

## Identification of astrovirus serotypes from children treated at the Hospitals for Sick Children, London 1981–93

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### SUMMARY

An enzyme immunoassay (EIA) for astrovirus type 1 together with immune electronmicroscopy (IEM) was used to type a collection of 162 astroviruses obtained from 1981–93 from children with diarrhoea. The EIA was found to be specific for astrovirus type 1. Astrovirus types 2–4 were typed by IEM.

Astrovirus type 1 was the prevalent serotype 107/125 (86%), followed by type 3 (8%), type 4 (6%) and type 2 (1%). Six samples containing astrovirus could not be typed or detected by EIA because they were coated with coproantibodies; 11 others were not identified. Virus particles could no longer be detected in 15/162 (9%) samples following storage for  $\geq 2$  years.

Selected samples containing astrovirus types 1–4 were passaged in CaCO<sub>2</sub> cells and their identity confirmed by one or both assays. One sample was shown to have remained viable for 10 years when stored as an aqueous suspension at  $-20^{\circ}\text{C}$ .

Two patients with severe combined immune deficiency disease (SCID) were shown to be excreting astrovirus type 1 for 32 and 102 days respectively. One child was simultaneously shedding rotavirus and the other child was excreting adenovirus.

### INTRODUCTION

Astrovirus was first described in 1975 as the result of electron microscopy (EM) studies in the UK on faeces from children with diarrhoea [1–3]. During the following decade astroviruses were experimentally transmitted in adult volunteers [4], propagated in human embryonic kidney cells [5] and serially passaged in a continuous line of monkey kidney cells, LLCMK2 [6]. Immunofluorescence studies showed that there were at least five antigenically distinct strains [7] and recently a further two strains, types 6 and 7 have been described [8]. The use of EM in other countries showed that astrovirus infection occurs in children throughout the world [9]. The production of a group specific monoclonal antibody in 1988 [10] in combination with polyclonal antisera raised in Oxford to five strains of astrovirus enabled the development of an enzyme immunoassay as an alternative to EM for screening faecal samples [11]. The application of this assay has recently enabled larger epidemiological studies to be carried out on samples from developing countries, e.g. Thailand [12] and Guatemala [13]. Although astroviruses have been

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recognized for almost 20 years there are few data on prevalence of different strains in children presenting with symptoms of diarrhoea. The present study was set up to evaluate the use of an astrovirus EIA and to type a collection of astrovirus samples which had been obtained from 162 children treated at the Hospitals for Sick Children, London from 1981 to 1993.

## MATERIALS AND METHODS

### *Samples*

Samples of faeces which were found to contain astrovirus particles by EM between 1981 and 1987 were emulsified in water and stored as suspensions in water at  $-20^{\circ}\text{C}$  until they were tested in 1991. Samples collected between 1988 and 1993 were stored undiluted at  $+4^{\circ}\text{C}$ – $8^{\circ}\text{C}$ . Samples containing small round structured viruses, calciviruses, coronaviruses and rotaviruses had been held under identical conditions, and were used as controls.

### *Electronmicroscopy (EM)*

All samples were stained with 2% potassium phosphotungstic acid and examined by direct electronmicroscopy.

### *Immune electronmicroscopy (IEM)*

IEM was performed by the serum in agarose method as described previously [14] using antisera to astrovirus types 1–5, diluted 1/100. A reaction was considered to be positive if virus particles were heavily coated with one of the antisera but not the other four. A virus control was included with each run to ensure that particles were not initially coated with coproantibodies. Samples which gave optical density (OD) readings in the range 0.1–0.15 in the EIA were typed by IEM to confirm that these were not low level cross-reactions with other serotypes.

### *Cell culture*

Several samples were inoculated and passaged in  $\text{CaCO}_2$  cells as described by Willcocks and colleagues [15]. Astroviruses were identified by EM and typed by IEM.

### *Enzyme immunoassay (EIA)*

Astroviruses were initially screened using an EIA for astrovirus type 1 as described previously [11], but with the following modifications. Immunolon II (Dynatech Ltd) microplates were coated with the group specific monoclonal antibody (ascites) 8E7 diluted  $10^{-4}$  in carbonate/bicarbonate buffer and then blocked for 1 hr at  $37^{\circ}\text{C}$  with tris/0.1% Tween/3% bovine serum albumen buffer. A type 1 astrovirus antiserum prepared in a guinea-pig and purified as described previously [16] was used at a dilution of 1/300 in phosphate buffered saline/tween/2.5% skimmed milk. Horseradish conjugated anti-guinea-pig IgG (Sigma Ltd) at a dilution of 1/20000 was used as conjugate and TMB as the substrate. Samples with an OD of  $\geq 0.1$  (Coated–uncoated well, C–U) and a ratio of  $\geq 2.0$  (OD coated/OD uncoated well, C/U) were considered to be positive.

