Winter vomiting disease caused by calicivirus

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SUMMARY   The clinical, epidemiological, and virological features of an outbreak of winter vomiting disease among London schoolchildren are described. Evidence is presented to support the view that this epidemic was caused by a human calicivirus, a virus not previously shown to be associated with this disease in man.

The term 'The Winter Vomiting Disease' was introduced 50 years ago by Zahorsky (1929) to describe what he believed to be a previously unrecognised disease. The predominant clinical features of the illness he described were vomiting, sometimes forceful, a proportion of victims also suffering abdominal cramp and diarrhoea. Symptoms rapidly disappeared, and complete recovery was the rule. The disease was highly infectious, attacking infants and young children, although adults were sometimes affected.

During the subsequent 40 years after Zahorsky's report, numerous outbreaks with features approximating closely to the original description have occurred (Rischel, 1935; Miller and Raven, 1936; Gray, 1939; Bradley, 1943; Reimann *et al.*, 1945; Silverton, 1952; Webster, 1953; Clarke *et al.*, 1972). Although a virus aetiology was strongly suspected in all these outbreaks, no virus was isolated, and in only one were transmission experiments with bacteria-free filtrates convincingly positive. More recently, however, electron microscopy has provided evidence indicating the possible association of several viruses with such outbreaks. Probably the most studied of these viruses has been the Norwalk agent, which was associated with an extensive outbreak of vomiting and diarrhoea in Norwalk, Ohio (Adler and Zickl, 1969; Thornhill *et al.*, 1977). This agent is described as a parvo-like virus, although antigenically distinct from other parvoviruses (Greenberg *et al.*, 1979). Several other viruses have been associated with outbreaks of winter vomiting disease or epidemic vomiting. The W (Clarke *et al.*, 1972; Appleton *et al.*, 1977), Harlow (Appleton and Higgins, 1975), and Ditchling agents (Appleton *et al.*, 1977) have been incriminated in outbreaks in

this country, and the Hawaii and Montgomery agents (Thornhill *et al.*, 1977) in the United States. None of these agents has yet been cultured nor has any been characterised to any great extent; they are usually referred to as 'small round viruses' or 'unclassified agents' (Andrewes *et al.*, 1978a).

In a preliminary report (McSwiggan *et al.*, 1978), we referred to an outbreak of winter vomiting disease among London schoolchildren caused by a virus not previously associated with this disease in man. We now give in more detail the clinical, epidemiological, and virological features of that outbreak.

Clinical features

The outbreak occurred in January 1978 in an infant and junior school in north-west London. The school accommodated 235 children aged 4 to 11 years in three infant and four junior classes. There were 26 teaching and ancillary staff. The distribution of the 13 cases which occurred at the school is shown in Table 1. The class most affected was reception, which contained the youngest pupils. Nearly one-third of the children and the teacher of this class developed overt illness (Table 1). The three affected children in the higher classes were all siblings of affected children in the reception class. Only one case is known to have occurred outside the school, a 19-month-old sibling of one of the affected pupils in the reception class. After onset the number of cases built up rapidly (Fig. 1). The three children in the higher classes demonstrated symptoms 24-48 hours after their younger siblings, and the one patient outside the school became ill 48 hours after his sister. Vomiting was the most frequent clinical feature, occurring in 10 out of 14 patients; colic
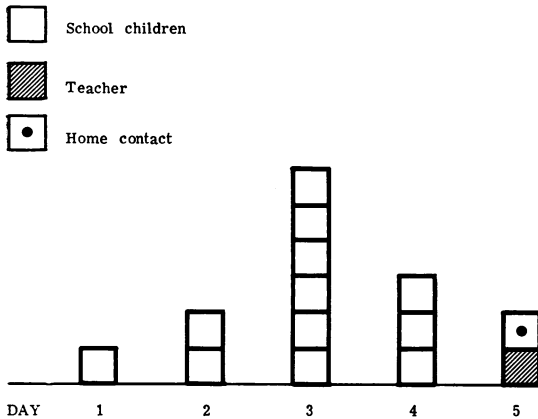
☐ School children

