

## **Epidemiology of enteric adenovirus infection in prospectively monitored Argentine families**

A. S. MISTCHENKO, K. H. HUBERMAN, J. A. GOMEZ AND S. GRINSTEIN

*Laboratorio de Virología, Hospital de Niños 'Ricardo Gutiérrez', Gallo 1330,  
1425 Buenos Aires, Argentina*

*(Accepted 6 May 1992)*

### SUMMARY

To examine the role of enteric adenoviruses (EAV) in an urban area of Buenos Aires (Argentina), we prospectively studied faecal samples from 49 families of newborns. These were monitored weekly for diarrhoea for 2 years.

A total of 180 samples from cases of diarrhoea and 766 samples obtained during diarrhoea-free periods were studied by dot-blot hybridization with an EAV-specific DNA probe. EAV were found in 6/180 (3·3%) cases of diarrhoea and 6/766 (0·8%) asymptomatic samples ( $P < 0\cdot015$ ). Incidence of EAV was 3·9 cases per 100 person-years in children < 60 months old. EAV-related diarrhoeas were slight and of short duration. In addition, 129 faeces from hospital out-patients, 1–30 months old, were also studied. EAV was identified in 7/129 cases (5·4%). These cases were  $9\cdot5 \pm 3\cdot5$  months old and the diarrhoea was mild or severe, of  $3 \pm 1\cdot5$  days of duration.

We suggest that EAV are low-risk causes of diarrhoea under natural conditions, although a few children may develop more severe diarrhoea. The diagnosis of EAV needs to be considered in these patients.

### INTRODUCTION

At present, 47 adenovirus (AV) serotypes have been identified. Group F comprises types 40 and 41; the enteric adenovirus (EAV). These have been implicated as the second most common cause of viral gastroenteritis in infants and young children, after rotavirus [1–3]. Most AV serotypes may be excreted in faeces, sometimes for long periods, as with AV group C [4]. The role of AV other than EAV in gastroenteritis has recently been suspected [5], although their causal role has not been proved.

EAV are difficult to culture and alternative methods, such as immunoassay techniques [6], DNA restriction analysis [7] or molecular hybridization [8], are currently used for diagnosis. EAV usually infect and cause symptoms in children up to 3 years old, but mainly in those 2 years old or younger [9]. Some aetiologic studies of hospitalized and outpatient subjects with diarrhoea have been carried out recently [10–12]. Although these studies are necessary in order to determine the importance of the aetiologic agent, the epidemiological results are relevant

only for those cases in which medical treatment is required. Community-based surveys are needed in order to determine the general risk of infection.

We organized a 2-year prospective study to establish the frequency of different common causes of viral infantile gastroenteritis in a normal population and some results on rotavirus have been reported [13]. As part of the study of EAV, we have included a group of children with community-acquired diarrhoea that required medical care. Here we present results related to the epidemiology of EAV in both populations.

## METHODS

### *Subjects*

*Community-based study.* Briefly, the study included 49 families (227 individuals, 52 adults and 175 children aged < 15 years) from the city of Avellaneda, 15 km south of Buenos Aires. To be included in the study each family had a child young enough (< 5 years old) to be seen by a paediatrician if it became ill, and the mother had to be in the last trimester of pregnancy. The newborn was studied together with its family until it reached the age of 2 years. These families were visited weekly for 2 years, and the mother was asked about the presence of gastrointestinal symptoms. Samples of faeces were collected and stored at  $-70^{\circ}\text{C}$  from all family members prior to the birth of the newborn. Another sample was collected from every member of the family before the newborn was 1 month old. Samples were collected from all family members when diarrhoea symptoms developed, and every 6 months, until the end of the study period. Diarrhoea was defined as a significant increase in the frequency, or a decrease in the consistency, of the stools, compared to the usual bowel habit. Between 1 May 1984, and 31 May 1986, stool specimens from a total of 766 asymptomatic individuals and 180 cases of diarrhoea were collected from the studied individuals. Our laboratory had previously studied the epidemiology of rotavirus infection under natural conditions in this population [13].

