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Viruses and diarrhoea in West Africa and London: a collaborative study

M. G. M. ROWLAND
Medical Research Council Unit, Keneba, Gambia

HEATHER DAVIES, S. PATTERSON, R. R. DOURMASHKIN
Division of Electron Microscopy, Clinical Research Centre and Northwick Park Hospital, Harrow, U.K.

D. A. J. TYRRELL, T. H. J. MATTHEWS, J. PARRY, J. HALL AND H. E. LARSON
Division of Communicable Diseases, Clinical Research Centre, and Northwick Park Hospital, Harrow, U.K.

Diarrhoea, particularly of children, is a major unsolved world-wide problem (ELLIOTT, 1976) and there is dispute about what precipitates and continues the diarrhoea. Rotaviruses and, in some cases, adenoviruses are found on electron microscopy of faeces of a substantial proportion of children with acute diarrhoea in various parts of the world and there is evidence that these organisms can cause the disease (BISHOP *et al.*, 1974; BRYDEN *et al.*, 1975; MIDDLETON *et al.*, 1974; SEXTON *et al.*, 1974; FLEWETT *et al.*, 1975; SCHOUB *et al.*, 1975). Particles resembling coronaviruses (CAUL *et al.*, 1975; MATHAN *et al.*, 1975) and small isometric particles (APPLETON & HIGGINS, 1975; MADELEY & COSGROVE, 1975, 1976) have also been seen although it is not clear whether they are pathogenic Viruses which are detected by inoculation of tissue cultures, such as enteroviruses and adenoviruses, are only occasionally cultured from patients with diarrhoea in Britain, but are common in children with and without diarrhoea in developing countries; in some studies there has been evidence that these viruses may be associated with diarrhoea (e.g. YOW *et al.*, 1966; RAMOS-ALVAREZ & OLARTE, 1964, and see CRAMBLETT & SIEWERS 1965) but they are not generally thought to explain diarrhoea of children in the tropics.

This study was undertaken to look systematically and by comparable techniques for the viral causes of childhood diarrhoea in a typical village in rural Africa and a comparison group of children and adults in north-west London. Patients in the village of Keneba in rural Gambia were examined clinically and faeces were collected for bacteriological examination on the spot and were frozen and transported to London for virological studies if no bacterial pathogens were found. A preliminary study was made in 1974 but the present study was made in the rainy season July-September 1975 when there is frequent diarrhoea which has a significant effect on nutritional status. Samples were collected during the first four days of an attack of diarrhoea which usually continued much longer. Harrow in N.W. London is served by our unit's paediatric and infectious disease services and between January 1975 and January 1977 a substantial proportion of those patients who presented with diarrhoea and/or vomiting as main symptoms were studied.

Patients' faeces were examined by routine bacteriological methods, including a search for serotypes and pathogenic *Escherichia coli* in children. Faeces were examined by inoculation into tissue cultures of human embryo lung fibroblasts, monkey kidney and HeLa cells and by electron microscopy. In the case of the patients from Keneba we employed immuno-electron microscopy using the patient's convalescent serum.

Though they are difficult to summarize concisely, most of the results are shown in Tables I and II. The bacteriological techniques used in Africa and the U.K. were almost identical and the virology was done in the same laboratory. The children less than two years old in Harrow roughly match for age the children in Keneba and the specimens were handled very similarly.

Pathogenic bacteria were recognized in 10 of 83 children (eight of 67 under two years) from Harrow, and comprised the expected mixture of Salmonella, Shigella and *E. coli* serotypes. An adeno- or enterovirus was isolated from 14 of 67 children under two. However, a higher proportion of faeces were positive by electron microscopy and in addition to 19 with rotaviruses, four specimens showed caliciviruses. While the Salmonellas were found in all age groups the viruses were found mainly in the age group under two years. Only in this group was a potential pathogen found in over half of the patients. Rotaviruses were found mainly in the early months of the year and altogether represented about half the viruses detected and one in eight of the patients studied. There were few older children but the pattern of isolation of viruses was roughly similar to that in the children under two, while in the 52 adults, nine bacteria and only three viruses were found.

The pattern in the children in Keneba was very different from that of children and adults in Harrow. Only one in six (12%) had bacterial pathogens, i.e. Salmonella, Shigella or pathogenic types of *E. coli*; of the remainder a high proportion, 60%, were excreting enteroviruses; rotaviruses were not found at all, though a small proportion were shedding particles resembling coronaviruses or small isometric viruses. The technique used, namely immuno-electron microscopy, is known to be more sensitive than that used for Harrow specimens, and in almost all the specimens it was clear that the patient's serum contained antibody against the isometric particles seen, but not against the corona-virus-like particles. Further there was no evidence that any virus was associated with the presence of diarrhoea.

