A three-year diagnostic and epidemiological study on viral infantile diarrhoea in Rome

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SUMMARY

Rotavirus infection was demonstrated in 168 (29·3%) of 573 children hospitalized for acute diarrhoea in Rome between January 1982 and December 1984. Laboratory diagnosis of these infections was made by transmission electron microscopy and enzyme immunoassay techniques with an overall agreement of 91·3%. Astroviruses, adenoviruses and small round viruses were detected in the faeces of 36 patients (6·4%). Whereas in 1982 rotavirus positive patients were clustered in the winter and following spring, in the following years cases were recorded all year round. The median age of patients with rotavirus infections was 17, 10 and 11·5 months in 1982, 1983 and 1984, respectively. In addition, a smaller number of rotavirus positive cases were admitted in 1983 when compared to those admitted during the previous as well as the subsequent years. It is suggested that a herd immunity was induced in the population by epidemic spread of rotavirus in the first half of 1982.

INTRODUCTION

Acute gastroenteritis is extremely common in childhood in both industrialized and developing countries (Monto & Koopman, 1980; WHO Scientific Working Group, 1980; Snyder & Merson, 1982; Cukor & Blacklow, 1984). Among the different infectious agents involved in the pathogenesis of disease, rotavirus plays a major role accounting for more than 50% of hospitalized cases in peak months (Kapikian *et al.* 1976; Konno *et al.* 1978). In addition to rotaviruses, several other candidate aetiological viral agents are known, including enteric adenoviruses (Albert, 1986), astroviruses (Madeley & Cosgrove, 1975), calicivirus (Madeley & Cosgrove, 1976), coronavirus (Caul, Paver & Clarke, 1975), the Norwalk group of viruses (Kapikian *et al.* 1982) and other 20–30 nm diameter small round viruses (Flewett, Bryden & Davies, 1974; Middleton, Szymanski & Petric, 1977).

Because of the difficulties still encountered in growing such viruses routinely in cell cultures, diagnosis of infection currently relies on direct visualization of viruses in the stools by transmission electron microscopy (EM). As a consequence, only a limited number of comprehensive longitudinal surveys on viral enteritis have been reported in the literature.

As part of a nationwide programme to evaluate the role of different viral agents in infantile diarrhoea in Italy, we report here the results of a diagnostic and epidemiological study on 573 children with acute diarrhoeal disease admitted to the main paediatric hospital in Rome.

MATERIALS AND METHODS

Subjects. These were patients from 0–3 years admitted with acute diarrhoea to the Bambino Gesù Hospital in Rome during the period between January 1982 and December 1984. All the cases studied had signs of dehydration and had passed three or more loose or watery stools daily for at least 1 day and for no longer than 5 days prior to admission.

Specimens. Faecal samples were obtained within 24 h of admission and were frozen and stored at -70 °C until processing. Then 20% suspensions of faeces in phosphate-buffered saline (pH 7·2) were clarified by centrifugation at 3000 **g** for 20 min and extracted twice with Freon 113. The aqueous phase was centrifuged at 200000 **g** for 2 h at 4 °C, and the pellet was suspended in a small volume of 0·1 M ammonium acetate (pH 7·2).

Transmission electron microscopy (EM). A drop of each specimen, prepared as described above, was placed onto a carbon-Formvar coated EM grid (400 mesh), and then stained with 1% phosphotungstic acid (pH 7·1) for 1 min. Excess fluid was removed, and the grid was examined in a Philips EM 430 transmission electron microscope at a magnification of 40000. For each sample, 10 different grid squares were scanned during 15 min.

Enzyme linked immunosorbent assay (ELISA). The faeces were also analysed by a commercial ELISA test for rotavirus (Rotazyme, Abbott Laboratories, Chicago, IL), according to the manufacturer's instructions. Readings were made spectrophotometrically at a wavelength of 492 nm.