*Hospital-based study.* Simultaneously with the community-based study, 129 specimens of faeces were collected from children 1–30 months old, within 5 days of onset of acute gastroenteritis, admitted to the San Justo Children's Hospital. Only one sample was studied from each patient. San Justo Children's Hospital is located in the West neighbourhood of Buenos Aires, and accepts patients from several peripheral cities.

### *Laboratory studies*

*ELISA.* A 10% suspension of faeces in 0.05 M Tris-HCl, pH 7.5, was clarified by centrifugation at 10000 g for 5 min. Supernatants were tested for AV group antigen by enzyme-linked immunoassay (ELISA) [14] with polyclonal rabbit antiserum against AV-2 hexon purified according to Pereira and colleagues [15] for capture, and guinea-pig antiserum to hexon for detection. This was followed by a peroxidase-conjugated serum anti-guinea-pig immunoglobulin (Dako Laboratories, Denmark).

*Dot-blot hybridization.* After screening by ELISA, positive samples were treated as

described by Hammond and colleagues [16]. Briefly, each faecal suspension (10% w/v stool in 0.05 M Tris-HCl, pH 7.5) was clarified in Eppendorf tubes at 10000 g for 5 min. Then 20  $\mu$ l of the suspension were treated with 4 M-NaOH and 100 mM-EDTA, followed by boiling for 10 min. Sodium acetate was then added to neutralize the mixture, and the suspension deposited directly onto a GeneScreen Plus<sup>™</sup> membrane (Dupont) previously placed on the dot-blot apparatus. After 30 min samples were subjected to vacuum through the membrane and air-dried before hybridization.

The DNA probe 41-27 (probe 41-27) was an 800 bp restriction fragment generated from AV-41 by digestion with *Bam*H1/*Eco*R1. It is specific for fastidious adenoviruses, detects only AV-40 and -41 [17], and was a kind gift from Christian Niel (INSERM, Lille, France). The probe was <sup>32</sup>P-labelled by the random primer method. Membranes were hybridized for 20 h at 65 °C with 10<sup>6</sup> cpm/ml of the labelled probe. Hybridization was carried out for 15 min at 65 °C in 5% dextran sulphate, 1 M-NaCl, 1% SDS. The membrane was then thoroughly washed under conditions of low salt and autoradiographed on X-OMAT AR film (Kodak) at -70 °C with an intensifying screen.

*Viral controls for hybridization.* Positive controls were DNA of AV-40 (strain Dugan ATCC VR-931) and AV-41 (strain Tak ATCC VR 930). Negative controls were DNA of AV-2 (strain Adenoid 6, ATCC VR-846), AV-3 (strain GB, ATCC VR-3), AV-4 (strain RI-67, ATCC VR-4), AV-7a (strain S-1058, ATCC VR-8), AV-1 (strain Adenoid 71, ATCC VR-1078) and AV-5 (strain Adenoid 75, ATCC VR-5). All these viruses were grown in 293 cell line (CRL 1573, ATCC) and viral DNA was obtained as described [19].

*Additional studies.* Dot-blot positive samples were further analysed by ELISA specific for subgroup F AV (Adenoclone type-specific 40-41, Cambridge Bio-Science) and by direct viral DNA extraction and restriction analysis with *Sma*-I treatment, as described by Buitenwerf and colleagues [7]. Five EAV-positive faeces were further identified as AV 40 or AV 41 in the Department of Virology of the Oswaldo Cruz Institute (Rio de Janeiro, Brazil) by Dr S. Gomes with a monoclonal-based ELISA.

*Epidemiological calculation and statistics.* The incidence (I) of EAV-related diarrhoeas was calculated as No. of EAV-diarrhoea  $\times$  Total No. of diarrhoea episodes / No. of observations  $\times$  No. of diarrhoea episodes studied, using additional data previously reported by us [13]. Chi-square test or Fisher exact test were used for the analysis of significance.

