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Restriction enzyme analysis of faecal adenoviruses in Newcastle upon Tyne

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SUMMARY

Adenovirus DNA was isolated directly from virus-containing stools and digested with restriction endonucleases. The resulting fragments were separated by polyacrylamide gel electrophoresis (PAGE) and visualized by silver staining. This enabled us to assign most of the viruses detected to subgenus, serotype and, sometimes, unique strains. Although less sensitive than electron microscopy, the method allowed more information about the infecting virus to be obtained and no cultivation was necessary. Comparison with culture also allowed dual infections to be recognized.

A 2-year survey of faecal adenoviruses in Newcastle upon Tyne showed that type 41 (strain 41a) was the predominant type and strain 41p was not recorded. Heterogeneity in strain 41a was also noted as found elsewhere. Adenovirus type 40 was common prior to 1985 but was absent during the last 2 years.

INTRODUCTION

Adenovirus are widespread infectious agents commonly associated with mild upper respiratory tract illness. Forty-two serotypes are now recognized and these have been grouped into six subgenera. Originally allocations to subgenera were based on the properties of the virus proteins, but similar groupings have been derived by comparisons of DNA base composition (Green *et al.* 1979) and, recently, by analysis of restriction enzyme digests of the virus genome (Wadell, 1984). Members of different subgenera have little DNA homology with members of the other subgenera, and each subgenus shows distinct patterns of disease and oncogenicity (Wadell, 1987).

There are differences in the guanine/cytosine content of the DNA from adenovirus of different subgenera and this has been exploited using the restriction endonuclease *Sma* I which recognizes the GC-rich sequence CCCGGG. Digestion of adenovirus DNA with this enzyme leads to the production of discrete fragments whose number and size depend on the sequence of the virus DNA. Each of the six subgenera of human adenoviruses displays a characteristic range of numbers of *Sma* I fragments (Wadell, 1984). Within each subgenus individual serotypes differ in the size and number of fragments produced (Adrian *et al.* 1986). Thus each

serotype and possibly each strain of the virus may be distinguishable. This approach to classifying viruses from clinical samples has been limited to those specimens from which virus can be propagated in cell culture to produce enough DNA for digestion, or to those which contain large quantities of virus.

Although adenoviruses were originally isolated from the upper respiratory tract, they also frequently infect the gut and the majority of adenovirus isolates are now made from faeces. Many of these gut infections are silent but some 16% of all adenovirus illnesses involve symptoms of gastroenteritis (Schmitz, Wigand & Heinrich, 1983). We wished to characterize the adenoviruses found in stools in Newcastle upon Tyne and to determine whether the serotypes identified have changed with time.

In Newcastle, as elsewhere, adenoviruses are found in about 8% of faecal samples by electron microscopy (EM), but recognition by EM does not identify the serotype involved. Determination of serotype by neutralization is time consuming and depends on the availability of specific antisera as well as susceptible cells in which the virus will grow. Adenoviruses implicated in acute enteric disease, grow poorly and often not at all in the cells routinely used for culture (Brandt *et al.* 1979). These viruses have been designated serotypes 40 and 41 (de Jong *et al.* 1983). In the absence of readily available type-specific antisera, and difficulties in virus culture, direct serotyping by neutralization or by other methods such as ELISA are inapplicable. In order to look at the prevalence of these 'fastidious' adenoviruses in the Newcastle area as well as the more readily propagated lower numbered serotypes, we decided to extend the polyacrylamide gel electrophoresis (PAGE) method, developed in Newcastle to identify rotavirus RNA in stools, and which can also detect adenovirus DNA (Moosai, Carter & Madeley, 1984). Adenovirus DNA was obtained from stool samples without prior growth in cell culture, and digested with the enzyme *Sma* I. The resultant fragments were analysed on polyacrylamide gels and visualized by silver staining (Herring *et al.* 1982). This detection method is considerably more sensitive than ethidium bromide staining which has been used in the past (Buitenwerf, Louwerens & de Jong, 1985). Silver-staining has not previously been used for the detection of nucleic acid fragments derived from direct digestion of adenovirus DNA in the stool without virus growth and should allow detection of adenoviruses present in much lower numbers. This method has been compared with conventional cell culture isolation for both fastidious and non-fastidious viruses.

