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Viruses demonstrated in children in Tanzania: studies in diarrhoea and measles

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Summary

Causes of diarrhoea with particular reference to viral agents were investigated in 123 infants and young children in Dar es Salaam, Tanzania. Twenty-six of the patients also had measles. Viruses were found in 52 of the 123 patients (43 per cent) and rotavirus occurred in 38 children (31 per cent). Enteroviruses were found in 10 patients, adenoviruses in nine and 'small round virus' in one (six patients had dual infection). Four patients died and only one of these children had viral particles in the stools. Breast milk formed part or all of the diet in 77 children (63 per cent) and virus isolation showed a similar pattern in breast fed infants and those not receiving breast milk. In 26 patients with measles only five were excreting viruses in their stools. Therefore no strong evidence was found to link the diarrhoea associated with measles in Tanzanian children to any particular virus. The pattern of virus infection causing infantile diarrhoea was similar in Dar es Salaam to other parts of the world.

Introduction

Diarrhoeal illness is a major cause of morbidity and mortality in developing countries. In the main hospital in Dar es Salaam it was the commonest reason for admission to the paediatric wards (children aged seven years and under) in 1976. In that year 2410 children were admitted with a diagnosis of gastroenteritis (Kigadye, Kimboi and Kimati, 1976) and 75 of them died (3.1 per cent). This study was carried out to examine viral aetiologial agents in diarrhoeal illness: bacterial diarrhoea is not included in this study. A number of patients with measles who also had diarrhoea were included in the study to see whether diarrhoea in measles was caused by a detectable viral agent. Such diarrhoea occurring in children in the tropics indicates severe measles and often these infants require parenteral fluids (Morley, 1969).

In order to provide a complete examination the study was a joint one between the University of Dar es Salaam and the University of Glasgow.

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Methods

Clinical methods
The patients in this study were selected randomly without any specific bias from the paediatric infectious disease ward in Muhimbili Hospital, Dar es Salaam, which serves mainly an urban African population. Stool samples were examined for viruses from children with diarrhoea irrespective of whether bacterial pathogens were isolated. The study was performed between March 1976 and September 1976, a period which covers the cooler months of the year. Studies in Europe have suggested that stool viruses are more common in autumn and winter (Flewett, Davies, Bryden and Robertson, 1974). A total of 123 children were investigated, of whom 26 had measles.

The following information was collected from each patient: age, sex, a short feeding history and the degree of dehydration assessed clinically, using the criteria of Ironside, Tuxford and Heyworth, 1970. Measles was diagnosed clinically by the agreement of two doctors who had considerable experience of paediatric infectious diseases in Tanzania. A sample of faeces was collected within the first 48 hours after admission and usually within the first 24 hours.

Virological methods
The faeces were stored at $-20^\circ\text{C}$ for between six and 12 months before being flown to Scotland by air freight. On receipt in Glasgow an extract of each stool in phosphate buffered saline was made and, after clarification at 3000 rev/min for 30 min in a bench centrifuge, was inoculated into cell cultures and prepared for electron microscopy (EM) as described by Madeley, Cosgrove, Bell and Fallon (1977). Briefly, the extracts were centrifuged at 100,000 g for one hour, the pellet resuspended in two drops of EM diluent (0.1 per cent bacitracin) and mixed with three per cent potassium phosphotungstate pH 7.0 as a negative stain.

Results
Stool samples were taken from 123 patients and viruses were detected either by electron microscopy or culture in 52 of these patients. The results are listed in Table I. Three morphological types of virus (rotavirus, adenovirus and ‘small round virus’) were observed by electron microscopy but no astroviruses, caliciviruses or coronaviruses were detected (Madeley and Cosgrove, 1975, 1976; Caul, Paver and Clark, 1975). Adenoviruses and enteroviruses (polioviruses of two types and echoviruses of six types) were isolated in culture. The adenovirus cultured was not detected by electron microscopy, and no virus was cultured from the stool in which the ‘small round virus’ was observed.

