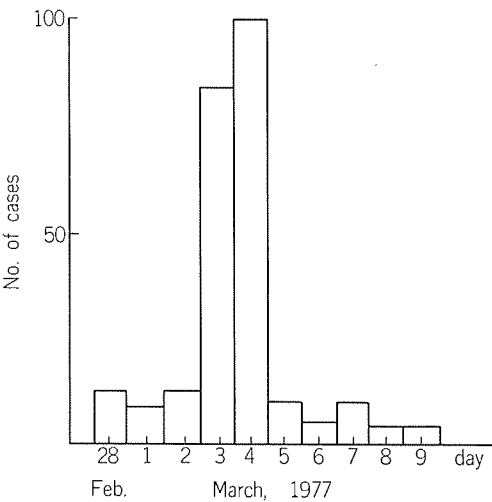


FIGURE 1. Outbreak of acute gastroenteritis in AK Primary School. The no. of cases is plotted against the day of incidence.

TABLE 2. Frequency of clinical symptoms in cases of acute gastroenteritis in AK and N Primary Schools^a

Clinical symptoms	AK School		N School	
	No. of cases	Frequency (%)	No. of cases	Frequency (%)
Abdominal pain	193	77.2	83	91.3
Diarrhea	96	38.4	38	41.3
Vomiting	104	41.6	6	6.5
Nausea	93	37.2	28	30.4
Anorexia	143	57.2	— ^b	—
Fever	45	18.0	—	—

^a The total no. of cases in AK and N Schools were 250 and 91, respectively.
^b Not investigated.

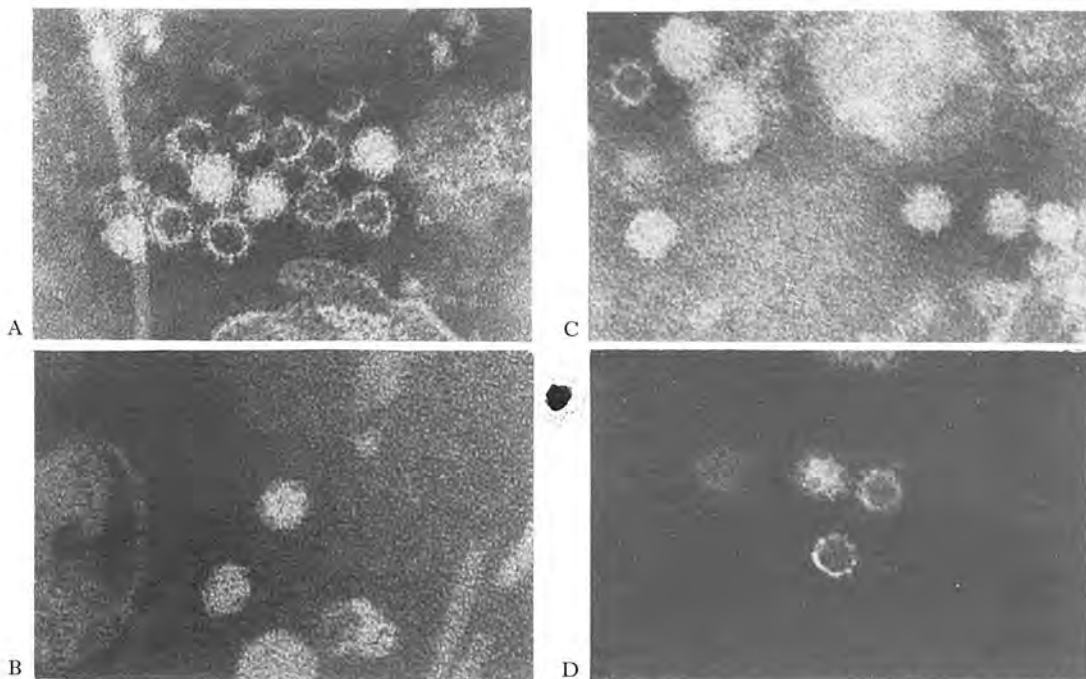


FIGURE 2. Calicivirus detected in fecal extracts of the patients. A and B from AK School, and C and D from N School ($\times 180,000$). Note a fringe-like structure and some black hollows on complete particles (A, B, C, and D) and some projections on empty shells (A and C).

