

Seroprevalence of astrovirus types 1 and 6 in London, determined using recombinant virus antigen

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SUMMARY

We have developed a microimmunofluorescence test (IF) which uses cells infected with a recombinant baculovirus which expresses the capsid proteins of astrovirus types 1 or 6. The IF test was sensitive and specific and the results for human astrovirus type 1 (HAst-1) were comparable to those obtained by immune electronmicroscopy and radioimmunoassay. Application of the test to a panel of 273 sera collected from patients and staff at two childrens hospitals in London showed that over 50% of the population were infected by HAst-1 between the age of 5 and 12 months rising to 90% by 5 years, whereas human astrovirus type 6 (HAst6) was relatively uncommon (10–30%) in all age groups.

INTRODUCTION

Astroviruses were discovered in 1975 as the result of an investigation of an outbreak of diarrhoea among babies in a maternity unit in the south of England [1]. However, until the production of a group specific monoclonal antibody in 1988 [2] and the subsequent development of an enzyme immunoassay (EIA) [3] diagnosis depended on electronmicroscopy (EM). The application of EIA has provided increasing evidence that astroviruses may be a more common cause of diarrhoea than previously supposed [4, 5] but a limited supply of reference typing antisera has meant that its application has been confined to one or two research laboratories in the USA and UK [6–8]. Data on the seroprevalence of astroviruses is even more sparse, being limited to two surveys; an immunofluorescence study on the prevalence of antibodies to human astrovirus type 1 (HAst-1) using 87 sera from children in the Oxford area of England [9] and human

astrovirus type 5 (HAst-5) in the community in the USA [10].

The present study describes the development of a simple microimmunofluorescence assay to measure IgG responses, using baculovirus expressed HAst-1 and HAst-6 capsid antigens. The efficacy of the test was evaluated using reference materials and samples from well documented cases of astrovirus infection and then applied to seroprevalence studies.

MATERIALS AND METHODS

Antisera

Reference sera

Hyperimmune rabbit antisera to human astrovirus type 1 (T59, T65); type 2 (T57); type 3 (T61); type 4 (T62); type 5 (T63); type 6, (T64); and Type 7 (T66) were obtained from T. Lee, Public Health Laboratory, John Radcliffe Hospital, Oxford, UK. A group specific monoclonal antibody, (8E7) raised in mice [2]

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was obtained from Dr J. Herrmann, University of Massachusetts, Worcester, USA.

HAst-1; Cambridge, UK outbreak, February 1985

Acute and convalescent phase sera were obtained from 12 residents in a home for the elderly who were involved in two successive episodes of gastroenteritis. Previous laboratory investigations [11] had shown that the cause of the initial episode was a human calicivirus and the second episode which followed a week later was due to astrovirus type 1. Significant IgM and IgG responses to astrovirus type 1 were demonstrated by radioimmunoassay in two patients who excreted virus particles in their stools [12].

HAst-1; Norfolk, UK outbreak, August 1983

Acute and convalescent phase sera were obtained from pupils at a high school where an outbreak of diarrhoea and vomiting occurred. Astrovirus particles were detected in faecal samples from three of the cases and identified by immune electronmicroscopy (IEM) as serotype 1 using reference antiserum (T59).

HAst-4; London, UK November 1982

Acute (day 4 post infection) and convalescent phase (day 18) sera were obtained from a female medical laboratory scientist, aged 30 years who developed severe symptoms of diarrhoea and vomiting, shown to be caused by astrovirus by direct electronmicroscopy. The strain was identified by immune electronmicroscopy and immunofluorescence as type 4 using Oxford reference antiserum T62.

HAst-5; (Marin County agent, USA)

Pre- and post-challenge sera were obtained from an adult volunteer (DA) infected with a faecal extract derived from material obtained from the original outbreak which occurred in California in 1978 [13]. Subsequent studies identified the causative agent as astrovirus type 5 [6, 10, 15]. All sera had been stored at -20°C .

Test Sera

Sera from 253 children (treated at Gt Ormond St Hospital for Children or Queen Elizabeth Hospital for Children) which had previously been screened for the presence of antibodies to Norwalk virus [16] and

from 20 members of the hospital staff aged 18–30 years were screened for the presence of IgG antibodies to astrovirus types 1 and 6 as described below. Samples from patients who were immune deficient or who had symptoms of diarrhoea were excluded from the study.

Baculovirus expressed HAst-1 and 6 capsid proteins

The astrovirus capsid gene was cloned into the baculovirus shuttle vector pVL 1392. The construct was combined with *Sau-1* linearized baculovirus (LAC-2) DNA and cotransfected into Sf21 cells with lipofectin. Virus yield was harvested and plaqued. White plaques were picked and tested for expression of astrovirus protein as described previously [14].