MATERIALS AND METHODS

Isolation of adenovirus DNA from faecal samples

Approximately 0·5 g of each stool sample was suspended in 5 ml of Hanks' balanced salt solution (HBSS), shaken well and maintained at 4 °C overnight. The samples were then clarified at 2800 g for 10 min in a bench centrifuge and the pellet of faecal debris was prepared for inoculation into cell cultures (see below). The supernatant was centrifuged at 100000 ***g*** for 1 h at 4 °C to sediment the virus. This second pellet was resuspended in approximately 0·3 ml 0·1% bacitracin in distilled water and a sample was negatively contrasted with 3% phosphotungstic acid (pH 7·0) and examined in a Philips EM 300 electron microscope.

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Moosai, Carter & Madeley (1984) found that treatment with 5% sodium dodecyl sulphate (SDS) was sufficient to release adenovirus DNA from this material. This DNA could be clearly identified as a single band on a 7·5% discontinuous polyacrylamide gel. However, we found that DNA prepared in this manner was an unsuitable substrate for restriction enzyme digestion and developed the following method. The virus pellet, resuspended after high speed centrifugation, was clarified for 2 min in an Eppendorf microfuge (10000 ***g***). The resulting pellet was washed by resuspension in 100 μl NTE (100 mM-NaCl, 10 mM-Tris pH 7·5, 1 mM-EDTA) and repelleting. The supernatants were pooled and a one-ninth volume of 10× proteinase K buffer (0·1 M-Tris pH 7·8, 50 mM-EDTA, 5% SDS), proteinase K enzyme (to a final concentration of 50 μg/ml) and 5 μg glycogen carrier were added. The samples were incubated at 37 °C for 20 min before protein was removed by two extractions with NTE-saturated phenol. The DNA was precipitated by the addition of 2·5 volumes of ethanol and sodium acetate to a final concentration of 0·1 M and collected by centrifugation for 12 min in the microfuge. The pellet was dried under reduced pressure and resuspended in 40 μl distilled water. The preparation of virus DNA by this method required less than 2 h.

A 5 μl sample was removed, mixed with an equal volume of 2× sample buffer (Laemmli, 1970) and applied to a 7·5% polyacrylamide discontinuous gel to detect and roughly quantify any adenovirus DNA present in the sample (Moosai, Carter & Madeley, 1984).

The remaining 35 μl of each sample was reprecipitated from 2 M ammonium acetate with ethanol (Okayama & Berg, 1982) and the pellet was resuspended in 80% ethanol and repelleted. DNA was resuspended in an appropriate volume of distilled water, between 10 and 50 μl depending on the intensity of the adenovirus DNA band on the gel. 10 μl aliquots were used subsequently for restriction enzyme digestion.

Isolation of DNA from cell culture grown adenovirus

One confluent 4 oz bottle of Bristol HeLa cells was infected with the appropriate adenovirus serotype. The viral DNA was extracted from infected cells approximately 4 days post-infection and purified by the method of Brown, Petric & Middleton (1984*a*). The final DNA pellet was resuspended in 25 μl distilled water and 1 μl aliquots were used for restriction enzyme digestion.

Restriction enzyme analysis of adenovirus DNA samples

Adenovirus DNA was digested with an appropriate restriction endonuclease (*Sma* I, *Eco* RI, *Hin* dIII; Northumbria Biologicals Ltd, Cramlington, Northumberland NE23 9HL). In each case 10 μl DNA suspension was incubated with 1·1 μl of the appropriate 10× strength buffer (for *Sma* I: 200 mM-KCl, 100 mM-Tris pH 8·0, 100 mM-$MgCl_2$, 10 mM dithiothreitol; for *Eco* RI and *Hin* dIII: 1 M-Tris pH 7·5, 100 mM-$MgCl_2$, 500 mM-NaCl) and 10 units of enzyme for 2 h at 37 °C. An equal volume of 2× sample buffer (50 mM-Tris, pH 6·8, 0·0024% bromophenol blue, 5% 2-mercaptoethanol, 4% SDS) was then added and the entire sample loaded on to a 7·5% polyacrylamide gel.