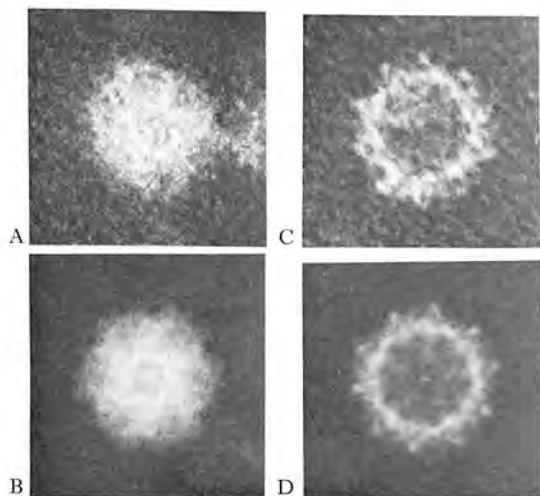


FIGURE 3. Calicivirus from patients seen by photographic rotation enhancement. A (complete) and C (empty particle) are the original electron micrographs by negative staining. B and D are the Markham rotation of A and C, with $n=3$ -fold and $n=5$ -fold rotation, respectively.

DISCUSSION

This paper reports that a virus resembling calicivirus was detected by EM observation during outbreaks of acute gastroenteritis among primary school-children. IEM examinations suggested that the gastroenteritis in AK School was associated with this virus. Furthermore, EM observations indicated that the outbreak in N School may also have been associated with this virus, because of the morphological similarity of the virus particles to those found in cases at AK School.

4. Virus cultivation

Attempts to cultivate virus from fecal extracts in some cultured cells and suckling mice were unsuccessful.

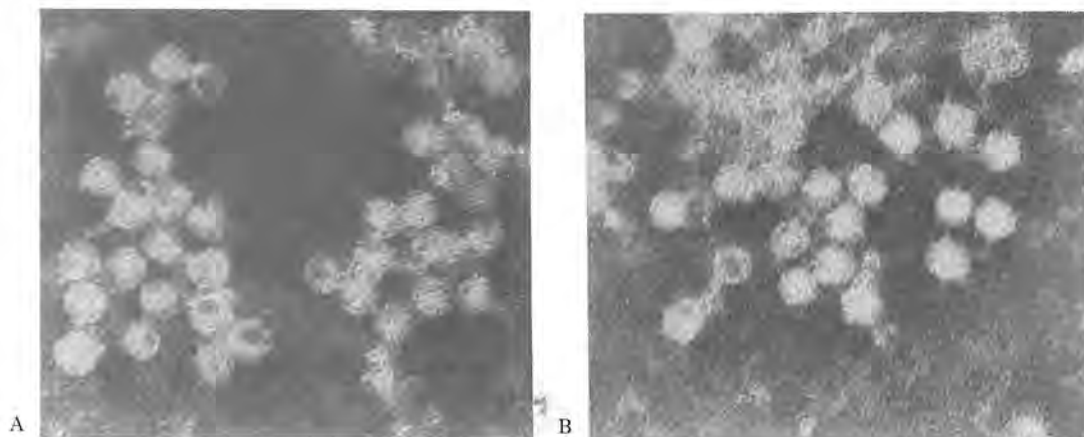


FIGURE 4. Aggregation of calicivirus particles in the IEM test with convalescent phase sera from cases (A; M. Y., and B; I. O.) at AK School. The quantity of specific antibody was scored as 2–3+ for both ($\times 150,000$).

Recent reports (Flewett and Davies, 1976; Madeley and Cosgrove, 1976; Kjeldsberg, 1977; Spratt et al., 1978; McSwiggan et al., 1978; Cubitt et al., 1979; Chiba et al., 1979; Suzuki et al., 1979) have suggested that infants and young children are the main targets of this virus, but the present work shows that school children aged 6–11 years and even adults may also be infected.

Examination of the clinical symptoms in the two outbreaks showed that the frequency of abdominal pain was higher than those of diarrhea and vomiting, but symptoms all seemed to be rather milder than those in cases in infants (Chiba et al., 1979) or in cases of rotavirus infection in similar age groups (Oishi et al., 1979). The latent period of the virus in the cases in AK School was 48 to 72 h, as evaluated on the basis of secondary infection among families.

The virus particles detected in our cases resembled feline calicivirus morphologically, but there was no antigenic cross-reaction between the two, as judged from the results of the NT test (data not shown).

Caliciviruses isolated from the cases could not be cultivated in vitro or in mice, as found by other workers (Madeley and Cosgrove, 1976; Kjeldsberg, 1977; McSwiggan et al., 1978; Cubitt et al., 1979; Chiba et al., 1979; Suzuki et al., 1979). In contrast calicivirus of other species, such as feline, pinniped and porcine virus, can grow readily and rapidly in vitro as reviewed by Studdert (1978).

It seems to be essential to cultivate the virus for detailed studies on it and for its use in epidemiological surveys on humans.

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