## RESULTS

### *Community-based study*

Between 1 May 1984 and 31 May 1986 stool specimens were collected from 946 study participants, of whom 180 had diarrhoea and 766 were asymptomatic. As shown in Table 1, 26/180 (14.4%) cases of diarrhoeas and 102/766 (13.3%) asymptomatic individuals excreted AV as detected by ELISA. These 128 AV-

Table 1. *Detection of adenovirus in prospectively monitored families and in outpatient children*

	Group		
	Community		Hospital O/P*
	Diarrhoea (n = 180)	Asymptomatic (n = 766)	Diarrhoea (n = 129)
ELISA	26 (14.4%)	102 (13.3%)	14 (10.8%)
Dot-Blot	6 (3.3%)	6 (0.8%)†	7 (5.4%)

\* O/P, outpatients.

†, Significant difference ( $P < 0.015$ ).

positive stools were analysed for EAV by dot-blot hybridization with probe 41-27. Each hybridization included DNA from positive and negative controls. In no case was cross reactivity between probe 41-27 and DNA of non-enteric AV observed. EAV were found in 6/180 diarrhoeas (3.3%) and 6/766 (0.8%) in asymptomatic individuals. The difference was statistically significant ( $P < 0.015$  by Fisher exact test), with a relative risk (RR) of being affected of 2.68 (95% confidence interval  $1.33 < RR < 5.43$ ). Individuals in which EAV were detected in diarrhoea were all children  $< 60$  months old. Vomiting did not occur and the diarrhoea was of short duration (2-3 days). In two of the children, EAV were still detected 5 days after the diarrhoea ceased. The incidence of diarrhoea associated with EAV was equivalent to 2.9 cases per 100 person-years for the overall population, 7.4 per 100 person-years in children 0-2 years, 3.9 per 100 person-years in children 0-5 years, and 0 in people  $> 5$  years. Asymptomatic excretion of EAV was detected in 6 individuals; 5 (including 1 mother)  $> 60$  months old, the other was a 2-month old child.

EAV excretion was detected in three children born during the study; infection occurred at 2, 9 and 14 months (Table 2). In two of these, slight acute diarrhoea developed and lasted 48 h. In the other case, excretion was asymptomatic and diarrhoea was not observed at least 15 days before or after sampling.

#### *Intrafamily spread*

The design of the study allowed us to detect EAV in family contacts of EAV-positive children. We studied faecal samples of the other members of the family as soon as one of the members was detected as EAV positive. Overall 29 siblings, aged between 1 month and 11 years ( $3.4 \pm 2.5$  years) and 4 mothers were analysed by dot-blot hybridization with probe 41-27. EAV were not detected in any sample, although concomitant diarrhoea developed in 12/29 (41%) siblings. The aetiology of diarrhoea in these cases was not identified.

#### *Outpatient children*

Among the 129 specimens studied, 14 (10.8%) excreted AV as detected by ELISA (Table 1). By hybridization, EAV were identified in 7/129 cases (5.4%) (Table 1). Thus 7/14 (50%) AV detected in children seeking medical attention for diarrhoea were enteric. In these children, a mild or severe gastroenteritis was found, with a mean duration of  $3 \pm 1.5$  days (mean  $\pm$  s.d.). The age was  $9.5 \pm 3.5$

Table 2. *Detection of enteric adenoviruses in children born during the study*

Age (months)	Duration of diarrhoea	Clinical features	Family members
9	3	Slight acute diarrhoea	Asymptomatic EAV negative
2*	0	Asymptomatic	Not studied
14	2	Slight acute diarrhoea	Increased frequency of bowel habit. EAV negative. Rotavirus negative.

\* Detected during routine surveillance of families.

Table 3. *Comparison of different methods for detection of 19 subgroup F adenovirus strains*

Method	Clinical category		Total
	Diarrhoea	Asymptomatic	
Dot-blot hybridization	13/13	6/6	19/19
ELISA for subgroup F	9/13	1/6	10/19
Restriction endonuclease analysis	10/10	5*/6	15/16

\* In one additional case this method provided a doubtful result.

months. Of the 7 EAV detected by dot-blot hybridization, AV-41 was recognized in 4 samples, and AV-40 in 1, using ELISA with an AV-40 and -41 specific monoclonal antibody. In the other two samples serotyping was not done.

