Proceedings of The 1st Asian Conference on Diarrhoeal Disease

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
DACCA, BANGLADESH
ROTAVIRUS AND OTHER UNCULTIVABLE ENTERIC VIRUSES IN THE

EPIDEMIOLOGY OF ACUTE DIARRHOEAL DISEASE IN SOUTHERN INDIA

M. Mathan, G. Kurian and V.I. Mathan
Wellcome Research Unit
Christian Medical College Hospital
Vellore 632004
India

ABSTRACT

The importance of the rotavirus as an aetiological agent in acute diarrhoeal disease in children in southern India has been well established by work during the last five years. At Vellore, an area 80 miles from the sea on the edge of the deccan plateau, rotavirus accounted for nearly 75% of children with diarrhoea seen in the hospital in the cooler months of the year, November to February during the three years of observation. In the hot dry summer months the prevalence of rotavirus related diarrhoea was much less giving an overall prevalence of rotoviral diarrhoea of 25% in a year. This pattern of rotoviral diarrhoea at Vellore is quite different from that observed at Calicut, 300 miles to the west of Vellore on the west coast of India. The overall prevalence of rotavirus in children hospitalized with diarrhoea at Calicut was 70% and during November to January when acute diarrhoea of childhood assumes epidemic proportions in the community nearly 100% of hospitalized children with diarrhoea were rota positive.

There was striking differences in the seroepidemiology of rotaviruses between these two regions. Using the simian agent SA-11 as the source of antigen in an enzyme linked immunosorbent assay (ELISA) for serum antibodies to rotaviruses, antibodies were detectable in high titres in all sera tested. At Vellore the antibody titres were highest in newborn (cord blood) and after the fourth decade of life. There was also a significant fall in the GMT of antibody titres during the hot dry summer (May). At Calicut by age of 5 rotavirus antibody GMT appeared to be falling and in adults the highest GMT was in the 19-20 year age group (mothers).

Further work needs to be done to explain these differences in the epidemiology of rotavirus associated diarrhoea. Preliminary data on copro-antibodies (IgA class specific) suggests that the pattern of rotavirus infection in the populations may be different. Electron microscopy, ELISA for detection of antigen and antibody, serotyping of rotavirus isolates and biochemical characterisation of the rotavirus are necessary to understand the problem.
further and plan effective control measures.

INTRODUCTION

Direct electronmicroscopic examination of suitably prepared faecal samples is now recognized as an essential method for detecting the uncultivable enteric viruses, which may be causally related to acute diarrhoeal disease. During the later half of the 70's, a large variety of virus particles have been reported from different parts of the world, and it is now well established that several of these could be causing acute diarrhoea both as endemic cases and in epidemics.

Since 1974 in Vellore a systematic search for the detection of these uncultivable enteric viruses, has been going on. This paper summarizes the current status of these studies at Vellore. Stool samples from patients with acute diarrhoea and appropriate age matched controls were collected. Twenty percent suspension of faecal samples in phosphate buffered saline was clarified by low speed centrifugation, the supernatant was spun down at 90,000xg for 30 minutes and the pellet appropriately applied to grids and examined in the electronmicroscope. This methodology was preferred 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 reporting to hospitals with acute diarrhoea in southern India in 1974. A one year hospital based study of children under two years with acute diarrhoea (carried out in 1974 and 1975) rotavirus could be identified in 25% of these children. However, in 66% of the children pathogenic bacteria were isolated which could be causally linked to diarrhoea. These included enterotoxigenic and enteropathogenic strains of E.coli. A subsequent one year survey of cases reporting to the 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 hospitalized children with striking seasonal prevalence. Rotavirus was detected during the colder months of the year, August to February and were not during the hot dry summer month of May. The seasonal difference in temperature in the region is - during the winter months temperature ranged from 15°C to 30°C and in the hot dry summer from 30°C to 45°C.

Calicut, about 300 miles west of Vellore on the west coast of India, has a high rainfall and humidity, as well as more or less uniform temperature
throughout the year. The findings in Calicut were a striking contrast to Vellore. At Calicut, among hospitalized children with acute diarrhoea, rotavirus was detected in 77%. Recognized enteropathogenic bacteria could be cultured only from about 10% of the stools. Rotavirus was detected throughout the year. During November to January, they were present in almost 100% of the children tested. The frequency of rotavirus detection was lowest during May which is a relatively hot and dry month.

A seroepidemiological survey was carried out both at Vellore and in Calicut. Enzyme linked Immunosorbent Assay (ELISA) was used for detecting serum antibodies of all three Ig classes, antibody titres in all age groups were found to be very high at Vellore. Antibody titres were significantly higher in adults than children and were found to be highest in those aged over 40, who were mostly grandparents. The antibody titre in children from the higher socioeconomic strata suggested that they were less exposed to rotavirus infections than children from a lower socioeconomic background. The 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 Vellore, in Calicut, antibody titres were found to be highest in children; and children with 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 different in the ecology there were differences in rotavirus prevalence in hospitalized 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. 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 668. Between 1st of January 1979 and 31st April 1979, 176 cases of acute diarrhoea were detected in this village (Fig. 1). All age groups were affected, with the highest attack rates in preschool children (Table 1). The patients affected were not febrile. They passed watery stools without blood and mucus for a few days without vomiting. Some of the patients complained of increased
Fig. 1: Epidemic curve of SVV. New cases occurring per week from January 1, 1979 till end of April.