Immune electronmicroscopy (IEM)

IEM was carried out by the serum in agarose method described previously [17] using antigen derived from the patient's stools. The end point was taken as the highest dilution at which antibody could still be seen binding to the virus particles.

Radioimmunoassay (RIA) for IgM and IgG to HAst-1

The results of tests for IgM and IgG responses to astrovirus type 1 on some of the sera have been reported previously [12]. The same assay was used to test samples from the other episodes of infection.

Immunofluorescence

A suspension of Sf21 cells infected with baculovirus expressed HAst-1 capsid protein was diluted 1:100 in phosphate buffered saline and 10 μl drops placed on each well of a 16 spot (5 mm, diameter) microscope slide (Hendley-Essex, Essex, UK) to produce a film containing discrete cells. Slides were fixed in acetone/alcohol, air dried and then stored at -70°C until used. Wells containing uninfected sf 21 cells acted as controls for non-specific fluorescence. Sera were titrated in a series of doubling dilutions from 1:10 through 1:1280 and a 10 μl drop placed on the fixed cells and incubated at 37°C for 30 min. Slides were washed for 15 min in PBS, dried and 10 μl of 1:40 rabbit anti human IgG γ -chain conjugated fluorescein isothiocyanate (Dako Ltd, High Wycombe, UK) added. This was allowed to bind for 30 min at 37°C in a moist chamber. Slides were then removed, washed

in PBS for 10 min and then in distilled water for 10 min. After air drying slides were examined under a UV fluorescence inverted incident light IMT-2 microscope (Olympus Optical Co, London, UK), for the presence of fluorescing cytoplasmic inclusions and the end point recorded for each serum. The protocol was identical for the reference rabbit hyperimmune sera and monoclonal antibody except that anti rabbit IgG-FITC at a dilution of 1:40 or anti mouse IgG-FITC at 1:100 (Dako Ltd, UK) were used as conjugates.

Seroprevalence studies were conducted using sera diluted 1:20 in PBS as non-specific fluorescence was observed when sera were tested at lower dilutions on uninfected cells. Bound antibody was detected using goat anti human IgG (gamma chain)-FITC conjugate (Dako Ltd).

RESULTS

Standardization of the recombinant antigen

Tests using the Oxford rabbit reference antisera and HAst-1 capsid protein expressed in baculovirus-infected sf 21 cells showed strong immunofluorescence up to a titre of 800 and 1600 respectively with the type 1 antisera T59 and T65 but no reaction at a dilution of 1:100 when antisera to types 2–7 were used. Use of a group specific monoclonal antibody 8E7 gave a strongly fluorescent signal with an end point of 1:4000.

In cells expressing HAst-6 capsid protein discrete granular fluorescence was observed using the reference sera raised to type 6 (T64) to a titre of 400 and with the group specific monoclonal antibody 8E7 at 800 but not with antisera raised to any of the other six serotypes.

Serological response following human infection

The results of tests on patients involved in the Cambridge outbreak are shown in Table 1. The IF results were directly comparable with those previously obtained by IEM, and RIA. The elderly patients (patients 1–3) involved in the first and second episode of diarrhoea produced antibody (> fourfold response) to HAst-1. Individuals involved only in the first wave of diarrhoea (4–9) and known to have been infected with a strain of human calicivirus and a further three patients (10–12) presenting with a rash resembling Fifth disease showed no evidence of recent astrovirus infection.

Table 1. *Antibody responses to astrovirus type 1 determined by immune electronmicroscopy (IEM), radioimmunoassay (RIA) and microimmunofluorescence (IF) following an outbreak in a residential home for the elderly in Cambridge, UK (A, acute; C, convalescent)**

Case†	IEM (total antibody)	RIA (IgM)	RIA (IgG)	IF (IgG)
1 A‡	< 20	< 80	< 80	< 10
C	> 640	> 2560	> 20480	> 320
2 A	< 20	< 80	< 80	NA§
C	320	2560	10240	> 160
3 A	< 20	< 80	640	10
C	> 640	320	> 20480	320
4 A	< 20	< 80	< 80	< 10
C	< 20	< 80	< 80	< 10
5 A	< 20	< 80	< 80	< 10
C	< 20	< 80	< 80	< 10
6 A	40	< 80	< 80	10
C	40	< 80	< 80	10
7 A	20	< 80	< 80	< 10
C	20	< 80	< 80	< 10
8 A	40	< 80	< 80	10
C	40	< 80	< 80	10
9 A	20	< 80	80	< 10
C	< 20	< 80	< 80	10
10 A	< 20	< 80	< 80	10
C	< 20	< 80	< 80	10
11 A	< 20	< 80	< 80	10
C	< 20	< 80	< 80	10
12 A	< 20	< 80	< 80	< 10
C	< 20	< 80	< 80	< 10

* Significant antibody response in **bold** type.
 † Patients 1–3 involved in first and second episode of diarrhoea. Patients 4–9 involved in first episode of diarrhoea caused by calicivirus. Patients 10–12 presented with a rash.
 ‡ Patient excreting Astrovirus.
 § NA, Not available.