#### *Independent identification of EAV*

In an attempt to further substantiate the usefulness of the dot-blot technique, we retested all dot-positive EAV samples by an ELISA test specific for subgroup F (i.e. enteric) AV. As shown in Table 3, all 7 samples from the outpatient study and 3/12 specimens from the community study were positive, including 1 sample from an asymptomatic excretor. The other nine samples positive by dot-blot remained unconfirmed by ELISA.

We reconfirmed our results (including 6 out of the 9 unconfirmed by ELISA) by restriction analysis of DNA obtained by direct extraction from faecal samples as described by Buitenwerf and colleagues [7]. Out of 16 tested, 15 were identified as EAV, and 1 gave equivocal results (Table 3). The 6 samples unconfirmed by ELISA were shown by restriction analysis to have an AV 40 profile in 4 cases, and an AV 41 profile in 1 case; the last remaining case produced an unclear profile.

#### DISCUSSION

We have investigated the importance of enteric adenoviruses (EAV) as aetiological agents in diarrhoea during a 2-year period in which we weekly surveyed 49 families from Avellaneda District, Buenos Aires. EAV were associated with 3.3% of diarrhoea cases and were detected during diarrhoea-free periods in 0.78% of samples.

EAV were thought to be aetiologic agents of diarrhoea in our population, as shown by the significant difference between the frequency of EAV detection in diarrhoea cases and asymptomatic individuals. In the same population we have shown that 10·5% of diarrhoea cases were associated with rotavirus in children < 2 years old [13]. However in 85% of the cases the aetiology remained unknown and probably enteric parasites and enteropathogenic bacteria have a relevant role.

The overall incidence of diarrhoea in the total study population was 0·9 cases per person-year and the peak incidence of diarrhoea in children aged < 2 years was 2·1 cases per person-year [13]. The incidence of EAV-associated diarrhoea in the overall population was 2·9 cases per 100 person-years. These figures are low and the clinical features of the affected individuals were relatively unimportant, being self-limited and of short duration.

The role of EAV in diarrhoeal disease has been investigated in various studies performed by different methods in different populations and unbiased comparison is difficult. The most valuable studies are those, carried out in children, in which the criteria for inclusion was diarrhoea. Although our data agree with several studies in such developing countries as Brazil, Thailand, India and Korea [14, 18–21], the results are not strictly comparable.

Two longitudinal studies, similar to ours, have been carried out. Rodriguez and colleagues in Washington DC, surveyed 70 families for 29 months and reported a diarrhoea incidence of 100 episodes per 100 person-years, with an incidence of EAV-related diarrhoea of 3 cases per 100 person-years in those 6–11 months of age, and 2 cases per 100 person-years in those 12–23 months of age [22]. These results are similar to ours (7·4 cases per 100 person-years in children 0–2 years old).

However, Cruz and colleagues [23], in a 1-year study in a rural population of Guatemala, restricted to children 0–24 months old, reported that 22% of rural children excreted EAV, and that 14% were associated with diarrhoea. In that population overall diarrhoea episodes were prolonged (1–2 weeks). These data differ from ours; our EAV detection rate in Avellaneda was lower (1·3%) and the mean duration of diarrhoea was 2–3 days. The reasons for the difference are not clear. The populations under study are different (urban *v.* rural), and one possibility is the higher general endemicity of EAV in Guatemalan rural areas than in urban areas of elsewhere in Latin America or USA. Another possibility is an outbreak of EAV infection in the Guatemalan study which may have temporarily increased the incidence. A third possibility is that the presence of other enteropathogens could modify the extent of EAV shedding.

Infection with EAV occurs worldwide and has been associated with 4–17% cases of diarrhoea in children. Our results in out-patients from similar environments to those surveyed elsewhere show the frequency of EAV-associated diarrhoea to be 5·4%. Studies on out-patients or hospitalized children elsewhere have shown similar values, with the range 3·3–8% [10–11, 24–27].

The frequency and incidence of EAV and rotavirus diarrhoea in the Avellaneda population was not substantially different. In the 180 cases of diarrhoea studied, rotavirus was detected in 8 cases (4·3%) and EAV in 6 cases (3·3%). Nevertheless a peak of rotavirus-related diarrhoea (10·5%) occurred during the first year of life, at an incidence of 25 episodes per 100 person-years [13]. Coinfections with the two viruses were not observed.