Extraction of rotavirus RNA. Faecal suspension of 0·2 ml was diluted with an equal volume of extraction buffer (Tris 0·01 M, NaCl 0·1 M, EDTA 0·001 M, 1% SDS, pH 7·5). Rotaviral double-stranded RNA (ds RNA) was extracted twice with phenol-chloroform as previously described (Herring *et al.* 1982), and precipitated with ethanol at -20 °C overnight. After centrifugation at 8800 **g** for 30 min, the pellet was washed with cold ethanol, centrifuged again, and stored desiccated at 4 °C.

Polyacrylamide gel electrophoresis of rotaviral RNA. Precipitated RNAs were resuspended in Laemmli sample buffer (Laemmli, 1970) containing 2·5% Ficoll 400. Electrophoresis was carried out in 10% polyacrylamide slab gels (Laemmli, 1970) at a constant voltage of 180 V for 18 h. The gels were silver-stained as described by Herring *et al.* (1982).

Complement fixation. (CF). Paired acute and convalescent phase sera were obtained on day of admission and 2 weeks later. CF anti-rotavirus antibodies were

determined by standard techniques with a rotavirus antigen from Istituto Behring, Scoppito, Italy.

Climatological data. Data on the outdoor temperature and relative humidity were obtained from the Central Office of Agrarian Ecology in Rome. Monthly mean values were averaged from four distinct measurements taken daily at 8 a.m. and 2 p.m.

RESULTS

Stools from 573 children with diarrhoea collected in Rome from January 1982 to December 1984 were screened from rotavirus by the Rotazyme ELISA test; 560 of them were also examined by electron microscopy. The results are summarized in Table 1. Overall, rotavirus was detected by EM and/or ELISA in the faeces of 168 patients (29·3 %); astroviruses, adenoviruses and 24–30 nm small round viral particles were observed by electron microscopy (Fig. 1) in a total of 36 out of 560 cases (6·4 %). In four patients, a dual infection with rotavirus and astrovirus was demonstrated.

In Fig. 2, the number of patients and the results of virological diagnosis are shown by month of admission to the hospital. In 1982, cases of rotavirus diarrhoea occurred mostly between January and July, and accounted for about 40 % of the 152 patients admitted during that period. In contrast, rotavirus infections occurred in 1983 and 1984 throughout the whole year, although the highest numbers were still recorded in the winter months.

Astrovirus infections were particularly frequent in 1982, being concentrated in the period from March to September during which they accounted for 8·5 % of all cases.

A possible relationship between the occurrence of rotavirus infection and climate was investigated. The monthly mean values for relative humidity and temperature recorded during the study period are shown in Fig. 3. In each year, the mean temperature registered minimum 7–8 °C levels in February and 25–27 °C peaks in July, varying consistently through the intermediate months. The relative humidity showed a slightly less regular course than the temperature. However, the two factors appeared to be inversely related.

The occurrence of rotavirus diarrhoea related to patient age is shown in Table 2. Rotavirus infection was found to occur with similar frequencies in all age groups from 3 months to 3 years, but was relatively uncommon in very young infants. The median age of rotavirus infected children was 17, 10 and 11·5 months in 1982, 1983 and 1984, respectively. In the same years, patients negative for rotavirus showed a median age of 9, 9 and 10 months, respectively.

A comparison of the rotavirus positive results obtained by EM and ELISA is made in Table 3 on the 560 samples which could be examined by both methods. Of the remaining 13 specimens, 9 were positive by the ELISA. Using electron microscopy as the standard for comparison, we found the Rotazyme test to have a sensitivity of 85·3 %, a specificity of 93 % and an overall agreement of 91·3 %. For 18 of the 30 ELISA positive and EM-negative cases, it was possible to test the CF antibody on paired acute and convalescent phase sera. A fourfold increase of anti-rotavirus antibody titre was found in three cases. Two additional patients,

Table 1. *Virus infections in children hospitalized for acute diarrhoeal disease in Rome, 1982–1984*

Year	Patients	Rotavirus	(%)	Astrovirus	(%)	Adenovirus	(%)	SRV	(%)	All viruses
1982	213	63*	(29·6)	14*	(6·6)	3	(1·4)	5	(2·3)	85
1983	190	41	(21·6)	5	(2·6)	2	(1·1)	3	(1·6)	51
1984	170	64	(37·6)	2	(1·2)	—	—	2	(1·2)	68
Total	573	168	(29·3)	21	(3·7)	5	(0·9)	10	(1·7)	204

* Four patients positive for both rotavirus and astrovirus.