The use of the three serological tests to monitor seroresponses to astrovirus following an outbreak involving pupils at a high school are presented in Table 2. The three pupils shown to be excreting virus developed significant IgM and IgG seroresponses to HAst-1. The other pupils had low or undetectable levels of antibody indicating that they were not infected.

The scientist infected with HAst-4 developed a strong antibody response to HAst-4 titres rising from 10 on day 4 of infection to 640 (day 18, post infection) as determined by IEM using antigen obtained from her stool, and showed a slight decline in titre to HAst-1 when her paired sera were tested by IEM, RIA and

Table 2. *Antibody responses to astrovirus type 1 in teenagers and adults infected with astrovirus types 1, 4 or 5/Marin County Agents (A, acute; C, convalescent; Pr, pre challenge; Po, post challenge)**

Norfolk High School (type 1)				
Case	IEM (total)	RIA (IgM)‡ counts/5 min	RIA (IgG) counts/5 min	IF (IgG)
*1 A†	< 20	870	565	< 10
C	320	1166	2329	320
*2 A†	< 20	861	433	< 10
C	40	1913	1873	40
*3 A†	80	957	733	< 10
C	> 160	2553	3565	160
4 A	40	919	536	10
C	> 160	1209	2447	160
5 A	< 20	850	699	< 10
C	< 20	959	692	20
6 A	40	981	848	10
C	40	957	812	20
7 A	20	647	1055	10
C	20	693	1142	20
8 A	< 20	554	793	10
C	20	860	659	10
9 A	40	526	920	40
C	40	631	1108	40
10 A	20	628	663	20
C	20	635	678	20
11 A	20	575	745	10
C	20	613	728	10
12 A	20	1096	783	10
C	20	598	661	10
13 A	< 20	491	610	20
C	< 20	717	698	40
Adult, sporadic case, UK (type 4)†				
A	160	490	1860	80
C	80	526	1386	40
Adult volunteer (DA) USA (Marin County agent/type 5)†				
Pr	20	942	893	20
Po	40	1482	2500	80

* Significant antibody response in **bold** type.

† Astrovirus detected in stools.

‡ RIA performed on sera diluted 1/80.

IF (Table 2). A fourfold response to HAst-1, 1/20 (acute phase)-1/80 (convalescent phase) was demonstrated by immunofluorescence in a volunteer challenged with HAst-5. Similar responses were previously demonstrated by IEM and RIA [6, 12].

Seroprevalence study

The results of screening 253 sera from children aged 1 day–13 years and 20 adults are shown in Figure 1. The

majority of neonates possessed maternal antibody to astrovirus 1, (58%) and Norwalk virus (64%) which correlated to the prevalence in women of childbearing age. Maternal antibody waned by 5 months followed by a period of rapid acquisition of antibodies to HAst-1 during the following 6 months rising to 90% by the age of 5 years. However, the prevalence in the age group 6–40 years was lower (50–60%). The prevalence of antibodies to HAst-6 in neonates was comparatively low (20%) and remained relatively constant in all age groups 1–3 years (10–30%). Many patients possessed antibodies to HAst-1 but not against HAst-6 indicating a lack of cross reactivity in one direction.

DISCUSSION

The use of HAst-1 capsid proteins expressed in baculovirus provides a simple, sensitive and type specific method comparable to IEM or RIA. The ease of producing large amounts of capsid protein creates for the first time the potential to perform large-scale seroepidemiological studies to ascertain the true prevalence and incidence of astrovirus infections worldwide. At present there is a paucity of information; the only studies that have been conducted are a small survey of antibodies to HAst-1 using sera from 87 children in the Oxford region of the UK [9] and a survey of the prevalence of HAst-5 in 182 children and adults in the USA [10]. The importance of obtaining such information is highlighted by recent reports of extensive outbreaks of astrovirus infection in Japan affecting many thousands of school children, related to consumption of contaminated food [8, 18, 19]; serological studies were limited due to the lack of suitable reagents. One of these outbreaks was caused by HAst-6 [18]. We are in the process of attempting to express the full range of astrovirus serotypes in baculovirus which will provide the potential to study such outbreaks in more detail as the period in which sufficient antigen is present for detection by EM and EIA tests is limited to a few days post infection [20]. Access to a full range of reference material will enable us to study the extent of cross reactivity between the proteins in a number of different assay formats.