Four patients (three per cent) died in hospital following admission for diarrhoea and one child died during a subsequent admission with intussus-
Table 1 Viruses found in patients' stools

| Viruses                  | (a) Electron microscopy: | (b) Culture: |
|-------------------------|--------------------------|--------------|
|                         | Rotavirus                | Adenovirus type | Echo type | Polio type | Total |
|                         | 38                       | 4            | 6           | 2          | 58*   |
|                         | Adenovirus               | 8            | 11          | 2          |       |
|                         | 'Small round virus'      | 1            | 11          | 31         |       |

*There were six cases of dual infection: five rotavirus (all detected by EM) with one each of the following: adenovirus (EM), echovirus type 8, echovirus type 14, echovirus type 17, poliovirus type 2; one adenovirus with a 'small round virus' (both by EM).

ception. Three had additional causes contributing to the severity of their illness: measles, measles and pneumonia, and marasmic kwashiorkor respectively. The fourth was severely dehydrated on admission.

All of the children except two were under four years of age when admitted to hospital. The remaining two were six and seven years old. The numbers were too small for further analysis of the age distribution to be possible, but they fell into the age group in which most viruses have been observed.

Seventy-seven of the children (63 per cent) were still receiving breast milk on admission, though 68 of these were being weaned. In this part of Africa, weaning, usually on to maize porridge, is a prolonged process, and children may be offered breast milk up to two to three years of age. A comparison of the type of feeding with the viruses identified is shown in Table II. It is apparent that breast feeding did not prevent the acquisition of viruses with rotaviruses being found most frequently (28 out of 77 (36 per cent)). In the three groups shown in Table II five out of nine children (56 per cent) in the 'breast fed' group had virus in their stools, 34 out of 68 children (50 per cent) in the 'breast and other food' group, and 13 out of 44 children (30 per cent) in the 'other food' group. The lowest rate was in the 'other food' group, which was also the oldest group with an average age of 22 months, compared with four months of the 'breast-fed' and the eight months of the 'breast milk with other food' group.

Though breast feeding did not prevent the acquisition of microorganisms it
Table II  Viruses in stools related to the type of feeding given to the child

| Type of feeding          | Breast fed | Breast fed and other food | Other food | Unknown |
|-------------------------|------------|---------------------------|------------|---------|
| Number of children      | 9          | 68                        | 44         | 2       |
| Average age (months)    | 4          | 8                         | 22         | 13      |
| Range (months)          | 2–7        | 3–21                      | 3–84       | 7–18    |

**VIRUSES**
- Rotavirus: 3, 25, 10, —
- Adenovirus: 2, 4, 3, —
- Small round virus: —, 1, —, —
- Echovirus: —, 6, 1, —
- Poliovirus: 1, 1, 1, —
- Total viruses: 6, 37, 15, —

Six children had dual infection (see footnote on Table I), one was breast fed, three were breast fed and had other food, and two were receiving other food.

Table III  Relationship of virus to the degree of dehydration of the patient

| Degree of dehydration* | Nil 0% | Mild 2.5% | Moderate 5% | Severe 10% |
|------------------------|--------|-----------|-------------|------------|
| Rotavirus              | 4      | 12        | 13          | 9          |
| Adenovirus             | —      | 3         | 4           | 2          |
| Small round virus      | —      | —         | 1           | —          |
| Echovirus              | —      | 3         | 3           | 1          |
| Poliovirus             | 2      | 1         | —           | —          |
| Total positive         | 6      | 19        | 21          | 12         |

*After criteria of Ironside, Tuxford and Heyworth, 1970.
Six of the 52 infected patients had a dual infection (see footnote on Table I).

might reduce the severity of the disease. Tables III and IV explore this possibility by comparing firstly, the organism with the severity of the disease and secondly, severity with diet. The numbers are small but there is no suggestion that any of the viruses were associated with a more severe disease. Rotavirus was the most commonly observed virus, and Table IV includes the figures for this virus in relation to disease severity (percentage of dehydration) and type of feeding. There is no suggestion that breast feeding protects nor that other forms of feeding aggravate the effects of rotavirus infection.

