Rotavirus infections in a maternity unit

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Totterdell, B. M., Chrystie, I. L., and Banatvala, J. E. (1976). *Archives of Disease in Childhood*, 51, 924. **Rotavirus infections in a maternity unit.** Between May and August 1975, rotaviruses were detected in the stools of 76 out of 174 (44%) newborn babies in the maternity unit at this hospital. Infection occurred less frequently in breast-fed than in bottle-fed babies ($P < 0.001$). However, only 7 out of 76 (8%) babies who excreted rotaviruses had symptoms and these were mild. Complement fixation tests did not show any apparent difference in the antibody titres or serological responses between mothers of rotavirus positive or negative babies. When 68 faecal extracts known to contain rotaviruses by electron microscopy were inoculated by centrifugation on to monolayers of continuous pig kidney cell cultures (IB-RS-2), rotavirus antigen was detected by immunofluorescence in 65 (95.5%) specimens, 58 being positive after centrifugation at 3000 g and a further 7 after centrifugation at 10 000 g . Antigen was first detected 6 hours after inoculation of specimens, maximum levels being detected at 24 hours.

Rotaviruses (reovirus-like particles, duoviruses) are important causes of gastroenteritis in children as well as in the young of many animal species (Mebus *et al.*, 1969; Woode *et al.*, 1974; *Lancet*, 1975). The accumulated data from studies conducted on children admitted to hospital in many parts of the world have shown that these viruses may be detected by electron microscopy in the faecal extracts of up to 50% of children with gastroenteritis but not in age-matched controls (Davidson *et al.*, 1975). In an outbreak of rotavirus infection among babies in a London maternity unit a technique was used for the detection of human rotaviruses by immunofluorescence (IF) in cell cultures which provides a suitable alternative to electron microscopy for establishing a diagnosis of infection by human rotaviruses.

Materials and methods

Stools were stored at -70°C before testing. For examination by electron microscopy (EM), a 10% suspension of faeces was made in phosphate-buffered saline and the preparation centrifuged at 1500 g for 10 minutes. The supernatant fluid was then centrifuged at 300 000 g for 30 minutes and the pellet suspended in 1–2 drops of distilled water. This preparation was then negatively stained with 3% potassium phosphotun-

stic acid ($\text{pH } 6.5$) and examined at a screen magnification of $\times 40\,000$ in a Philips 200 EM.

For IF tests, a 20% faecal suspension was made in Eagle's minimum essential medium containing 2% fetal calf serum (Flow Laboratories, Ltd.) and agitated on a Rotamixer shaker for 1–2 minutes. After standing for 15 minutes, the faecal suspension was filtered through a 450 nm millipore filter and 0.1 ml of the filtrate inoculated onto confluent pig kidney monolayer cell cultures (IB-RS-2) grown on 12 mm coverslips (Chance) contained in 13 mm $\times$ 94 mm plastic flat bottomed tubes (Searle Diagnostics). The inoculated samples were then centrifuged in an MSE Mistral 4 l centrifuge at 3000 g for 2 hours at 4°C . After incubation for 24 hours at 37°C , the coverslip cell culture preparations were harvested and fixed in acetone for 10 minutes at 4°C . Preparations were then absorbed for one hour at 37°C with 1:20 dilution of human rotavirus antiserum raised in gnotobiotic pigs and, after washing, treated with a rabbit anti-swine fluorescein-labelled conjugate (Nordic Diagnostics). To confirm the specificity of this test, some preparations were treated with a calf rotavirus antiserum raised in gnotobiotic calves and a rabbit anti-bovine conjugate (Nordic Diagnostics). Coverslips were dried, mounted in Polaslip B and examined for IF.

Samples positive by EM but negative by IF were retested, but by centrifugation at 10 000 g in a SW27 rotor of a Beckman L-3-50 ultracentrifuge. Flat surface coverslip supports were made by polymerizing 1 ml of an Araldite resin/marble flour mixture in cellulose nitrate tubes. In order to determine the time se-

quence of the development of rotavirus antigen, a faecal extract rich in virus particles was inoculated into a number of coverslip preparations of IB-RS-2 and examined for IF at varying intervals after inoculation.

Attempts to detect serotypes of enteropathic *Esch. coli*, commonly associated with enterotoxin production in the UK, *Shigella* and *Salmonella* sp., and staphylococci were made, using routine diagnostic procedures.

Whenever possible, paired sera were obtained from mothers, the first sample being collected 4 days after delivery and the second at their postnatal visit 6 weeks later. These sera were tested for rotavirus antibodies by complement fixation, antigen being prepared from a calf faecal sample rich in rotavirus particles (Kapikian *et al.*, 1974). The complement fixed antigen was used at its optimal dilution.