All samples giving a negative result in the astrovirus type 1 EIA were retested to ensure that the initial result was correct. If the samples gave repeatedly negative results they were re-examined by electronmicroscopy for the presence of astrovirus. Samples found to contain viruses were typed by IEM as described above.

### *Controls*

155 faecal samples referred to the laboratory between January and February 1991 were examined independently under code by electronmicroscopy and in the astrovirus type 1 EIA. Samples previously shown by EM to contain other viruses were also used. These comprised human calciviruses, 14; small round structured viruses, 10; adenoviruses, 12; rotaviruses, 63; coronavirus-like particles, 3.

## RESULTS

### *EIA*

Faecal samples from 107/162 (66%) patients which had previously been shown to contain astroviruses by direct EM were found to be positive when tested in the astrovirus type 1 EIA (Table 1). IEM tests on six samples with low OD (0.1–0.15) confirmed that the astrovirus present was serotype 1. Re-examination of the 55 samples which gave negative results by EM revealed the presence of typical astrovirus particles in 35. Astrovirus particles were not detected in the other 20 but bacteriophage particles (20–30 nm) were detected in 6 which may have been previously misidentified. Eight of 14 samples which were negative had been stored at  $-20^{\circ}\text{C}$  and 6 at  $+4^{\circ}\text{C}$ .

### *IEM*

IEM using 'Oxford' typing sera to serotypes 1–5 enabled 18/35 samples to be identified which were positive by EM but negative in the type 1 astrovirus EIA (Table 1). Ten samples were serotype 3, 7 samples serotype 4, and 1 sample serotype 2. Further confirmation of their identity was obtained by IEM after serotypes 1–4 were passaged several times in  $\text{CaCO}_2$  cells. Some of these samples had been stored for over 10 years at  $-20^{\circ}\text{C}$  and were still viable.

The specificity of the astrovirus type 1 EIA was confirmed by the failure of serotypes 2, 3 and 4 to react in the assay, OD (0.042–0.093). False positive reactions were not encountered when samples containing other enteric viruses (30, rotaviruses; 12, calciviruses; 10, small round structured viruses; 3, corona-like viruses) were tested in the EIA.

The results of testing 155 coded samples by EM and EIA are shown in Table 2. There were 2 samples which were negative by EM but positive by EIA and 1 sample which contained astrovirus particles but was negative by EIA (OD, 0.027). The use of IEM identified the latter virus to be astrovirus type 3. None of the 41 samples containing other enteric viruses gave a positive reaction in the EIA.

The results of testing serial samples from two children with severe combined immune deficiency disease are shown in Fig. 1. Patient 1 was first found by EM to be excreting rotavirus and astrovirus type 1 on 12 May 1981 but the sample gave an equivocal result in the astrovirus EIA ( $C-U = 0.147$ ,  $C/U = 1.94$ ).

Table 1. *Astrovirus serotypes detected in London, 1981-93*

Year	No. patients	Serotype					UT*	VND†
		1	2	3	4	5		
1981	14	14	—	—	—	—	—	—
1982	10	5	—	1	2	—	—	2
1983	NA‡							
1984	18	13	—	—	—	—	2, 1§	2
1985	18	11	—	2	—	—	4	1
1986	21	15	—	—	—	—	1§	5
1987	8	6	—	—	—	—	1	1
1988	4	3	—	—	—	—	—	1
1989	11	8	—	—	—	—	1§	2
1990	26	18	—	—	2	—	—	6
1991	16	10	—	1	3	—	2	—
1992	9	1	1	4	—	—	2, 1§	—
1993	7	3	—	2	—	—	2§	—
	162	107	1	10	7	0	11, 6§	15, 5

\* UT, untyped.

† VND, virus not detected.

‡ NA, samples not available for testing.

§ Samples coated with antibody.

|| Bacteriophage detected.

Table 2. *Results of examination by EM and astrovirus type 1 EIA of 155 faecal samples from children with diarrhoea January-February 1991*

	EM+		EM-
	Astro	Other	
EIA+	6	0	2
EIA-	1*	41†	105

\* Astrovirus identified as serotype 3 by IEM.

† 33, rotavirus; 5, adenovirus; 3, calicivirus.

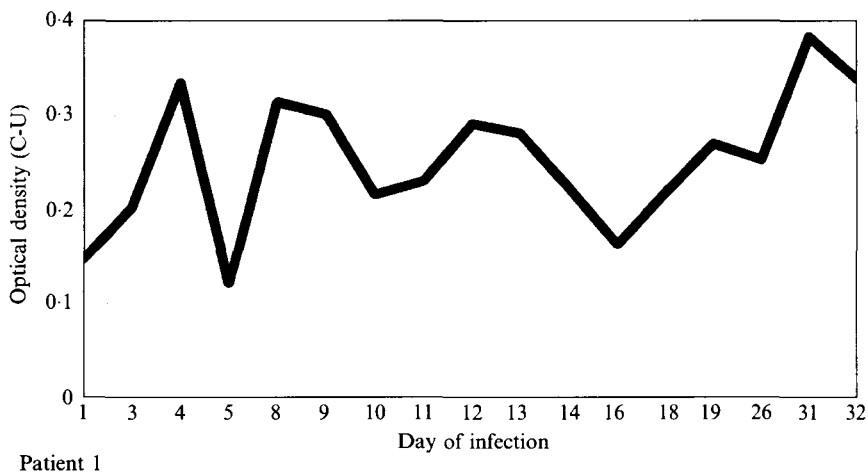


Fig. 1. Astrovirus excretion determined by enzyme immunoassay in two S.C.I.D. children.