Table 2. *Rotavirus infections by patient age in children hospitalized for acute diarrhoeal disease in Rome, 1982–1984*

Year ...	1982		1983		1984		Total	
Age (months)	No. tested	No. rotavirus positive (%)	No. tested	No. rotavirus positive (%)	No. tested	No. rotavirus positive (%)	No. tested	No. rotavirus positive (%)
0–3	39	4 (10·3)	29	2 (6·9)	29	3 (10·3)	97	9 (9·3)
4–6	29	6 (20·7)	32	7 (21·9)	22	13 (59·1)	83	26 (31·3)
7–9	22	5 (22·7)	32	10 (31·3)	23	9 (39·1)	77	24 (31·2)
10–12	19	5 (26·3)	21	5 (23·8)	21	11 (52·4)	61	21 (34·4)
13–15	12	4 (33·3)	15	4 (26·7)	12	3 (25·0)	39	11 (28·2)
16–18	23	11 (47·8)	17	6 (35·3)	10	4 (40·0)	50	21 (42·0)
19–21	14	5 (35·7)	18	4 (22·2)	19	11 (57·9)	51	20 (39·2)
22–24	19	10 (52·6)	8	1 (12·5)	12	5 (41·7)	39	16 (41·0)
25–30	11	4 (36·4)	9	1 (11·1)	10	2 (20·0)	30	7 (23·3)
31–36	17	6 (36·4)	4	1 (25·0)	10	3 (30·0)	31	10 (32·3)
n.d.*	8	3 (37·5)	5	—	2	—	15	3 (20·0)