Results

On 29 May 1975, faecal extracts from three 7- to 9-day-old babies with loose, offensive stools on one maternity ward were examined by EM and found to contain rotaviruses. Stools from apparently healthy infants in the same ward, two other maternity wards (one adjacent, the other two floors below), and the Newborn Special Care Unit (NSCU), were therefore examined. During the next 16 weeks, 76 of 174 (44%) 4- to 9-day-old babies in these wards, including the NSCU, were found to be excreting rotaviruses. No virus was detected in any of 34 specimens from babies 1-2 days old though it was detected in stools of 2 of 26 3-day-old babies. Rotaviruses were detected more frequently in the stools of bottle-fed than breast-fed babies ($P < 0.001$, Table I).

TABLE I

Relation between method of feeding and rotavirus excretion in 4- to 9-day-old babies, May-July 1975

	Rotavirus positive	Rotavirus negative	Total
Breast fed	31	39	70
Bottle fed	42	11	53
Total	73	50	123

$\chi^2 = 13.9$, $P < 0.001$.

Of the 76 babies excreting rotaviruses, only 7 (8%) had diarrhoeal symptoms (i.e. passage of a dozen or more loose and often offensive stools in 24 hours) and of these 5 were bottle fed. Only one baby required treatment (administration of clear fluids by mouth for 12 hours). Though many of the newborn babies, particularly those who were given breast milk, had 2-3 loose stools per day, only 8 of 21 (38%) with such symptoms excreted rotaviruses.

In contrast, in those bottle-fed babies who produced occasional loose stools, rotaviruses were detected in 20 out of 23 (87%). There was no difference in the pattern of weight gain between rotavirus-positive or -negative babies, nor in the age at which they were discharged from hospital. No bacterial pathogens were isolated from the stools of 45 rotavirus-positive and 36 rotavirus-negative babies.

EM studies showed that many of these babies excreted very large quantities of virus (e.g. up to 10^{10} virus particles per ml faecal extract) and, since rotaviruses are stable at ambient temperatures (Woode, 1976) and no precautions were taken to limit spread of infection, we were surprised that the epidemic ended abruptly during the second week in August (Fig.).

FIG.—Percentage of 4- to 9-day-old babies excreting rotavirus.

A surveillance programme was instituted in which the stools of 5-day-old babies in one maternity ward were examined monthly. During the second and third weeks in November, rotaviruses were detected in 3 children aged between 6 months and 2 years in the general children's ward, this being on the same floor, but at a distance of about 100 metres from one of the maternity wards. During the first week in December, the stools of an asymptomatic 3-day-old baby were found to contain rotaviruses. However, weekly examination of the stools of 5-day-old babies in the maternity unit during the next 4 weeks showed, in contrast with findings during the summer, that infection was not transmitted to any other newborn infants.

Sixty-eight of 76 faecal specimens in which virus had been detected by EM were inoculated into IB-RS-2 cell cultures, and these were then examined by IF. Rotavirus antigen was detected in 58 of

the 68 (83%) specimens after centrifugation at 3000 g. However, 7 of the 10 specimens in which no rotavirus antigen could be detected were positive when inoculated by centrifugation at 10 000 g. Very few virus particles were detected in these specimens by EM. It was therefore possible to detect rotavirus antigen by IF in 65 out of 68 (95%) of specimens which were positive by EM (Table II).

TABLE II

Comparison of electron microscopy and IF for detection of rotaviruses

Total positive	EM positive	IF positive		IF negative
		3000 g	10 000 g	
68	68	58	7*	3

*Only the 10 specimens negative at 3000 g were tested at 10 000 g. EM, electron microscope; IF, immunofluorescence.

None of 42 faecal specimens negative by EM when tested by IF gave positive results. 10 specimens tested using bovine reagents provided identical results. Rotavirus antigen became detectable 6 hours after inoculation, the maximum number of antigen-positive cells being detected 24 hours after inoculation (Table III). Fluorescence was intra-

TABLE III

Development of rotavirus antigen in IB-RS-2 cell cultures

Time after inoculation (h)	1	4	6	8	10	24	48	72
No. fluorescent cells per $\times 80$ field	0	0	1	7	15	98	37	16

cytoplasmic and particulate and closely resembled the pattern of fluorescence occurring in calf-rotavirus infected cultures (Bridger and Woode, 1975).

Serological studies showed that rotavirus excretion did not appear to be related to maternal CF antibody, since there was no significant difference in the levels of antibody of mothers of rotavirus positive and negative infants (Table IV).