▨ Teacher

▣ Home contact

Fig. 1   *Distribution of cases by day of onset.*

Table 1   *Distribution of illness in school*

Subjects	Total No.	No. ill
Reception class	37	9
2nd year infants	36	—
3rd year infants	36	1
1st year junior	35	2
2nd–4th year junior	91	—
Teaching and ancillary staff	26	1
Total	261	13

Table 2   *Clinical features in 14 cases*

Vomiting	6
Vomiting and colic	3
Diarrhoea and colic	2
Diarrhoea	2
Vomiting, diarrhoea and colic*	1

*Adult

occurred in six and diarrhoea in five (Table 2). Symptoms lasted 24-72 hours, and all affected made a complete and rapid recovery.

In view of the features of the outbreak, stool samples from the first cases were examined concurrently for bacterial and viral agents. *Salmonella*, *Shigella*, and *Campylobacter* species and *Clostridium perfringens* were not detected, but the initial faecal specimens revealed calicivirus in large numbers (Fig. 2) in a number of the affected children. As a result of this finding, serial specimens of faeces were sought from all the affected children and from unaffected contacts for comparison. Capillary blood was collected on filter paper on at least one occasion from all those affected. Specimens were obtained from eight children between 8 and 10 days after onset of illness and further specimens from all the 14 cases four weeks later. It was not thought desirable to seek capillary blood from well children,

but, in the event, several samples were taken from unaffected children in the reception class due to a misunderstanding. Venous blood was obtained from unaffected teachers and ancillary workers.

Laboratory investigations

1 EXAMINATION OF FAECES

(a) An emulsion of faeces was prepared in 3% potassium phosphotungstic acid (KPTA), pH 6·4, and placed on a formvar carbon-coated grid; excess fluid was removed, and the grids were examined in a Phillips 201 electron microscope.

(b) A 10% suspension of faeces was made in phosphate buffered saline (PBS), pH 7·4, and mixed in a Rotamixer (Hook and Tucker); this emulsion was then centrifuged at 1500 g for 20 minutes; 0·8 ml of the supernatant was layered onto 4·0 ml of 30% sucrose and centrifuged at 40 000 rpm for 2 hours in a SW50:1 rotor on a Beckman L5-50 centrifuge. Grids were prepared from the resulting pellet after being resuspended in a few drops of distilled water, and were then stained and examined.

2 BUOYANT DENSITY DETERMINATIONS IN CAESIUM CHLORIDE

Several pellets, prepared as described above, were pooled in 0·3 ml of PBS. This suspension was layered onto CsCl, density 1·38 g/l, and centrifuged at 40 000 rpm for 18 hours at 4°C in a SW50:1 rotor on a Beckman L5-50 centrifuge. Twelve fractions were collected from each gradient. The density of each fraction was determined by weighing 0·1 ml aliquots. 20 μl aliquots were removed from each fraction and placed onto 2% agarose contained in the wells of a microtitre plate. A formvar carbon-coated grid was placed on the fluid and left for 1 hour to enable the CsCl to diffuse into the agarose. The grids were then stained with KPTA and examined for the presence of virus particles in the electron microscope.

Feline calicivirus was grown in feline lung cells until the entire cell sheet showed cytopathic effect. The cells and tissue culture fluid were harvested and pelleted in the ultracentrifuge. This material was used as a reference preparation and treated in an identical manner to the virus obtained from the faecal specimens.