Samples to be run on agarose gels were prepared in a similar manner except that

a double volume reaction mixture was prepared and an equal volume of 2 × TBE sample buffer (Wadell *et al.* 1985) was added before loading.

Preparation of DNA size markers

Markers with short, protruding, single-stranded termini (sticky ends) were produced by digesting bacteriophage lambda DNA with both *Eco* RI and *Hin* dIII to generate the following standard DNA fragments: 21·7, 5·15, 5·0, 4·27, 3·48, 1·98, 1·9, 1·59, 1·37, 0·94, 0·83 and 0·5 kilobase pairs (kbp). Blunt ended markers were produced by digesting bacteriophage lambda DNA, first with *Sma* I then with *Nru* I, to generate the following sizes of DNA fragments: 14·81, 8·65, 7·48, 6·69, 4·49, 3·57, 1·92 and 0·7 kbp. To cover the required size range more completely a second set of blunt ended markers was produced by digesting lambda DNA with *Pvu* II to generate the following size fragments: 21·29, 4·42, 4·27, 4·19, 3·92, 3·64, 2·29, 1·71, 0·63 and 0·58 kbp. After digestion an equal volume of the appropriate double strength sample buffer was added. We routinely used 0·2 μg digested DNA marker on polyacrylamide gels and 0·8 μg DNA on agarose gels.

Gel analysis of DNA fragments

Polyacrylamide gel analysis was performed using the Laemmli (1970) discontinuous polyacrylamide gel system as described by Moosai, Carter & Madeley (1984), except that PAG Gel Bond (Miles Laboratories Ltd, Slough, England) was used to provide a rigid support for the gel to facilitate handling. The gel was subsequently silver-stained by the method of Herring *et al.* (1982) using the modifications described by Moosai, Carter & Madeley (1984). This method stains nucleic acids black in a light brown gel matrix.

Agarose gel analysis was performed as described by Wadell *et al.* (1985). DNA was subsequently stained for 30 min in 0·5 μg/ml ethidium bromide and viewed under ultraviolet light. Following agarose gel electrophoresis samples for Southern blotting were processed and hybridized as described by Maniatis, Fritsch & Sambrook (1982). Adenovirus genome DNA for use as a probe was excised from an agarose gel and radioactively labelled by nick-translation as described (Maniatis, Fritsch & Sambrook, 1982).

Virus identification by serology and culture

The faecal debris (produced in sample processing for electron microscopy) was resuspended in 5 ml of HBSS and disrupted by freeze/thawing. Antibiotics (penicillin and streptomycin) were added and the specimen was clarified at 2800 ***g*** for 10 min and 0·2 ml volumes of the supernatant were inoculated into HEp-2 and baboon MK cell cultures and incubated at 37 °C. The cell cultures were examined daily for cytopathic effect (cpe). Any adenovirus-induced cellular degeneration was confirmed by immunofluorescence (Gardner & McQuillin, 1980) and virus passage was attempted from all cultures showing cpe. Virus was released from infected cells by one cycle of freeze/thawing and 0·2 ml of the resulting lysate used to infect a fresh culture. Viruses which grew under these conditions were identified by a neutralization test using specific adenovirus antisera (Public Health Laboratory Service, Colindale, London). Antiserum (0·1 ml) was mixed with an equal volume of infected cell lysate and incubated at room temperature