Subgroup F-specific ELISA and dot-blot hybridization are probably the most suitable methods for rapid detection of EAV. However correlation between these methods is not always adequate. Tiemessen and colleagues [28] reported 41 samples (13.2%) positive, by dot-blot, in diarrhoea from rural African children, of which 10 (25%) were confirmed by subgroup F-specific ELISA. In our outpatient study there was a strong correlation between both methods. However, in samples from asymptomatic children in the community, unmatched results were obtained with both methods. EAV DNA was detected in these samples, and the low titre shedding of EAV in asymptomatics and/or antigen degradation during long-time conservation of faeces are possible reasons.

We conclude that EAV are low-risk aetiological agents of diarrhoea in our population because: (i) the incidence calculated for the population in its natural environment was low; (ii) the episodes of diarrhoea associated with EAV were mild and short, and hospitalization was not required; (iii) no intrafamilial spread was detected although the contacts of those that shed EAV were mainly < 5 years old. Nevertheless, EAV may be an important cause of severe gastrointestinal illness in hospitalized children and in some cases laboratory diagnosis of EAV will aid both patient management and control of nosocomial infection.

#### ACKNOWLEDGEMENTS

We thank Christian Niel for the gift of probe 41-27, Flavia Thompson for technical assistance, and Selma Gomez, from the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, for identification of EAV in outpatient samples. This research was supported by WHO grant C6/181/117 (A) and by CONICET PID 3033900/85. ASM is a member of the *Carrera del Investigador* of the CIC (*Comisión de Investigaciones Científicas*).

#### REFERENCES

1. Uhnoo I, Wadell G, Svensson L, Johansson ME. Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. *J Clin Microbiol* 1984; **20**: 365-72.
2. Vesikari T, Maki M, Sarkkinen HJ, Arstila PP, Halonen PE. Rotavirus, adenovirus, and non-viral enteropathogens in diarrhea. *Arch Dis Child* 1981; **56**: 264-70.
3. Gary GW Jr, Hierholzer JC, Black RE. Characteristics of noncultivable adenoviruses associated with diarrhea in infants: a new subgroup of human adenoviruses. *J Clin Microbiol* 1979; **10**: 96-103.
4. Adrian T, Schafer G, Cooney MK, Cox JP, Wigand R. Persistent enteral infections with adenovirus types 1 and 2 in infants: no evidence of reinfection. *Epidemiol Infect* 1988; **101**: 311-14.
5. Adrian T, Wigand R, Richter J. Gastroenteritis in infants, associated with a genome type of adenovirus 31 and with combined rotavirus and adenovirus 31 infection. *Eur J Pediatr* 1987; **146**: 38-40.
6. Johansson ME, Uhnoo I, Kidd AH, Madeley CR, Wadell G. Direct identification of enteric adenovirus, a candidate new serotype, associated with infantile gastroenteritis. *J Clin Microbiol* 1980; **12**: 95-100.
7. Buitenwerf J, Louwerens JJ, De Jong JC. A simple and rapid method for typing adenovirus 40 and 41 without cultivation. *J Virol Meth* 1985; **10**: 39-44.
8. Takiff HE, Seidlin M, Krause P, et al. Detection of enteric adenovirus by dot-blot hybridization using a molecularly cloned viral DNA probe. *J Med Virol* 1985; **16**: 107-18.
9. Brandt CD, Kim HW, Rodriguez WJ, et al. Adenoviruses and paediatric gastroenteritis. *J Infect Dis* 1985; **151**: 437-43.