The results of the tests on the adult volunteer challenged with HAst-5 confirmed our previous observations [6, 12] and the findings of Midthun and colleagues [10] that an anamnestic response or cross

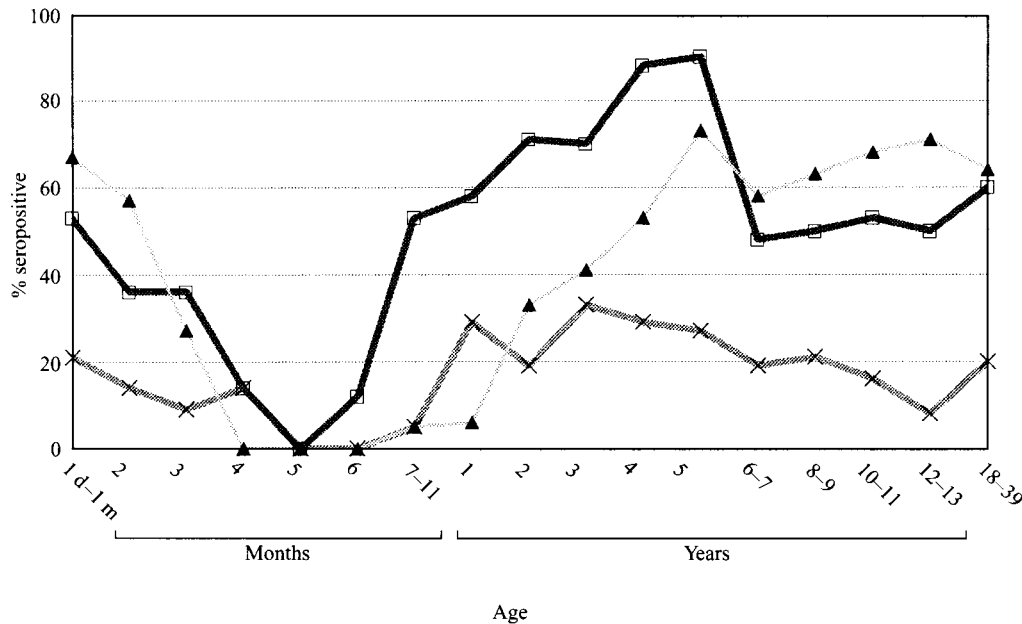


Fig. 1. Seroprevalence of antibodies to astrovirus types 1 and 6 and Norwalk virus in 253 children aged 1 day–13 years and 20 adults in London. ▲, NV; □, HAst-1; ×, HAst-6.

reaction occurs between HAst-5 (Marin County agent) and other strains of astrovirus. A similar cross reaction between HAst-1 and 5 was demonstrated by IEM using reference materials [21]. In contrast we found no such response to HAst-1 in an adult naturally infected with type 4 who had high levels of antibody to HAst-1 in her acute phase sera. This suggests that there may be shared epitopes between type 1 and 5 which do not exist with type 4. Considerable differences are known to exist between the capsid protein of type 4 and 1 [22] but these are confined to relatively precise regions of the protein.

The seroprevalence studies indicate that HAst-1 is prevalent in London and that infants become infected once maternal antibody has waned. The rapid acquisition of antibodies over a 6 month time span suggests that astrovirus must be maintained at high levels in the environment. It is therefore of interest that astrovirus has been detected by RT-PCR in samples of drinking water obtained from the mains supplies from several locations in the UK (S. Myint, personal communication) and that it appears that the prevalence has increased judging by the high seropositivity in the age range 2–5 year olds compared with patients and staff aged between 6–40 years. In contrast antibodies to HAst-6 in the same population of individuals were relatively uncommon in all age groups, suggesting that this serotype may have only recently emerged in the UK. It would be of interest to conduct a similar study on Japanese sera in view of

recent reports of an epidemic of HAst-6 affecting individuals of all ages [18]. At present little is known about the incidence of HAst-6 worldwide as this serotype was first described last year; to date only one confirmed case has been reported in the UK [23]. Further studies are now in progress to express the capsid proteins of the other serotypes in baculovirus which will enable seroprevalence studies to be carried out in the UK and elsewhere.

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