Table 1. Attack Rates

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Population</th>
<th>Rate per 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 4.9</td>
<td>86</td>
<td>51.1</td>
</tr>
<tr>
<td>5 - 11.9</td>
<td>120</td>
<td>28.3</td>
</tr>
<tr>
<td>12 - 17.9</td>
<td>72</td>
<td>22.2</td>
</tr>
<tr>
<td>18 +</td>
<td>370</td>
<td>21.8</td>
</tr>
</tbody>
</table>

The prodromal period was 9 days. More than 1 out of 2 patients had diarrhea, and control patients had 11 out of 13 stools positive for SVV. The control group included 17 healthy individuals. Other controls were not detected.
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; among them, there were episodes of remission and relapses but the second attacks could not definitely be identified as a relapse or a reinfection.

Stools for bacteriological and virological studies were obtained from 62 patients (34 during the first seven days of illness) 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. Human enteric corona virus was present with equal frequency among the patients and controls (Table 2). A small round virus particle (Fig. 2) was present in 11 out of 34 patients during the first week of diarrhoea and in 1 two-year-old control who developed diarrhoea 2 days after the stool was collected. It was not detected in any patient who had diarrhoea longer than 7 days or any of the other controls (Table 2).

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>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</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>Viruses</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>
</tr>
</tbody>
</table>

Bacterial pathogens included 4 Shigellae and 2 Salmonellae. Corona = Human enteric Corona virus, SRV = Small round virus as shown in figure 2.

The prevalence of this small virus particle only in patients during the early period of the illness, strongly suggests 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 epidemics over 16 weeks does not suggest a
common source epidemic and in fact no common vehicle such as water supply could be identified in the village. 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 in faecal sample by direct electronmicroscopic examination are now well recognized 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 cannot be yet implicated in the etiology of acute diarrhoeal disease\(^1\). It has been suggested that they may
be causing the syndrome of neonatal necrotizing enterocolitides\textsuperscript{12}. It has not been possible to determine the aetiological roles of the other viral particles that are seen in the stool. Antibody titre level in serum obtained in convalescent cases compared to serum from acute cases strongly suggests an aetiological role. In the absence of such proof, the association of a single morphological type of virus particle with diarrhoea of short duration epidemics, and the absence of such particles in controls give 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 and in other parts of the developing world. Electronmicroscopy of suitably prepared faecal samples is a powerful new tool in the search for the aetiological 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 is possible to treat the majority of such cases with oral maintenance of hydration. However this is purely a therapeutic approach. 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 can only be achieved if the aetiological agent is identified and the mechanism of their transmission in the community known. Direct EM examination of faecal samples in addition to detailed bacteriological studies is an essential part of epidemiological investigations of diarrhoeal diseases, endemic and epidemic in developing countries.

ACKNOWLEDGEMENT

The work of the Wellcome Research Unit is supported by the Wellcome Trust, London, U.K. The technical help of Mr. S.P. Swaminathan, Miss K.M. Roshni and Mr. R. Chandrasekaran is gratefully acknowledged.

REFERENCES


of acute gastroenteritis in infancy and early childhood in southern India. 

5. Paniker CKJ, Mathews S, and Nathan M: Rotavirus and acute diarrhoeal 
disease in children in a southern Indian coastal town. Submitted for 
publishation, 1981.


7. Mathan VI and Baker SJ: Epidemic tropical sprue and other epidemics of 
1968.

epidemic of bacterial diarrhoea with subsequent person to person 
transmission. Submitted for publication, 1981.

Shiga bacillus dysentery in a southern Indian village. Submitted for 
publishation, 1981.

10. Mathan VI, Kurian G, Rejan DP and Albert MJ: Shigella dysenteriae type I 
infestation in southern India during the 1970’s. Proceedings of regional 
acute diarrhoeal disease conference, ICDDR,B, Bangladesh, 1981.


coronavirus infection with hemorrhagic enterocolitis in newborn infants). 

Discussion

Q. Dr. Ganguly

Inverse relationship between rotavirus incidence and antibodies was shown 
in your area? In Calicut a three year spread has shown upto 92 percent 
incidence what was the status of antibodies? What was the incidence in 
children below six months in your area when Cord blood titres were high?

A. Children get placental antibody from the mothers. We don't know the 
exact causes why it is higher in Vellore than Calicut.
Q. Dr. Sazeem (SA)  
When did you find the maximum seroconversion in rotavirus in your patients?  
A. No particular age group was found to have high seroconversion rates.

Q. Dr. Sanyal  
What antigen do you use for ELISA test for detection of rotavirus infection?  
A. Antigen prepared in our own laboratory.

Q. What is your opinion about the role of viruses other than rotavirus (causing) diarrhoea?  
A. Yes, it is possible that other viruses cause diarrhoea.

Q. Dr. Nazma Hizvi  
Does colostrum give any immunity to rotavirus?  
A. Yes, it does.