10. Kotloff KL, Losonsky GA, Morris JG Jr, Wasserman SS, Singh-Naz N, Levine MM. Enteric adenovirus infection and childhood diarrhea: An epidemiologic study in three clinical settings. *Pediatrics* 1989; **84**: 219-25.
11. Richmond SJ, Wood DJ, Bailey AS. Recent respiratory and enteric adenovirus infections in children in the Manchester area. *J R Soc Med* 1988; **81**: 15-18.
12. Cevenini R, Mazzaracchio R, Rumpianes F, et al. Prevalence of enteric adenovirus from acute gastroenteritis: a five year study. *Eur J Epidemiol* 1987; **3**: 147-50.
13. Grinstein S, Gomez JA, Bercovich JA, Biscotti EL. Epidemiology of rotavirus infection and gastroenteritis in prospectively monitored Argentine families with young children. *Am J Epidemiol* 1989; **130**: 300-8.
14. Leite JPG, Pereira HG, Azeredo RS, Schatzmayr HG. Adenovirus in faeces of children with acute gastroenteritis in Rio de Janeiro, Brazil. *J Med Virol* 1985; **15**: 203-9.
15. Pereira HG, Valentine R, Russell W. Crystallization of an adenovirus protein (the hexon). *Nature* 1968; **219**: 946-7.
16. Hammond G, Hannan C, Yeh T, Fischer K, Mauthe G, Straus SE. DNA hybridization for diagnosis of enteric adenovirus infection from directly spotted human fecal specimens. *J Clin Microbiol* 1987; **25**: 1881-5.
17. Niel C, Gomes SA, Leite JPG, Pereira HG. Direct detection and differentiation of fastidious adenovirus in stools by using a specific nonradioactive probe. *J Clin Microbiol* 1986; **24**: 785-9.
18. Cardoso DD, de Brito WM, Martins RM, et al. Ocorrência de rotavirus e adenovirus em amostras fecais de crianças com gastroenterite na cidade de Goiânia. *Rev Soc Bras Med Trop* 1989; **22**: 67-71.
19. Herrmann JE, Blacklow NR, Perron-Henry DM, Clements E, Taylor DN, Echeverria P. Incidence of enteric adenovirus among children in Thailand and the significance of these viruses in gastroenteritis. *J Clin Microbiol* 1988; **26**: 1783-6.
20. Bhan MK, Raj P, Bhandari N, et al. Role of enteric adenoviruses and rotaviruses in mild and severe acute enteritis. *Pediatr Infect Dis J* 1988; **7**: 320-3.
21. Kim KH, Yang JM, Joo SI, Cho YG, Glass RI, Cho YJ. Importance of Rotavirus and Adenovirus types 40 and 41 in acute gastroenteritis in Korean children. *J Clin Microbiol* 1990; **28**: 2279-84.
22. Rodriguez WJ, Kim HW, Brandt CD, et al. Fecal adenoviruses from a longitudinal study of families in metropolitan Washington, D.C.: Laboratory, clinical and epidemiological observations. *J Pediatr* 1985; **107**: 514-20.
23. Cruz JR, Cáceres P, Cano F, Flores J, Bartlett A, Torún B. Adenovirus types 40 and 41 and rotavirus associated with diarrhoea in children from Guatemala. *J Clin Microbiol* 1990; **28**: 1780-4.
24. Pavone R, Schinaia N, Harst CA, Getty B, Molyneux M, Borgstein A. Viral gastro-enteritis in children in Malawi. *Ann Trop Paediatr* 1990; **10**: 15-20.
25. De Paerregaard A, Hjelt K, Genner J, Moslet U, Krasilnikoff PA. Role of enteric adenoviruses in acute gastroenteritis in children attending day-care centers. *Acta Paediatr Scand* 1990; **79**: 370-1.
26. Uhnoo I, Olding-Stenkvis E, Kreuger A. Clinical features of acute gastroenteritis associated with rotavirus, adenovirus and bacteria. *Arch Dis Child* 1986; **61**: 732-8.
27. Shinozaki T, Araki K, Fujita Y, Kobayashi M, Tajima T, Abe T. Epidemiology of enteric adenovirus-40 and adenovirus-41 in acute gastroenteritis in infants and young children in the Tokyo area. *Scand J Infect Dis* 1991; **23**: 543-7.
28. Tiemessen CT, Negerhoff FO, Erasmus MJ, Kidd AH. Infection by enteric adenoviruses, rotaviruses, and other agents in a rural African environment. *J Med Virol* 1989; **28**: 176-82.