3 IMMUNE ELECTRON MICROSCOPY

Faeces from a child collected on the first day of illness, shown to contain large numbers of single particles, was chosen as the source of antigen (Roth strain). Material prepared from this specimen, as described under (1b) above, was used as a source of antigen in all serological tests. A section measuring
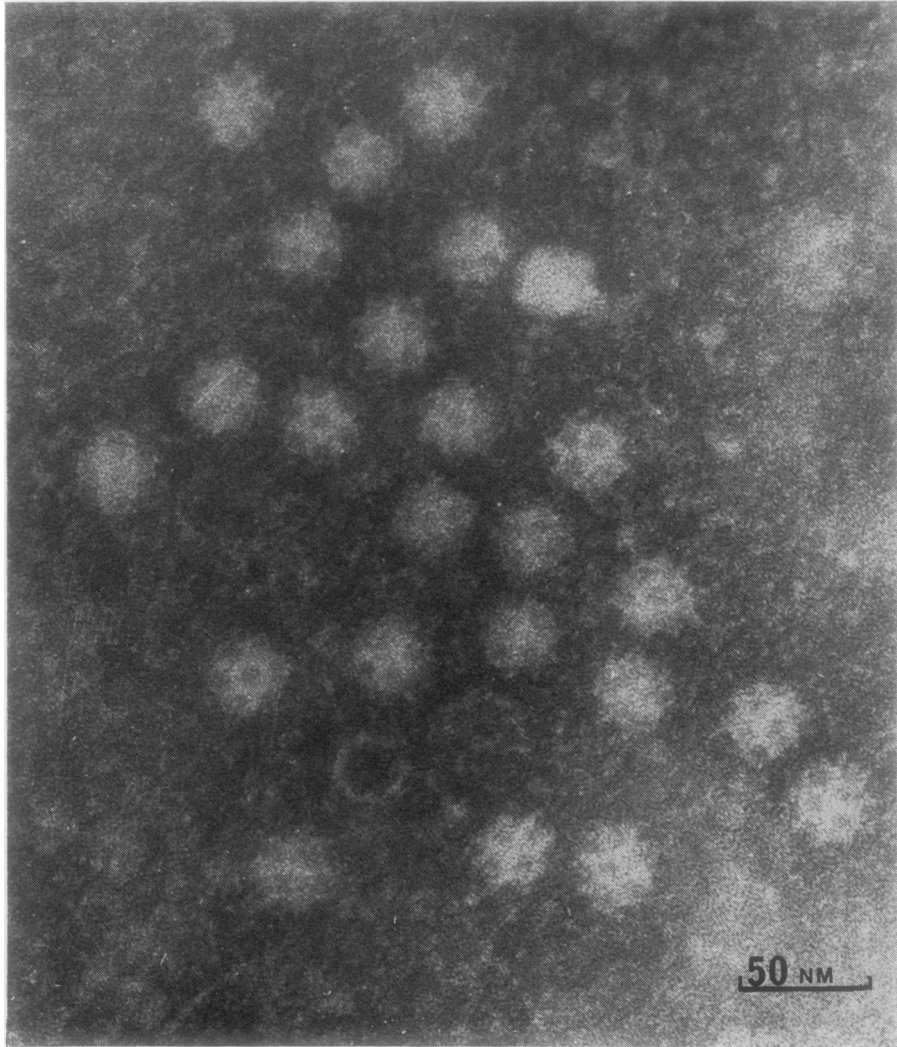
Fig. 2   *Electron micrograph of virus particles in stool (Roth)* × *348 000*

5 mm² and completely covered with blood was cut out of each filter paper, and the blood was eluted in 0·1 ml complement fixation test (CFT) buffer for 2 hours at room temperature. An equal amount of 'neat' eluate was mixed with 25 μl of antigen suspension; further tests were made with doubling dilutions of the eluate, up to 1 in 8, prepared in CFT buffer. Mixtures were placed onto 2% agarose contained in the wells of a microtitre plate, and formvar carbon-coated grids were inverted over each drop. After incubation at room temperature overnight the grids were removed from the agarose and stained with 3% KPTA and examined in the electron microscope. The serum from adults was examined neat and in doubling dilutions by the same method as that described for capillary blood; 25 μl of (Roth) antigen mixed with 25 μl of PBS was used as a control preparation.