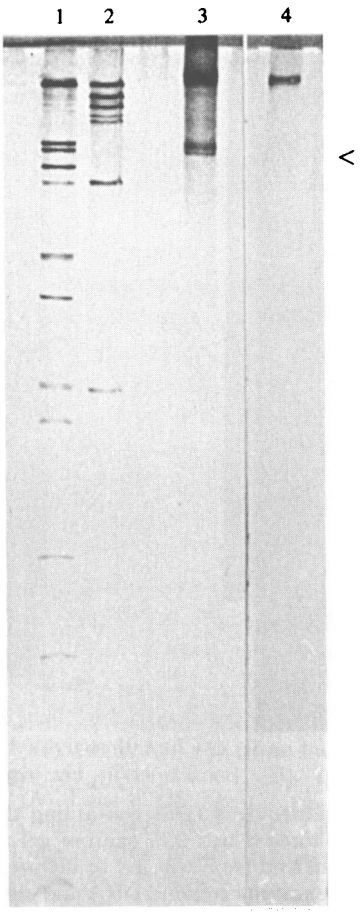

Fig. 1. Purified, undigested adenovirus DNA extracted from faecal samples electrophoresed in 7·5% polyacrylamide gel and silver-stained. Lane 1, DNA size markers (lambda, *Eco* RI *Hin* dIII digest): lane 2, DNA size markers (lambda, *Sma* I *Nru I* digest); lane 3, undigested adenovirus DNA from sample also containing three extra bands; lane 4, undigested adenovirus DNA.

for 1 h. Cell cultures were inoculated with this mixture and incubated at 37 °C. A positive control lacking antiserum was processed identically. The neutralization test cultures were examined daily until degeneration in the positive control was observed.

RESULTS

DNA was extracted from all clinical samples by the method described and was first analysed in its undigested state ($\frac{1}{8}$th total sample) on 7·5% discontinuous polyacrylamide gels. This allowed an estimate of the quantity of sample required for restriction enzyme analysis to be made. Although most previous reports (Kidd, 1984; Wadell, 1984; Buitenwerf, Louwerens & de Jong, 1985; Adrian *et al.* 1986) have used agarose gels followed by ethidium bromide staining, polyacrylamide gels were chosen for this study as they allow the restriction digestion patterns to be visualized by silver staining without having to amplify virus samples by

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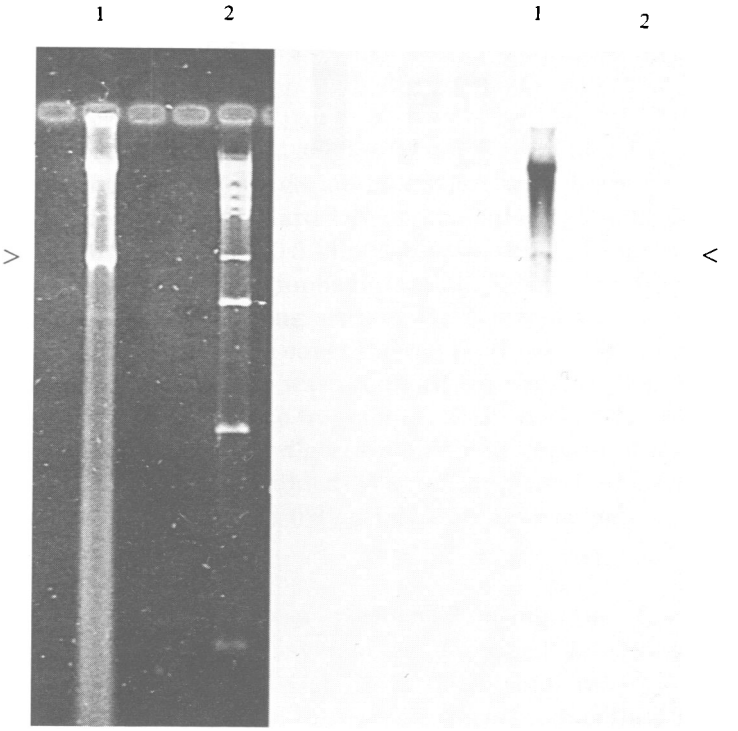

