“Uncultivable” Enteric Viruses and Acute Diarrhoeal Diseases in Tropical Countries

MINNIE M. MATHAN

The Wellcome Research Unit, Christian Medical College Hospital, Vellore 632004, India

Acute diarrhoeal diseases are now recognised as one of the leading causes of death especially in preschool children in tropical developing countries. The importance of this public health problem is underlined by the initiation of the Control of Diarrhoeal Diseases programme by the World Health Organization, described in detail elsewhere in this publication. The magnitude of the problem is seen by statistics from India which estimate that at least 1.5 million children below the age of 5 years die every year due to non-cholera diarrhoea (India, Registrar General and Census Commissioner, 1972). Sophisticated laboratory techniques are necessary to identify the etiological agents causing diarrhoea, and these techniques have to be applied in prospective longitudinal studies involving cohorts of children to understand the dynamics of infection in the community to enable the planning of adequate control measures. Although it is now well accepted that the maintenance of fluid and electrolyte nutrition can significantly reduce the mortality and possibly the morbidity associated with diarrhoea, efforts at control and prevention have been hampered because “Science is not available where the diarrhoea is”.

It is necessary to emphasise that several carefully carried out prospective studies which looked for viruses as agents of diarrhoea using conventional methods of tissue culture showed that while enteroviruses could be cultured from about 30% of the children below 5 years of age at any one time, these could not be causally related with episodes of diarrhoea. But since bacterial pathogens could be cultured only from less than 10 to about 40% of children with acute diarrhoea, it was generally assumed that the rest of the cases were due to viral infection. It was this large hiatus in our understanding of the pathogenesis of acute diarrhoea that has been filled in by electron microscopy. Direct electron microscopic examination of faecal samples suitably prepared show viral particles which have now been proven to be causal agents. Subsequent to EM demonstration of these viral particles, other techniques particularly microscale enzyme linked immunosorbent assay (ELISA) have been developed to screen large number of stools for the presence of some of these agents. Since the mid 1970s although a large number of publications from different parts of the world have implicated a wide variety of uncultivable viruses in the pathogenesis of acute diarrhoeal diseases, there is little information from tropical developing countries. This review is an attempt to put together the available data on viral diarrhoeas in tropical developing countries, and to highlight some of the areas where further research work is needed.

The uncultivable enteric viruses

At present under this group of viruses are included the Rotavirus, Norwalk group and similar small round viruses — Astrovirus and Calicivirus, Adenovirus, and Coronavirus. Table 1 lists the viruses which were detected by electron microscopic examination of faecal samples obtained from 368 children with acute diarrhoea admitted in a hospital at Calicut in south India. Rotaviruses were identified with greatest frequency (71%) but the results in this group showed that all the viruses which have been

<table>
<thead>
<tr>
<th>Virus</th>
<th>Count</th>
<th>%</th>
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<tbody>
<tr>
<td>Rotavirus</td>
<td>260</td>
<td>70.7</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>35</td>
<td>9.5</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>17</td>
<td>4.6</td>
</tr>
<tr>
<td>Calicivirus</td>
<td>12</td>
<td>3.2</td>
</tr>
<tr>
<td>Small round viruses</td>
<td>6</td>
<td>1.6</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

TABLE 1. Viruses detected by Electronmicroscopy in Faeces of 368 children with acute diarrhoea in Calicut.
reported to give rise to acute diarrhoea in the temperate parts of the world, do occur in tropical countries. The morphological and other characteristics of these viruses have now been extensively documented and the reader is referred to the excellent review by Holmes. Diarrhoea due to rotavirus in tropical countries

The role of rotavirus as a causative agent of acute diarrhoea in childhood has been described from several tropical developing countries. The methodology for the preparation of the stool sampled for EM in these studies differed. In some of the studies, only serological evidence of infection was available. The frequency of identification of rotavirus ranged from none in Gambia in west Africa, to 71% at Calicut in southern India. Eight of these studies were hospital based (Table 2) and covered at least one year. Two studies reported from India in two areas with differences in the climate show some epidemiological differences. At Vellore, 80 miles inland from the sea and with about 15°C temperature difference between summer and winter and a low rainfall, rotavirus was detected mainly during the cooler months of the year. At Calicut, 300 miles to the west of Vellore in a high rainfall area on the sea coast with relatively uniform temperature during the year, rotavirus was detectable throughout the year (overall detection 71%) with almost 100% of tested samples containing the virus during November to January. A similar pattern was seen in hospital based studies from Argentina, Bangladesh, Costa Rica, Indonesia, Mexico and Venezuela with an increase in cases in the cooler months (Table 2). In a community based study in Guatemala, 24 infants were followed for three years. 183 episodes of diarrhoea were detected, of which 14.2% were associated with rotavirus in the stool. There did not appear to be any particular seasonal pattern, another community based study in Salvador showed a similar pattern.

Studies for shorter periods of time (2 to 8 months) reported from Central Australia, Gambia, Indonesia, Northern Nigeria, Philippines, Salvador, Taiwan and Tanzania also showed that rotavirus is prevalent in all the areas except the Gambia. In Gambia, a serological survey showed that by the age of 2 years 86% of the children had antibodies to rotavirus in serum suggesting that, although in the months of July to September, no rotavirus were seen in 43 faecal samples from children below 2 years, rotavirus infection is present in the community. Serological surveys have shown evidence of rotavirus infection in Bangladesh, India, Kenya and Taiwan.