ESTIMATION OF DILUTION FACTOR OF ANTIBODY FROM WHOLE BLOOD WHEN ELUTED FROM FILTER PAPER

Venous blood was dropped onto Whatman's filter paper No. 1 and allowed to dry; 5 mm² areas covered with blood were cut out and placed in 0·1 ml of CFT buffer, and the blood was allowed to elute at room temperature for 2 hours. Doubling dilutions of eluate were prepared in CFT buffer, and

10 μl aliquots were placed in the wells of a 1% agarose gel slide. Serial dilutions of the same venous blood were prepared in tubes, and 10 μl aliquots were placed in wells of an agarose gel. The respective end points at which IgG could be detected in the eluate and the 'whole' venous blood were compared by allowing dilutions to diffuse towards rabbit anti-human IgG and examining the gels for the presence of precipitin lines.

TISSUE CULTURE STUDIES

Pellets prepared from faeces (1b) were resuspended in 0·5 ml PBS, and 0·1 ml of the suspension was inoculated into primary monkey kidney, primary baboon kidney, primary human embryo intestine, human embryo kidney, MRC5, Hep, Vero, and feline lung cell cultures. These were incubated at 35 °C on roller drums and examined daily for cytopathic effect. Cultures were maintained for three weeks through two passages. Cells and tissue culture fluid were examined by electron microscopy at each passage.

ANIMAL STUDIES

One-day-old mice were inoculated intracerebrally and subcutaneously with material prepared as for tissue culture inoculation. The mice were examined daily for 14 days for any signs of illness.

Results

The results of stool and antibody tests on the 14 symptomatic cases are shown in Table 3. Virus was seen in the stools of seven and antibody was detected in 13 of the 14 affected people. Three showed evidence of an increase in antibody between the first and second sera. The eluted blood was calculated to have been diluted 1 in 64 (geometric mean titre). This would be the equivalent to a titre of 1 in 128 in serum from a clotted blood specimen obtained by conventional venepuncture.

Owing to a misunderstanding, stools were obtained from three unaffected children in the reception class. In addition, stools were obtained by request from 10 other unaffected children and six teachers. These were all taken within six days of the last case showing symptoms. Virus particles were not detected in any of these specimens. (Many more specimens from unaffected children were examined and found to be negative, but these were not considered valid for control purposes for reasons stated below.)

Antibody was not detected in the capillary blood from the three unaffected children, nor in the venous blood of the six well adults.

The rate of detection of virus correlated closely

Table 3 *Results of electron microscopy of stools and immune electron microscopy of sera in 14 patients*

Case	Virus particles seen by EM	Immune electron microscopy on eluted sera*	
		1st Specimen	2nd Specimen
1	+	NA	+ Neat
2	+	+ Neat	+ 1 in 4
3	+	NA	+ 1 in 4
4	+	+ 1 in 4	+ 1 in 4
5	+	NA	+ 1 in 4
6	+	NA	+ 1 in 4
7	+	+ Neat	+ 1 in 4
8	—	+ Neat	+ Neat
9	—	NA	+ 1 in 4
10	—	Negative	+ Neat
11	—	NA	+ Neat
12	—	+ 1 in 4	+ 1 in 4
13	—	+ Neat	+ Neat
14 (Adult)	—	Negative	Negative

*Neat eluate = serum diluted 1 in 128
NA = NOT available

Table 4 *Relation between time after onset of illness and ability to detect virus in stool of seven virus-positive cases*

Day	No. positive	
	Unconcentrated specimen	Concentrated specimen
(a) *Day after onset first specimen obtained from the 14 cases*		
1	1/1	1/1
2	—	—
3	2/4	2/4
4	0/1	0/1
5	0/3	2/3
6	0/3	2/3
7	0/2	0/2
Total	3/14	7/14*

(b) *Subsequent specimens from 7 initially positive patients*
| >6th day | 0/35 | 0/35 |

with the time after onset at which the stool was obtained (Table 4). Direct examination of stools demonstrated virus in three specimens obtained within three days of the onset of symptoms. Differential centrifugation before electron microscopy revealed virus in a further four stools; however, virus particles were not detected in any specimen taken after the sixth day of onset. The rapid disappearance of virus from the stool by the sixth day was confirmed when virus could not be detected in subsequent stools from any of the seven previously positive cases.