Table I—Pathogens detected in faeces of patients with acute diarrhoeal disease in North West London (17.2.75 to 17.1.77)

Methods used and pathogen found	Number of positive results in indicated group Age (years) and number of patients studied				
	< 2 67	2—15 16	>15 52	Total 135	
Bacterial culture and microscopy					
Salmonella	3	2	9	14	
Shigella	0	0	0	0	
E. coli pathogenic serotype	5	0	0	5	22
Giardia	0	1	1	2	
Strongyloides and *Trichostrongylus*	0	0	1	1	
Virus culture					
Adenovirus	11	3	1	15	
Enterovirus	3	2	1	6	22
Influenza (throat swab)	1	0	0	1	
Electron microscopy					
Adenovirus	5	1	0	6	
Enterovirus	1	1	1	3	
Rotavirus[ø]	19	4	0	23	40
Calicivirus	4	0	0	4	
Coronavirus-like	1	0	0	1	
" Virus-like particles "†	3	0	0	3	
Total detected	56	14	14	84	

In most specimens only one pathogen was detected by one technique but in 6 cases two viruses were found — adeno + (4 rota, 1 calici, 1 corona), and in one adeno + influenza + rota.
[ø]Three of 4 viruses found in patients aged 2-15 were from children under 5.
†Small isometric particles (see Table 2).

Table II—Viruses detected in faeces of children aged 1 to 2½ years with and without diarrhoea in West Africa, June—October 1975

Method used and pathogen found	Number of positive results in indicated group Clinical state		
	Diarrhoeal illness	Well or non-diarrhoeal illness*	Total[ø]
	32	18	50
Virus culture			
Adenovirus	1	3	4
Enterovirus	20	10	30
Immune electron microscopy			
Adenovirus	0	0	0
Coronavirus-like particles	3	2	5
Virus-like particles†	2	2	4
Rotavirus	0	0	0
Total detected	26	17	43

*These specimens matched those collected earlier or later when the patient had clinical recognized diarrhoea.
[ø]Of 150 specimens collected 9 were excluded because a Shigella or Salmonella was isolated and 16 because a pathogenic serotype of *E. coli* was isolated—5 of the latter from patients without diarrhoea. Samples for virological study were drawn from the remainder. Tests for *Giardia* were done but were technically unsatisfactory—subsequent study indicates that at least 20% of the study population are affected.
†Small isometric particles (see Table I).

The results we have obtained with children under two in this country are very similar to those obtained by others, in particular the finding of the importance of rotaviruses. This shows that the electron microscope techniques we use are adequate to detect these particles; in fact, we have also detected caliciviruses and enteroviruses which are smaller and more difficult to recognize. It is therefore significant that we usually failed to detect viruses by electron microscopy in adults in Harrow. This may be because the above organisms are present in concentrations too low to be detected but it is more likely that other organisms are responsible for their illnesses—for example, smaller viruses like the Norwalk agent (KAPIKIAN *et al.*, 1972). We therefore hope to investigate these possibilities further, for instance by using the improved techniques referred to by APPLETON *et al.* (1977). Furthermore, toxin-producing *E. coli*, Campylobacter and other pathogenic bacteria would have been missed by the bacteriological tests used, and we are now looking for these. Further serotyping of *E. coli* might also be informative (ROWE *et al.*, 1977) and will be included.

The picture in the African children differs from that of both adults and children in Harrow. There is a high rate of enterovirus infection in Keneba which is hardly surprising because the environment and the way of life offer numerous opportunities for faecal-oral spread and, as expected, these viruses were found equally often in patients with and without diarrhoea. However, when we planned the study we expected that by electron microscopy we would detect rotaviruses, at least at the peak period of disease prevalence, as others have done since in other parts of the developing world, e.g. Bangladesh (RYDER *et al.*, 1976) or Java (SEBODO *et al.*, 1977). We were therefore surprised to find no rotaviruses and there were coronavirus-like and other virus-like particles in only a few specimens—furthermore their presence was not obviously related to diarrhoea (Table II). Indeed although coronaviruses from human faeces have apparently been propagated in organ cultures (CAUL & EGGLESTONE, 1977) some workers believe the particles seen may represent fragments derived from normal cells, while the isometric particles could be bacteriophage even though the patients had antibody against them.

Thus, examining the African faeces for bacteria or viruses yielded a possible cause for diarrhoea in only a small proportion of children though *E. coli* must be studied further by testing further isolates for serotype, toxigenicity and other possible markers of virulence. We suggest that the disease in these children may be largely due to colonization of the upper intestinal tract, especially with toxin-producing bacteria, and evidence for this will be reported soon (ROWLAND & MCCOLLUM, 1977). It is nevertheless likely that rotaviruses and other viruses associated with diarrhoea are present at some time in this community and we are collecting specimens throughout the year to look for them, and a serological survey is also needed.

It is absolutely essential to clarify our ideas on the nature of diarrhoea in Africa if we are to intervene effectively to prevent the disease with the slender resources available. Likewise if we are to do anything about diarrhoea in adults in London we should start by facing the fact that we do not know what causes most cases.

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