Diarrhoea is often associated with measles in the tropics. Twenty-six of the children in the study had clinical measles and the viruses identified in their stools are listed in Table V. In five stools a virus was detected.
Viruses in diarrhoea and measles

Table IV  Degree of dehydration related to the type of feeding

| Degree of dehydration | Breast fed | Breast fed and other food | Other food | Unknown |
|------------------------|------------|---------------------------|------------|---------|
| Nil                    | 2 (0)*     | 13 (4)                    | 7 (0)      | 0       |
| Mild                   | 2 (2)      | 19 (7)                    | 9 (3)      | 1 (0)   |
| Moderate               | 2 (0)      | 25 (9)                    | 16 (4)     | 1 (0)   |
| Severe                 | 3 (1)      | 11 (5)                    | 12 (3)     | 0       |
| Total                  | 9 (3)      | 68 (25)                   | 44 (10)    | 2 (0)   |

*Number in parenthesis are the numbers of patients with stools positive for rotavirus in that group.

Table V  Viruses found in the stools of measles patients

| Number of patients with measles | 26 |
|--------------------------------|----|
| Viruses identified (method):   |    |
| Rotavirus (EM)                 | 3  |
| Adenovirus (EM)                | 1  |
| Echovirus type 6 (culture)     | 1  |

Discussion

There have been many reports of rotavirus-associated diarrhoea from different parts of the world particularly from Europe, North America and Australasia. Reports from Africa have been less frequent and have been mostly confined to those from South Africa. The present study attempted to investigate the viruses associated with diarrhoea in Dar es Salaam and, since electron microscopy was considered essential, the study was limited to the number of stools that could be sent in one consignment by air to Scotland. Examination of stools from 123 children resulted in the detection of 58 viruses of which 38 were rotaviruses.

Viruses were isolated in similar patterns both in breast fed and weaned babies. Any antibody or other inhibitory substance in breast milk (Matthews, Nair, Lawrence and Tyrell, 1976) should present an immediate barrier to any organism attempting to colonise the gut. That it failed to do so in a number of cases suggests that later breast milk may be more deficient in inhibitors, antibody or otherwise, than colostrum and investigations into the content of breast milk after prolonged lactation have yet to be done; our numbers would not permit detailed analysis by age. It is also possible that in an area where protein malnutrition is common the quality of breast milk may be poorer than elsewhere.

Chrystie, Totterdell and Banatvala (1978) have reported that breast-fed babies excrete fewer rotavirus particles than bottle-fed babies in symptom
free infections. The amount of virus seen in the stools of our breast-fed infants appeared to vary considerably but we were unable to standardise our methods enough for any conclusions to be drawn.

There was no evidence that any one of the viruses detected caused a more severe disease than the others, judged by the amount of dehydration. Rotavirus was the commonest organism identified and it was found in all degrees of severity from no dehydration to severe (10 per cent) dehydration. Since this virus as well as others found in stools has also been observed in normal healthy babies (Totterdell, Chrystie and Banatvala, 1976; Madeley, Cosgrove and Bell, 1978) any observations must be interpreted with caution. Recent reports (Zissis and Lambert, 1978; Thouless, Bryden and Flewett, 1978) have suggested that there may be more than one serotype of rotavirus and, if so, the apparent variations in pathogenicity of the virus in this study may be due to a multiplicity of serotypes. No serotyping was attempted.

Examination of stools from 26 cases of measles failed to implicate any particular virus as a likely cause of the associated diarrhoea. Since diarrhoea will be due to a disturbance of gut equilibrium this may occur through the considerable systemic upset caused by measles, which in malnourished infants may be sufficient to depress cellular function. There is evidence that measles virus may be found in the cells of the gut (Fraser and Martin, 1978) but there has been little investigation into its relationship to the associated diarrhoea which occurs in children with measles in the tropics. However the diarrhoea associated with measles in Tanzanian children does not appear to be caused by any of the electron microscopically detectable viruses.

The absence of suitable electron microscopy in Tanzania meant that the stools had to be flown to Scotland. The virus identification rate was similar to a local study (Madeley, Cosgrove, Bell and Fallon, 1977) but no astrovirus, calicivirus or coronavirus was identified. Whether these were present and failed to survive the journey or whether their absence reflects different viral flora in different parts of the world is unknown at present.

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