The particle size, measured from furthest point to furthest point, showed a mean value of 30·5 nm (Fig. 3). Feline calicivirus was used as a reference for the measurement of size and buoyant density. The results of measurement on the feline calicivirus are in agreement with those previously established (Burroughs and Brown, 1974; Love and Jones, 1974). The distinct morphology of the virus particle, when
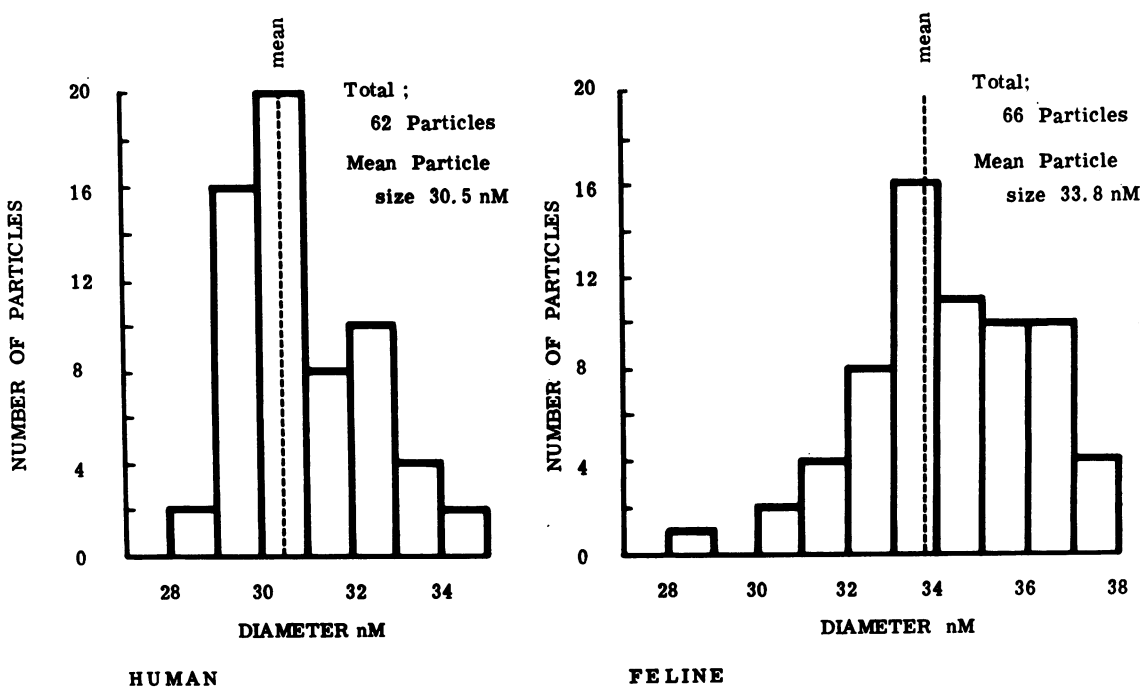
Fig. 3   *Histogram showing distribution and mean of particle size of human and feline calicivirus.*
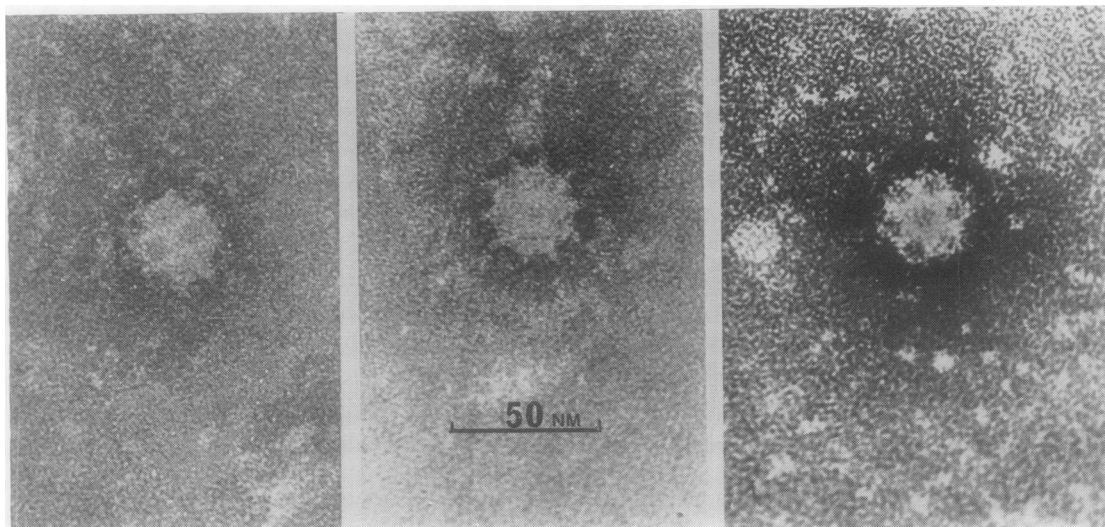