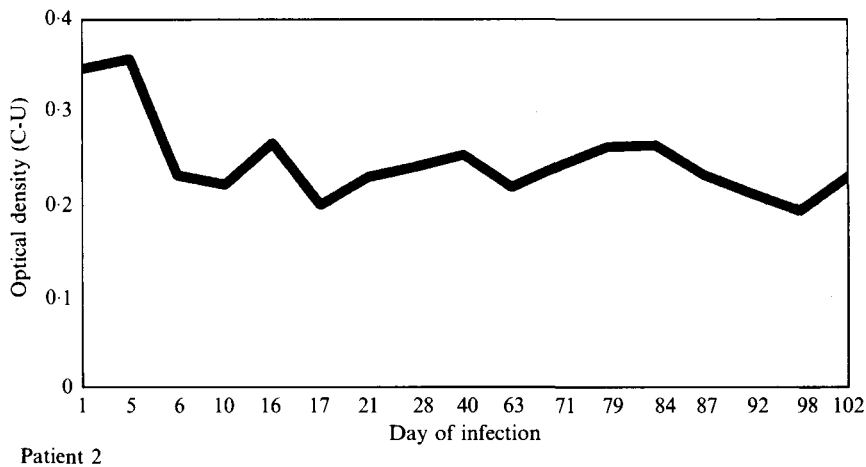


Fig. 2.

Subsequent samples collected on 16 occasions between 22 May and 2 July were all positive by EIA and shown by EM to contain rotavirus and astrovirus. Patient 2 was first shown to be excreting astrovirus type 1 on 13 January 1986 and was still excreting virus 102 days later on 25 April, at which time adenoviruses were also detected in his stools (Fig. 2). There were two samples in which virus was detected by EM but which were negative by EIA; both these samples gave high OD values on the coated and uncoated wells.

#### DISCUSSION

The results confirm the value and specificity of an astrovirus EIA as an alternative to electronmicroscopy for the diagnosis of infections in the UK. However, it was fortuitous that astrovirus type 1 was the predominant serotype because our EIA was type 1 specific. The development of an astrovirus antigen EIA for types 1–5 would provide a reliable alternative to EM. However attempts to develop a type-specific EIA using the 'Oxford' reference antisera to types 1–4 failed due to the high degree of cross-reaction between serotypes, as shown previously [11]. However, IEM clearly distinguished between types 1–4, presumably because the type-specific epitopes are displayed on the surface of intact particles. The present results confirm that EM remains the best method for detecting astrovirus until more specific antisera become available. Recent advances in sequencing astrovirus [17], development of probes [18], and the application of reverse transcription PCR should result in the availability of highly sensitive and specific diagnostic tests in the next few years. Serotype 1 was shown to be the predominant type associated with infection in children accounting for 107/125 (86%) strains which were identified. This is similar to the results of a study carried out in Oxford, UK between 1976 and 1992 on 291 astrovirus positive stools in which 65% of the isolates were type 1 [8]. In contrast to them we found no evidence of biennial peaks of infection. Serotype 3 was identified in 10 cases (8%), 7 of which occurred in the period 1991–3. In 1992 serotype 3 was the

predominant type accounting for  $\geq 4/9$  cases which coincides with the peak incidence of type 3 found in the Oxford study [8]. Only one case was shown to be associated with serotype 2 and no cases of astrovirus type 5 were encountered.

Re-examination of samples which had previously been identified as containing astroviruses but were EIA negative suggested that a few samples contained phages. The duration of storage and temperature ( $+4\text{ }^{\circ}\text{C}$  or  $-20\text{ }^{\circ}\text{C}$ ) had no significant detrimental effect on the identification of astroviruses by EIA and we were surprised to find that an astrovirus sample was still viable after 10 years storage at  $-20\text{ }^{\circ}\text{C}$  when it was inoculated into  $\text{CaCO}_2$  cells. The results of EIA and EM on serial samples collected from several patients showed that astroviruses were often excreted over periods of 3–5 days. Examination of samples from two patients with Severe Combined Immunodeficiency Disease showed that they were both infected with astrovirus type 1 and coinfecting with other enteric viruses (rotavirus or adenovirus). Prolonged excretion occurred in both patients, 32 days in one child and 102 days in the other. Studies are now in progress to determine whether any variation in the genomic sequence occurred over this period; a feature which has been noted in rotavirus isolates obtained from SCID children who have excreted virus over several months [19].

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