ROTAVIRUS AND OTHER UNCULTIVABLE ENTERIC VIRUSES
IN THE EPIDEMIOLOGY OF ACUTE DIARRHOEAL DISEASE
IN SOUTHERN INDIA

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RUNNING TITLE: Uncultivable viruses and diarrhoea.
Direct electronmicroscopic examination of suitably prepared faecal samples is now recognised as an essential method to detect a group of agents, the uncultivable enteric viruses, which may be causally related to acute diarrhoeal disease (1). During the later half of the 70s, a large number of reports on a variety of virus particles have come from different parts of the world, and it is now well established that several of these agents could be causative in producing acute diarrhoea in children and adults, both as endemic cases and in epidemics (2).

Since 1974, a systematic search for these uncultivable enteric viruses have been carried out at Vellore and the current status of our studies is summarised in this paper. The methodology used consists of obtaining stool samples from patients with acute diarrhoea as well as from appropriate age matched controls. 20 per cent suspension of faecal samples in phosphate buffered saline is clarified by low speed centrifugation, the supernatant spun down at 90,000g for 30 minutes and the
pellet appropriately applied to grids and examined in the electronmicroscope. This methodology has been used in preference to adding agents like Freon 113 or PEG since these tend to obscure or destroy the details of some of the uncultivable viruses.

The role of rotavirus in acute diarrhoeal disease of childhood

Rotavirus was identified in the stools of children presenting to hospital with acute diarrhoea in southern India in 1974\(^3\). A one year hospital based study of children under two years presenting to hospital with acute diarrhoea carried out in 1974 and 1975 at Vellore showed that in 25% of these children rotavirus could be identified. However, in 66% of the children pathogenic bacteria which could be causally linked to diarrhoea were isolated. These included enterotoxigenic and enteropathogenic strains of \(E\).\( coli\)\(^4\). A subsequent one year survey of cases presenting to hospital with acute diarrhoeal disease at Vellore confirmed the earlier observations. At Vellore, which is an area of relatively low rainfall (90 cm a year) at an elevation of about 700' above mean sea level and 80 miles inland from the coast, rotavirus was detected
in hospitalised children with striking seasonal prevalence. These viruses were detected during the colder months of the year, August to February and were not detected during the hot dry summer month of May. In this region there is a considerable seasonal fluctuation in temperature, the winter months having temperatures ranging from 15 to 35°C and in the hot dry summer from 30 to 45°C.

The findings at Calicut about 300 miles west of Vellore on the west coast of India, in an area of high rainfall and relative humidity, as well as more or less uniform temperature throughout the year were a striking contrast to Vellore(5). At Calicut, 77% of hospitalised children with acute diarrhoea had rotavirus in their stools. Recognised enteropathogenic bacteria could be cultured only from about 10% of the stools. Rotavirus was detected throughout the year and during November to January, they were present in almost 100% of the children sampled. The lowest frequency of rotavirus detection was during May which is a relatively hot and dry month in this part of India.

A seroepidemiological survey was carried out both at Vellore and in Calicut(6). Using Enzyme linked Immunosorbent Assay (ELISA) for detecting serum antibodies of all three Ig classes,
antibody titres were found to be very high at Vellore in all age groups. Adults had antibody titres which were significantly higher than children and the highest antibody titres were in those above the age of 40 who were mainly grand-parents. The antibody titre in children from a protected background suggested that they were less exposed to rotavirus infections than children from a lower socioeconomic background. A marked seasonal variation in rotavirus antibody titres suggested that constant infection was a possible factor in the genesis of the very high titres observed. In contrast to the findings at Vellore, in Calicut antibody titres were highest in children and children who were having rotavirus diarrhoea had a lower antibody titre than unaffected children. The highest antibody titres were in the mothers of children with rotavirus diarrhoea while all older age groups had lower geometric mean titres. The geometric mean titres in children without diarrhoea at Calicut and at Vellore were comparable.

The results of these preliminary studies show that in two areas of southern India with striking differences in the ecology there were differences in rotavirus prevalence in hospitalised children with
diarrhoea. To understand these differences further, it is essential to carry out prospective studies in the community.

Epidemics of diarrhoea possibly associated with uncultivable enteric viruses

Several epidemics of acute diarrhoea have been studied in this region especially those caused by a variety of bacterial enteric pathogens (7-10). During 1979, an acute diarrhoeal epidemic in a village 43 km southwest of Vellore was detected and was probably due to an uncultivable enteric virus. The epidemiological and clinical features of this outbreak are described here.

SVV is a predominantly agricultural village with a population of 648. Between 1st of January 1979 and 31st April 1979, 176 cases of acute diarrhoea were detected in this village (figure I). All age groups were affected with the highest attack rates in preschool children (table 1). The patients affected by this illness were in general not febrile. They passed watery stools without blood and mucus for a few days without vomiting. Some of the patients complained of increased borborygmi. The median
duration of illness was 4 days and the mean duration was 9 days. Only 23 of the affected individuals had diarrhoea lasting for more than 15 days and even among them there were episodes of remission and relapses so that the second attack could not definitely be identified as a relapse or a reinfection.