Fig. 4   *Electron micrograph showing virus morphology when viewed along the two-fold axis (square cross), five-fold axis (ten spiked sphere), and three-fold axis (Star of David) × 410 500*
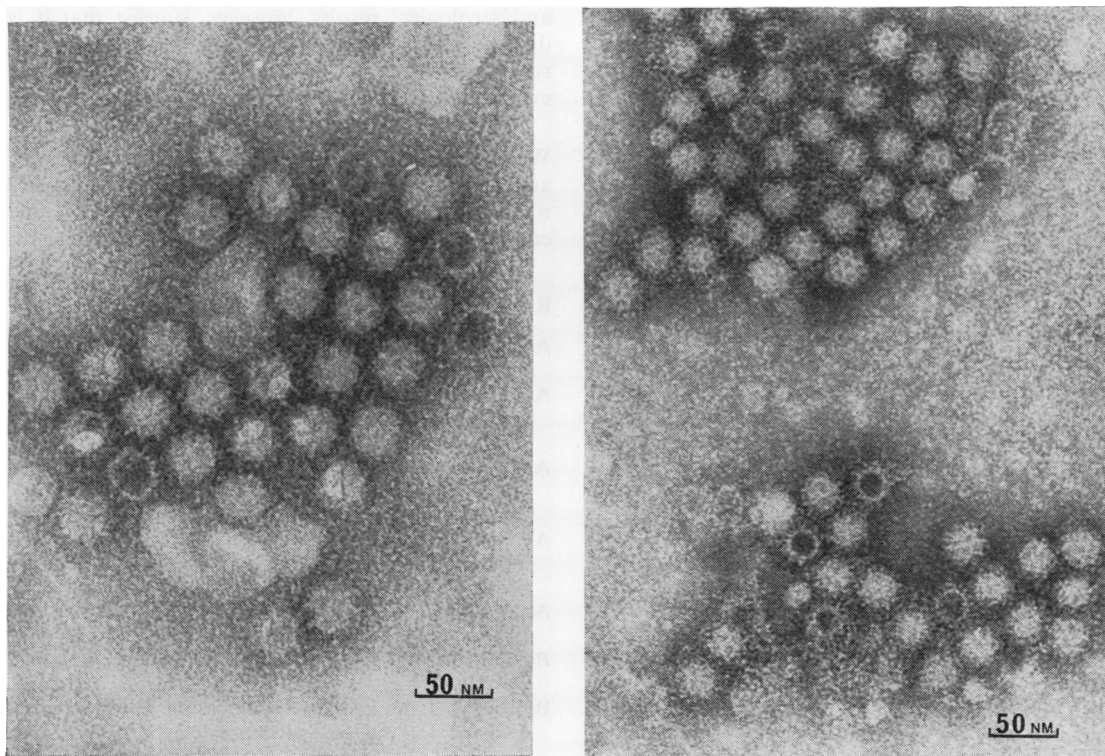
Fig. 5 *Electron micrograph of human (right) × 183000 and feline (left) × 203 500 calicivirus aggregated by homologous antibody.*

viewed along the two-fold, three-fold, and five-fold axes of symmetry, is clearly shown (Fig. 4). The three particles shown are all taken from one stool sample; similar particles were seen in all the other positive specimens. We have not so far been able to demonstrate evidence of viral replication in any of the cell culture systems tested or to show any effect on day-old mice.