It is clear from this data that widely separated regions of the tropics have significant incidence of rotavirus diarrhoea. The studies from India, suggest that there are regional differences which may be related to differences in the climate or other environmental factors. A full understanding of the epidemiology of rotavirus diarrhoea in tropical countries and the dynamics of infection in the community leading to diarrhoea is essential to

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**Table 2. Prevalence of rotavirus and adenovirus in infantile gastroenteritis in tropical region**

<table>
<thead>
<tr>
<th>Country and reference</th>
<th>Total No. studied</th>
<th>Period of study</th>
<th>Overall prevalence %</th>
<th>Peak season Time %</th>
<th>Adenovirus prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Hospital based study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venezuela (40)</td>
<td>293</td>
<td>Nov 1975–Dec 1976</td>
<td>41</td>
<td>Nov-Feb</td>
<td>72</td>
</tr>
<tr>
<td>Costa Rica (16)</td>
<td>130</td>
<td>Apr 1976–Nov 1977</td>
<td>38</td>
<td>Dec-Jan</td>
<td>60</td>
</tr>
<tr>
<td>Bangladesh (3)</td>
<td>2641</td>
<td>Feb 1978–Jan 1979</td>
<td>46</td>
<td>Dec</td>
<td>60</td>
</tr>
<tr>
<td>Argentina (28)</td>
<td>141</td>
<td>Aug 1978–Aug 1979</td>
<td>32</td>
<td>July</td>
<td>80</td>
</tr>
<tr>
<td>Indonesia (38)</td>
<td></td>
<td>July 1978–June 1979</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Community based study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guatemala (42)</td>
<td>24</td>
<td>1964–67 (183 episodes)</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salvador (39)</td>
<td>74</td>
<td>Jan 1977–Oct 1977</td>
<td>6.8</td>
<td></td>
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</tbody>
</table>
plan adequate measures for its control and prevention.

**Adenovirus infection**

Adenoviruses are detectable in the stools of some children with diarrhoea. Among the EM surveys in Costa Rica, Guatemala, India, Indonesia, Mexico, Northern Nigeria, Tanzania, Thailand, Venezuela, the major isolates during May, the warmest month (33% adenovirus positive) when rotaviruses were lowest in prevalence. It has been reported that diarrhoea associated adenoviruses detectable by EM are in general unculivable.

**Norwalk group and other small round viruses and diarrhoea**

A group of 26-27 nm diameter small round virus particles with a buoyant density of 1.35 to 1.41 which are presumed to be DNA viruses were first discovered in an epidemic of gastroenteritis in the town of Norwalk in USA. Before these agents were identified by EM, their pathogenicity was established in volunteer studies using filtered stool extracts. Radio-immunoassays for detecting these agents have been developed. Several similar agents have been reported from other parts of the world, of which the best recognized tropical one is the Hawaii. Studies by Cukor and his colleagues have shown that antibodies to this agent are present in the serum of children from Taiwan and the Philippines. Antibodies to Norwalk like agents have also been found in the serum of children in Bangladesh. Children in tropical regions appear to acquire antibodies earlier than children in temperate zones. The role of these and other small round viruses in diarrhoeal diseases in other tropical regions is not yet known.

**Human enteric coronaviruses**

Workers in Vellore in southern India first reported particles with fringes resembling coronaviruses, in human faeces in 1974 (Figure 1). These particles are 40-800 nm in size, markedly pleomorphic and have a fringe (approximately 18-22 nm in length) surrounding them. Diarrhoeal illness in the young of a wide variety of animals including calves, dogs, foals, and swine are caused by coronaviruses. There are some morphological differences between the coronavirus particles which have been seen in faecal samples and the human respiratory coronaviruses. Antigenic cross reactivity between these 2 groups has not been demonstrated.

![Figure 1. Enteric coronavirus-like particles. Bar = 100 nm.](image)

The human enteric coronavirus has now been seen in stool samples obtained from Australian aboriginals and in cases with acute diarrhoea in UK. However the pathogenicity of these organisms is not yet determined. In southern India, these particles have a wide prevalence in faecal samples obtained from asymptomatic individuals (Table 3) and some patients with chronic diarrhoea and malabsorption excrete these viruses for many months and have antibodies against them.

**TABLE 3. Prevalence of coronavirus in stool samples from healthy rural population**

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. tested</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 4.9</td>
<td>81</td>
<td>27</td>
<td>33.3</td>
</tr>
<tr>
<td>5 - 11.9</td>
<td>90</td>
<td>54</td>
<td>60.0</td>
</tr>
<tr>
<td>12 - 17.9</td>
<td>41</td>
<td>24</td>
<td>58.5</td>
</tr>
<tr>
<td>18+</td>
<td>161</td>
<td>99</td>
<td>61.5</td>
</tr>
</tbody>
</table>

**The future**

Acute diarrhoea will continue to be a major public health problem of tropical developing countries in the foreseeable future. Improvement in nutritional and sanitary factors which should come with improvement in socioeconomic status will ultimately relegate this illness to the status of just a nuisance. However, this is not likely in the near future and it is essential to try and see if infectious diarrhoea can be eradicat ed or at least controlled. The available data shows that "unculivable" enteric viruses are a major agent causing diarrhoea. Carefully planned prospective studies using electron microscopy of faecal samples to
identify agents and correlate them with clinical features, identification of serological and other characteristics of these viruses and the development of simpler methods of detection that can be used in developing countries appear to be major priorities. Ultimately it is hoped that vaccines could be developed to be used in the control pro-

grammes. Acute "noncultivable" viruses have now been adapted to in vitro tissue culture.14)

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