Stools for bacteriological and virological studies were obtained for 62 patients (34 during the first seven days of illness and 28 with longer duration) and 23 age matched controls. Using a variety of media pathogenic bacteria were isolated from 4 patients and two controls (3 shigellae and 2 salmonellae). Rotavirus was not detected in any stools. Human enteric corona virus was present with equal frequency among the patients and controls (table 2). A small round virus particle (figure 2) was present in 11 of 34 patients during the first week of diarrhoea and in 1 two year old control who developed diarrhoea 2 days after the stool collection. It was not detected in any patient who had diarrhoea longer than 7 days or any of the other controls (table 2).

The prevalence of this small virus particle only in patients during the early period of the illness, is strongly suggestive of a causal role. Unfortunately it was not possible to obtain blood samples from acute
and convalescent cases to confirm the infection by the presence of antibodies. This particle morphologically resembles the calcivirus, known to cause acute diarrhoeal epidemics. The spread of the epidemic over 18 weeks does not suggest a common source epidemic and in fact no common vehicle could be identified in the village, such as water supply. The median case to case interval in families with multiple cases was 9 days suggesting a person to person or vector mediated transmission. The epidemic subsided with the onset of the hot dry summer.

**DISCUSSION**

Viral agents detectable by direct electronmicroscopic examination of faecal samples are now well recognised as the aetiological agents of acute diarrhoeal disease \(^{1,2}\). Among these, the rotavirus has now been well established as possibly the single most important cause of this problem \(^2\). The data presented here establishes that the magnitude of rotavirus infection in the community in southern India is very large judged either by detecting the agent in children with diarrhoea or by seroepidemiological methods and that there are regional differences in epidemiology. However, the role of other uncultivable viruses is not yet clear. Human enteric coronaviruses
are widely prevalent in southern India (table 2) but as yet they cannot be implicated in the etiology of acute diarrhoeal disease\textsuperscript{(11)}. It has been suggested that they may be a cause of the syndrome of neonatal necrotizing enterocolitis\textsuperscript{(12)}. Determining the aetiological roles of the other viral particles that are seen in stool is even more fraught with difficulty. Unequivocal rise in antibody titre in serum obtained in convalescent cases as compared to serum from acute cases would be strongly suggestive of an etiological role. In the absence of such proof the association of a single morphological types of virus particle with short duration cases in a definable epidemic, without such particles being present in controls gives presumptive evidence of an aetiological role.

Acute diarrhoeal diseases continue to be a major cause of morbidity and mortality, especially in the rural areas of southern India as in other parts of the developing world. Electronmicroscopy of suitably prepared faecal samples has added a powerful new tool in the search for the etiological agents causing acute diarrhoea. It may be argued by some that the cost involved in a proper electronmicroscopic survey for viral aetiological agents is not justified when it
would be possible to treat the majority of such cases with oral maintenance of hydration. However this is purely a therapeutic approach and the ideal way to combat acute diarrhoeal diseases in the developing world would be to develop vaccines or devise means of interfering with the infection cycle in the community. This would be possible only if the variety of etiological agents and the mechanism of their transmission in the community are known. Direct EM examination of faecal samples in addition to detailed bacteriological studies is a very useful and essential part of epidemiological investigations of diarrhoeal disease endemic and epidemic in developing countries.

Acknowledgement

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References


# Table 1. Attack rates

<table>
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<th>Age groups</th>
<th>Population</th>
<th>Rate per 100</th>
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<tr>
<td>0 - 4.9</td>
<td>86</td>
<td>51.1</td>
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<tr>
<td>5 - 11.9</td>
<td>120</td>
<td>28.3</td>
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<tr>
<td>12 - 17.9</td>
<td>72</td>
<td>22.2</td>
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<tr>
<td>18+</td>
<td>370</td>
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### Table 2. Detection of pathogenic bacteria and viruses

<table>
<thead>
<tr>
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<th>Diarrhoea 1-7 days</th>
<th>Diarrhoea 7+ days</th>
<th>Controls</th>
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<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number studied</td>
<td>34</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>Pathogens</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number studied</td>
<td>34</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Corona</td>
<td>21</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>SRV</td>
<td>11</td>
<td>0</td>
<td>1</td>
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</table>

Bacterial pathogens included 4 *Shigella* and 2 *Salmonella*. *Corona* – Human enteric Corona virus, *SRV* – Small round virus as shown in figure 2.
LEGEND TO FIGURES

Figure 1. Epidemic curve of SVV. New cases occurring per week from January 1, 1979 till end of April.

Figure 2. The 30 nm small round particle detected in the stool of early cases from SVV. Full and empty particles are seen. Some of them show scalloping resembling Calci viruses. Bar shows 100 nm.