Discussion

The clinical and epidemiological features of the illness in the London schoolchildren correspond closely with the original description of winter vomiting disease (Zahorsky, 1929). Although the mode of transmission is not apparent, the distribution of cases among the youngest children in the school and the very limited spread (one case) to home contacts, might be taken as evidence of widespread acquisition of immunity to the causal agent shortly after the age of school entry. Only two of the 12 affected schoolchildren had *younger* siblings; one of these two was the affected home contact. As the virus has not yet been grown in tissue culture, insufficient antigen is at present available to allow investigation of the antibody status of the various age groups. This problem may be circumvented if it can be shown that animal calicivirus antigen can be used to test human sera, but this has not yet been established. Immunity in the older group is not necessarily the only explanation for the age-related distribution in this school outbreak. Shigella dysentery is more common in younger schoolchildren owing to their lower personal hygiene, particularly in relation to handwashing after defaecation. A similar mechanism might have influenced the age-related epidemiological pattern of spread in this outbreak.

It has been pointed out that the availability of electron microscopy for clinical investigations has resulted in an 'embarras de richesses' (Flewett, 1976) in terms of putative aetiological viruses in gastrointestinal infections. A number of criteria have therefore been proposed (Patterson *et al.*, 1975; Flewett, 1976), which should be satisfied before an agent detected by electron microscopy is accepted as the cause of a particular disease. These criteria have been sufficiently satisfied in this instance to justify the claim that the virus demonstrated was the

cause of the illness described. Its presence at the time of symptoms, and rapid disappearance thereafter, have already been referred to; indeed, this may be one reason why it has not been detected in earlier outbreaks of winter vomiting disease. On the other hand, the virus was not seen in those in the same population who were not ill. Similarly, antibody was detected (and, in several cases, shown to rise) at the appropriate time in all but one of those affected (the only adult with symptoms) and was *not* detected in any unaffected person. The use of capillary blood with an unavoidably high dilution factor (due to elution) proved satisfactory in this instance. Antibody determinations subsequently on *venous blood* specimens from three of the children with 'eluted' antibody titres confirmed the accuracy of the dilution factor calculations. Feeding experiments in volunteers (in conjunction with Dr S. E. Reed) and in chimpanzees (in conjuction with Professor A. J. Zuckerman) are being arranged, and the results will be reported.

Caliciviruses (Andrewes *et al.*, 1978b) are well known animal pathogens; indeed, the prototype virus, vesicular exanthema virus, is classified as a category A pathogen (Department of Health and Social Security, 1975). They have not, however, previously been shown to cause disease in man. Madeley and Cosgrove (1976) reported finding 'apparently typical' caliciviruses in the stools of 10 children during a survey in Glasgow. Although these workers were, however, unable to give any significance to the presence of these viruses, the finding of calicivirus in the small bowel of an infant who had died from gastroenteritis (Flewett and Davies, 1976) indicated a possible association with human disease. These workers also reported caliciviruses in the stools of a child with gastroenteritis. However, on this occasion adenoviruses were also present in large numbers. We believe that the agent causing the outbreak of winter vomiting disease in the London schoolchildren is a human calicivirus. The particle size and buoyant density are consistent with this conclusion, although these features are compatible with several other small viruses, for example, parvoviruses and some 'small round' viruses. Caliciviruses, however, have a very distinctive morphology, demonstrating cuplike depressions (calex = cup) on their surface and presenting three characteristic forms, depending on whether the virus is visualised along its two-fold, three-fold, or five-fold axes of symmetry (Burroughs *et al.*, 1978). The agent we have identified demonstrates the size and very distinctive morphology of the Caliciviridae, which strongly suggests that it is a member of this group. (A feline calicivirus is shown for comparison with the human strain (Fig. 5).) Preliminary cultural and serological results, however, suggest that it is distinct from the three established animal caliciviruses and likely to be of human origin. Further evidence for this is being prepared.

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