* Not known.
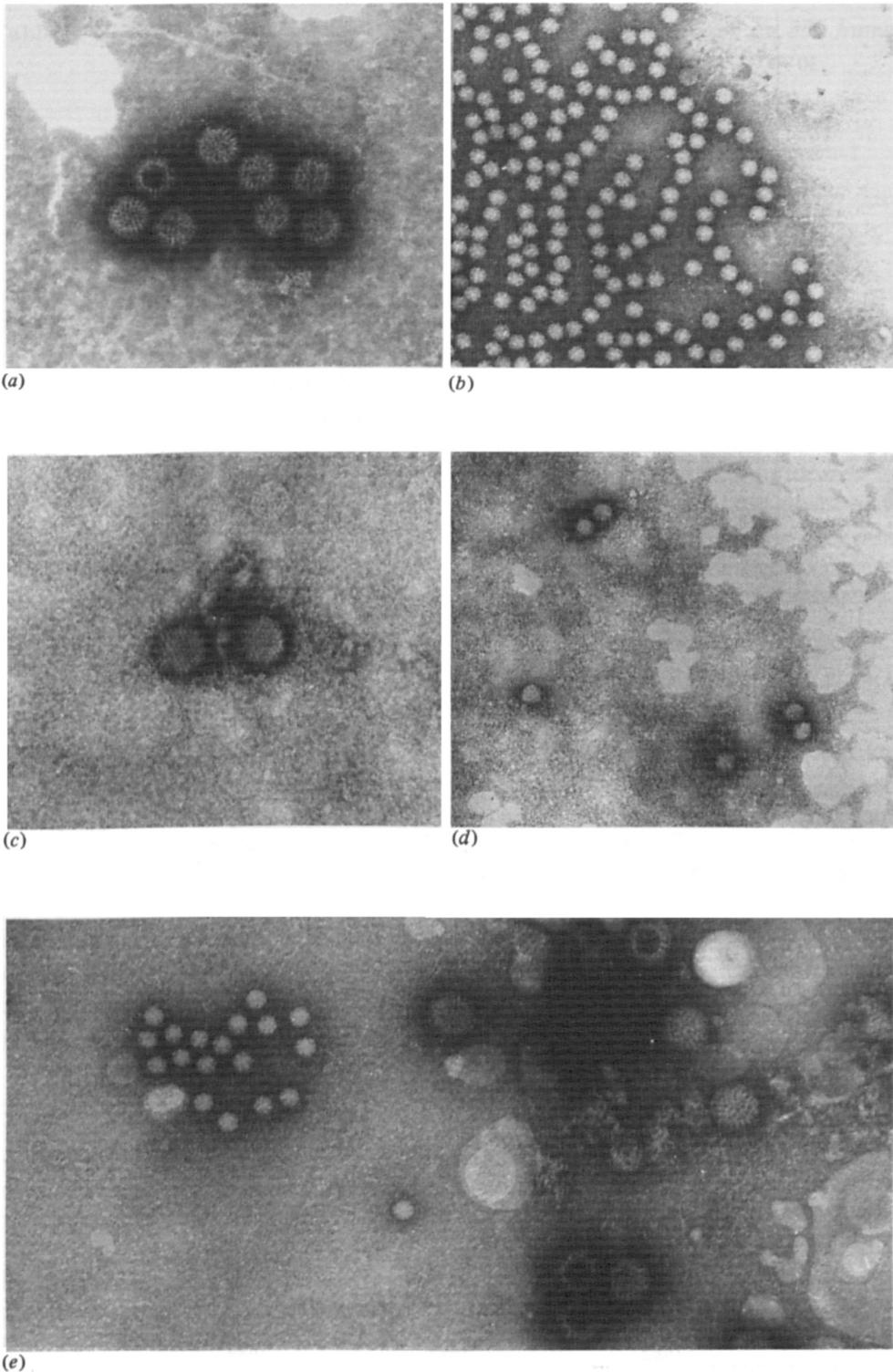
Fig. 1. Viral particles detected by EM in the faeces of children with diarrhoea. (*a*) Rotaviruses; (*b*) astroviruses; (*c*) adenoviruses; (*d*) 28 nm SRVs; (*e*) rotavirus-astrovirus mixed infection. (× 100000.)