Fig. 2. Purified, undigested adenovirus DNA containing three extra bands extracted from faecal sample, electrophoresed in 1·2% agarose gel and stained with ethidium bromide (A). DNA was transferred from this gel to nitrocellulose. The filter was then hybridized to ^{32}P-labelled adenovirus genome DNA and autoradiographed (B). Panels (A) and (B). Lane 1, adenovirus DNA; lane 2, DNA size markers (lambda, *Sma* I *Nru* I digest). Arrows point to the extra bands found in some samples.

culture. We have found that the silver stain is 10–100 times as sensitive as ethidium bromide in detecting DNA (data not shown) and by this method a clear restriction pattern could be seen with less than 25 ng of virus DNA. It is not possible to use the silver stain with agarose gels as it stains the entire gel black.

Undigested adenovirus DNA migrated as a single band near the top of the resolving gel (Fig. 1, Lane 4) and there was generally little contaminating material in the tracks. However, a small number of the samples (4/112) showed three additional bands close together (see Fig. 1, Lane 3, arrowed) with sizes of approximately 4·19, 4·09 and 4·03 kbp. The intensities of these bands were independent of the amount of adenovirus DNA seen in the sample. Selective enzymic digestion, using DNAse, RNAse A, S1 nuclease and restriction endonucleases has shown that these bands are double-stranded DNA and contain recognition sites for *Eco*RI and *Hin* dIII but not *Sma* I (data not shown). On an agarose gel these three species migrate as a single band at 4·5 kbp (Fig. 2A, arrowed). These molecules share some sequence homology with adenovirus 41a since nick-translated gel-extracted adenovirus genomic DNA hybridizes to this

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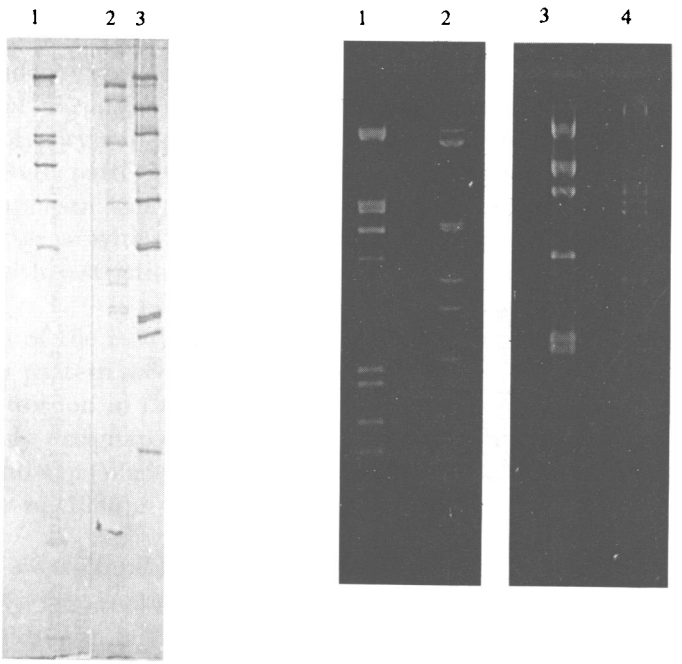

Fig. 3. *Sma* I digested adenovirus 40 and 41a (from faecal samples) electrophoresed in (A), 7·5% polyacrylamide gel silver-stained, (B), 1·2% agarose gel stained with ethidium bromide. Panel (A). Lane 1, DNA size markers (lambda, *Pvu* II digest); lane 2, adenovirus 40; lane 3, adenovirus 41a. Panel (B). Lane 1, DNA size markers (lambda, *Eco* RI *Hin*dIII digest); lane 2, adenovirus 41a; lane 3, adenovirus 40; lane 4, DNA size markers (lambda, *Pvu* II digest).

band in Southern blots (Fig. 2B arrowed) but this hybridization appeared lower then expected from the relative amounts of DNA on the gel.