Discussion

Rotavirus infection in maternity units may not be uncommon and might perhaps explain periodic episodes of loose stools among babies for which no cause has hitherto been found. Since our preliminary report, rotavirus infection has been detected in maternity wards in Australia (Cameron *et al.*, 1975; Murphy, Albrey, and Hay, 1975) but in these outbreaks gastroenteritis occurred more frequently than at St. Thomas's Hospital, though symptoms were generally mild. Asymptomatic excretion of rotaviruses by neonates has also been observed in Glasgow, and infection may have been introduced by contact with other children (C. R. Madeley, personal communication, 1976). Though there is no obvious explanation as to how infection was first introduced into our maternity wards, it is possible that it was prevalent some time before mid-May. None of the nursing staff or mothers had gastroenteritis, and virus particles were not detected in the stools of the mothers of the first 3 infants excreting rotaviruses. Nevertheless, infection may have been introduced by an asymptomatic carrier, for example a mother, a visitor, or a member of the hospital staff.

Studies in the USA have provided serological evidence of infection in 35% of adults who were family contacts of children with asymptomatic rotavirus (Kapikian *et al.*, 1976). Most of these adults were asymptomatic and were not excreting rotaviruses, at least at a threshold detectable by EM. However, rotaviruses have been detected in the stools of adults with gastroenteritis (Flewett *et al.*, 1974; Zissis *et al.*, 1976, Kapikian *et al.*, 1976). Transmission of infection from adults to their offspring may occur as there is evidence

TABLE IV

Rotavirus of antibodies in mothers of rotavirus CF-positive and CF-negative infants

	Serum 1			Serum 2			No. seroconverted
	No. tested	No. <4	GMT	No. tested	No. <4	GMT	
Infants rotavirus + ve	34	5	7.4	42	5	9.1	2/20*
Infants rotavirus - ve	15	1	9.2	29	1	10.1	2/13*

*Numbers of paired sera available.
CF, complement fixed.

suggesting that calves are infected from the adult population (Woode, 1976). Alternatively, infection may have been transmitted to the maternity unit by hospital staff by direct contact; the re-introduction of infection into the maternity ward after rotavirus infection in the general children's ward suggests this. As the infection was mild, no special precautions were taken and, since rotaviruses are stable at ambient temperatures and aerosols containing large numbers of virus particles were almost certainly produced when nappies were changed, it is difficult to explain why the epidemic terminated abruptly in early August. The only change in environmental conditions we know of was a rise of approximately 8 °C in the daily maximum temperature recorded by the London Weather Centre during the last week in July and first week in August. The abrupt end of rotavirus outbreaks among calves has also been observed in the field by veterinary workers (C. A. Mebus, personal communication, 1976).

Rotavirus infections occurred less frequently among breast-fed babies and this may have been due to either transmission of specific antibody in breast milk or perhaps a recently demonstrated antiviral factor, distinct from antibody or interferon, found in breast milk (Tyrrell, 1976). Though there was no apparent difference in the antibody levels between the mothers of babies who excreted rotavirus and those who did not, maternal antibody may have in some way been responsible for infection in bottle-fed newborn babies also being mild or asymptomatic. Complement fixed antibodies are usually time-dependent and our finding that most adults have these antibodies suggests that immunity may be maintained by frequent exposure to infection. It is perhaps surprising that passively acquired transplacental serum antibody rather than local gut antibody may protect against rotavirus infection in newborn babies. However, circulating antibodies can protect the gut against such infections as cholera (Pierce, 1976), and the respiratory tract against influenza (Edmonson *et al.*, 1971).

Alternatively, the high proportion of mild or asymptomatic infections recorded in this study may have resulted from infection by avirulent rotavirus strains and recent studies suggest that both pig and mouse rotaviruses vary in their virulence (Woode, 1976; Flewett, 1976).

Human rotaviruses may be detected *in vitro* in human embryonic organ cultures (Wyatt *et al.*, 1974), as well as by a technique in which inoculated human embryonic gut cultures are layered with further human embryonic gut cells and tested for

rotavirus antigen by immunofluorescence, antigen being detected some 18 days after inoculation (Purdham *et al.*, 1975). Recently, Wyatt and his colleagues (1976) adapted a rotavirus strain to replicate in human embryonic kidney cell cultures, but it is unlikely that this system will be of diagnostic value. We feel that our technique, which detects rotavirus antigen within 24 hours of inoculating specimens, compares favourably in sensitivity with EM and may provide a suitable alternative for those laboratories which do not have access to an electron microscope. Using this technique we have also shown human rotavirus antigen in both primary human and bovine embryonic kidney cell cultures, but these cultures are considerably less sensitive.

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