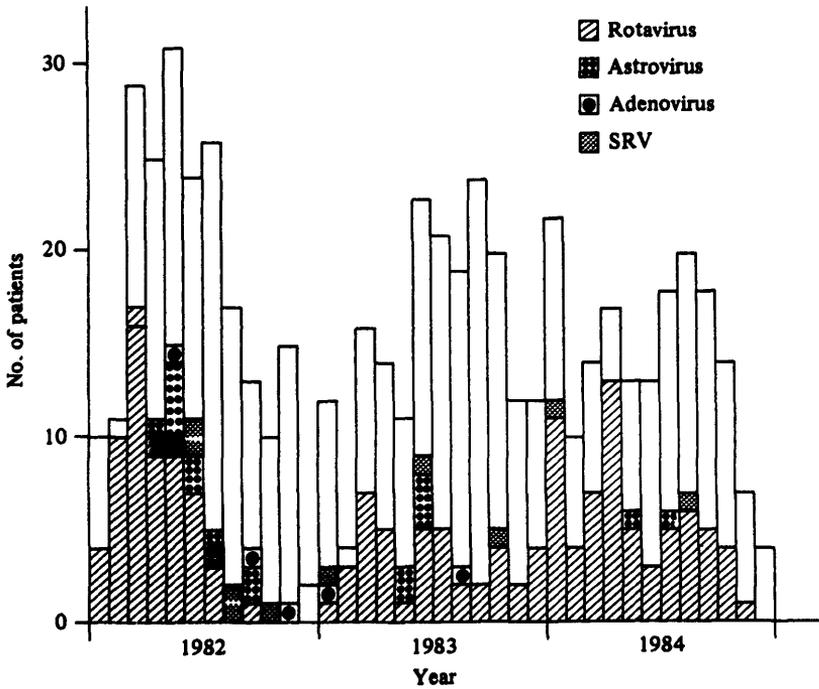
Fig. 2. Rotavirus, astrovirus, adenovirus and SRV infections in children with diarrhoea admitted to the Bambino Gesù Hospital (Rome, Italy) between January 1982 and December 1984.
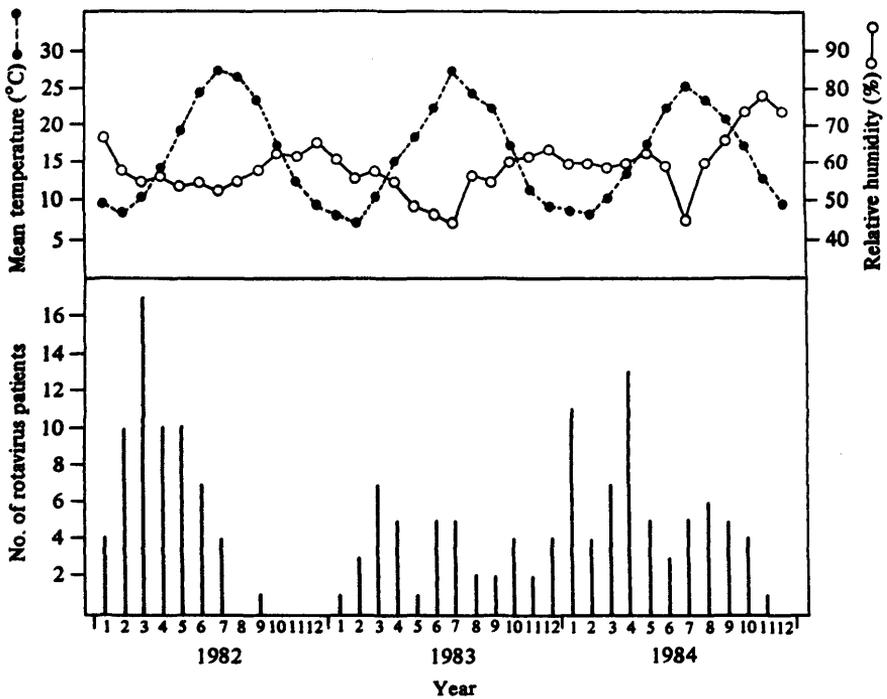