Restriction enzyme (*Sma* I) digestion of adenovirus DNA samples produced readily recognizable and reproducible patterns on polyacrylamide gels (Fig. 3*a*). The group of serotypes (subgenus) of the virus in the sample could be identified by the number of restriction fragments generated. The sizes of these restriction fragments determined on agarose gels have been correlated with serotype (Wadell, 1987; Adrian *et al.* 1986; Buitenwerf, Louwerens & de Jong, 1985). However, DNA fragment sizes determined in polyacrylamide gels in this study were not the same as those derived from agarose gel analysis. This could be due to a relatively greater influence of DNA tertiary structure on migration through polyacrylamide gels than through agarose. To minimize this the sizes on polyacrylamide gels were calculated using appropriate DNA markers, either blunt or sticky-ended, depending on the enzyme used to digest the virus DNA. Nevertheless this failed to resolve the discrepancy completely as shown in Table 1. This table compares the sizes obtained for *Sma* I digested samples of adenoviruses types 40 and 41a determined in both agarose and polyacrylamide gels, and lists the fragment sizes derived from published data determined on agarose gels. Polyacrylamide gels have the ability to resolve closely spaced bands more efficiently than agarose gels (Fig.

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Table 1. *Comparison of fragment sizes of Sma I digested adenovirus 41a and 40 obtained in agarose and polyacrylamide gels (kbp)*

Sma I restriction fragment sizes for Ad41 in 1·2% agarose (calculated from Wadell, 1987)	Sizes of sample L9016 in 1·2% agarose gel	Sizes of sample L9016 in 7·5% polyacrylamide gel	*Sma* I restriction fragment sizes for Ad40 in 1·2% agarose (calculated from Wadell, 1987)	Sizes of sample H1226 in 1·2% agarose gel	Sizes of sample H1226 in 7·5% polyacrylamide gel
11·56	11·5	13·8	11·04	11·1	* 14·13, 11·22
4·37	4·36	5·01	5·9	† 5·9, 5·4	5·75
4·32	4·26	3·89	4·3		
3·26	3·13	2·88	4·2	4·17	3·47
2·75	2·72	2·51	2·75	2·6	2·51
2·14	2·08	2·07	1·74	† 1·64, 1·59	1·78, 1·69
1·51	1·42	2·02	1·7		
1·29	1·30	1·49	1·62	† 1·51, 1·49	1·55, 1·51
1·19	1·15	1·45	1·59		
0·93	0·92	1·41	0·6	0·58	0·58
0·80	0·81	1·00	—	—	—

* The 11·1 kbp band has the appearance of a closely spaced double band on polyacrylamide gels.

† Possible double bands resolved poorly on agarose gels. Resolution of the smaller two pairs is improved on polyacrylamide, whilst the upper pair migrates as a single band.

3 and Table 1). This greater resolving capacity leads to the production of a closely spaced double band (14·13/11·22 kbp) from the 11·1 kbp agarose gel band observed in adenovirus 40. This is a reproducible observation and may reflect conformational variation in this DNA rather than a new band. Since results obtained in polyacrylamide gels were not directly comparable with those obtained in agarose, PAGE patterns were assigned to their adenovirus serotypes either by running a duplicate sample on an agarose gel (Fig. 3B) and comparing the fragment patterns with the sizes calculated from published figures, or by comparison with restriction digests of known adenovirus serotypes analysed by PAGE.

Comparison of the results obtained in agarose with published data shows the most common pattern seen in our specimens is that of adenovirus 41a. There has been some confusion in the literature over nomenclature, since this pattern has been previously designated both 41a (Kidd, 1984; Buitenwerf, Louwerens & de Jong, 1985) and 41p (Wadell, 1987). This has now resolved in favour of adenovirus 41a (Adrian *et al.* 1986).

Evaluation of the electrophoretic method in diagnosis

In this study 112 stool samples obtained between 30 August 1985 and 18 June 1987 from children in hospital in the Newcastle upon Tyne area were examined. Most had diarrhoea and all the samples were positive for adenovirus in the electron microscope. Adenovirus DNA could not be detected in 32 samples when $\frac{1}{8}$ of the undigested DNA was analysed on the initial gel. However results could be obtained for five of these samples when the total remaining sample was digested with *Sma* I and electrophoresed, while the other 27 samples remained negative. Thus the polyacrylamide gel electrophoresis and silver staining method detected about 75% of the EM-detectable adenoviruses. Ten of the 27 PAGE-negative samples were only borderline positives by electron microscopy, with only one or two particles being seen.