Fig. 3. Monthly distribution of rotavirus patients (lower) in relation to mean outdoor temperature (upper, dotted line) and relative humidity (upper, full line) in Rome between January 1982 and December 1984.

Table 3. *Comparison of Rotazyme ELISA and electron microscopy on 560 human stool specimens*

	No. of samples tested by EM		Sensitivity* (%)	Specificity† (%)	Overall agreement‡ (%)
	Positive	Negative			
Rotazyme					
Positive	110	30	85·3	—	91·3
Negative	19	401	—	93·0	—

* Sensitivity: ELISA and EM positives/EM positives × 100.
† Specificity: ELISA and EM negatives/EM negatives × 100.
‡ Overall agreement: (ELISA and EM positives)+(ELISA and EM negatives)/(EM positives + EM negatives) × 100.

aged 23 and 27 months respectively, had a stable high antibody titre (128) in their sera. Antibody to rotavirus was not detected in any of the other children.

Extraction of rotavirus RNA was attempted from 16 ELISA positive-EM negative faecal samples. In 5 of these, bands characteristic of the migration pattern of the segmented genome of rotavirus were detected. Of these 5 patients, 2 had previously been confirmed as positive by serology, whereas the other 3 had undetectable levels of anti-rotavirus antibody.

DISCUSSION

Our study provides further evidence that rotavirus is responsible for a large proportion of the hospitalized cases of acute diarrhoea in childhood, even in countries with high socioeconomic levels and good sanitation (Tufvesson & Johnsson, 1976; Konno *et al.* 1978; Brandt *et al.* 1983; Riepenhoff-Talty *et al.* 1983). Even though the sample we examined is not a statistically valid sample, it was collected each year by the same criteria and from the same population. The Bambino Gesù Hospital is the main paediatric hospital for Rome, and patients hospitalized there come from a well-defined catchment area. Our data may thus supply comparative data on the epidemiology of severe acute diarrhoea in Rome and its surroundings.

Each year, we diagnosed rotavirus infection in over 20% of our patients with a minimum of 21·6% of cases in 1983 and a maximum of 37·6% in 1984. The average was 29·3% of the 573 patients examined. As in other areas with a temperate climate (Bryden *et al.* 1975; Kapikian *et al.* 1976; Konno *et al.* 1978), relatively higher rates of infection were observed in late winter and spring, with monthly peaks which could exceed 50% of the cases of diarrhoea. However, differences emerge when comparing the monthly distribution of cases in 1982 to that found in the following 2 years of the study. In the first year, rotavirus infections appeared to be strictly concentrated between January and July, whereas in both 1983 and 1984, cases occurred all year round. It has been reported that climatic factors, particularly temperature, can influence the frequency of rotavirus infections (Brandt *et al.* 1982; Konno *et al.* 1983). However, we were not able to show any consistent variation in the annual pattern with either temperature or relative humidity during the survey period. Instead, the

occurrence of a single high peak of infection in 1982 followed by 6 months absence of rotavirus might indicate that a herd immunity had developed in the population. During the second year of the study, the introduction of new susceptibles would have allowed a gradual reintroduction of the virus into a still partially immune population. This is consistent with the succession of minor epidemic waves observed in the course of the two later years of the survey. We cannot rule out the possibility that high immunity levels during 1982 may have favoured the selection of rotaviruses which were antigenically different (Steering Committee, 1984) from the previously predominant strain(s). In either case, it is clear that a smaller number of rotavirus positive patients were admitted in 1983 compared to those admitted during the previous as well as the subsequent years. Moreover, we found a lower median age of rotavirus positive cases in 1983 (10 months) compared to 17 months in 1982.

Adenoviruses, which have been frequently involved in diarrhoea in several countries (Madeley *et al.* 1977; Brandt *et al.* 1979; Uhnoo *et al.* 1984), appear to be uncommon in our population and were detected in only 0·9% of cases.

Astrovirus infections, which were present in an overall 3·7% of patients, were relatively more frequent. Epidemic spread of this agent was seen between March and September 1982, when it was associated with 8·5% of cases. Even though 4 of the 21 patients with astrovirus infection were also excreting rotaviruses, our findings strongly suggest that astroviruses may be a significant cause of infantile diarrhoea in Italy.

In agreement with previous reports (Hammond *et al.* 1982; Miotti, Eiden & Yolken, 1985), our data indicate that for epidemiological purposes at least, rotavirus detection can be satisfactorily made by an enzyme immunoassay. In our hands, the immunoassay and electron microscopy gave the same result in 91·3% of the 560 samples examined by both methods. When the two tests were compared directly, the immunoassay registered sensitivity and specificity values of 85·3 and 93%, respectively. However, 8 of the 30 EM negative and ELISA positive cases could be confirmed as genuinely positive by demonstrating rotaviral RNA in the faeces and/or detection of anti-rotavirus antibody in the sera from the patients. Taking this into account, the actual specificity of the immunoassay test would be even higher than that we estimated. Nonetheless, since it allows recognition of viruses other than rotavirus, electron microscopy remains the method of choice in diagnostic and epidemiological studies on viral diarrhoea.

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