The same 112 samples were also screened by virus cultivation and serology. A total of 25 samples grew in culture and were identified by neutralization as specific serotypes. Two further samples grew but could not be typed. These 27 samples included 12 of those which were borderline in the EM. The remaining 85 samples failed to grow in the cell cultures described, although cpe which failed to passage was observed in 45 of them. Consequently those culture methods could only identify adenovirus with certainty in 25/112 (22%) of the samples. These viruses were shown by serology to represent lower numbered serotypes, 1–12.

This compares with a detection rate of 85/112 (76%) by PAGE, of which all but seven could be assigned to serotype by restriction endonuclease digestion. Six of these samples failed to give a clear pattern with *Sma* I and therefore no serotype designation could be made. One yielded a *Sma* I pattern which could not be identified from any published data. The five-band pattern observed indicated that this virus belonged to subgenus A (Wadell, 1987). However the sizes obtained on a 1·2% agarose gel were not fully compatible with any member of this group (serotypes 12, 18, 31) although it bore most similarity to adenovirus 12.

These seven samples were found by culture to consist of three low serotype viruses (one type 1, two type 2) and four which did not grow. Three of the four

Table 2. *Adenovirus serotypes identified over a 2-year period (1985–87)*

Serotype detected	Number (percentage) of detections
Ad1	4 (3·4)
Ad2	13 (10·9)
Ad3	2 (1·68)
Ad4	1 (0·84)
Ad5	2 (1·68)
Ad7	1 (0·84)
Ad12	2 (1·68)
*Ad31	1 (0·84)
*Ad41	74 (62·2)
Negative	19 (16)
Total	†119 (100)

* Adenoviruses 31 and 41 were identified by PAGE alone, other samples were identified from a combination of serology and PAGE.

† Total numbers of serotypes detected (119) exceeds total number of samples (112) because seven samples contained a fastidious virus detected by PAGE as well as a non-fastidious detected by culture.

non-cultivable viruses did produce cpe in culture but could not be passaged. These included the sample which yielded the unidentified *Sma* I digest pattern. The remaining sample was negative in culture. The detailed findings from these three diagnostic approaches are summarized in Table 2.

Adenoviruses associated with diarrhoea in Newcastle

Our survey of samples from the last 2 years has clearly shown that 41 is by far the most commonly detected adenovirus serotype in children with diarrhoea in the Newcastle area (Table 2). The 112 samples of which stored specimens remained, represented 88% of the total number of samples positive for adenovirus by electron microscopy over the 2-year period.

Adenovirus serotype 41 has been divided into two strains 41a and 41p on the basis of *Sma* I digestion patterns (Kidd, Banatvala & de Jong, 1983). All adenovirus 41 detected in Newcastle throughout the 2-year period of the survey were 41a strains. No other fastidious adenoviruses were identified. However strain 41a is not itself homogeneous, although all 41a viruses yield the same pattern of *Sma* I digestion products, DNA variants within these viruses can be recognized by cleavage with other restriction enzymes.

To determine whether the samples all contained an indistinguishable adenovirus 41a, digestion with two further restriction endonucleases was performed. *Hin* dIII, which cuts at AAGCTT sequences and *Eco* RI, which cuts at GAATTC sequences were used. Both these enzymes recognize AT-rich sequences, in contrast to the GC-rich sequence recognized by *Sma* I.

On digestion with *Hin* dIII two distinct fragment profiles were seen (Fig. 4A) profile H1 with 12 fragments and profile H2 with 13. DNA which yields profile H2 has an extra cleavage site for *Hin* dIII within band 4 (3·09 kbp) of profile H1. This band is no longer seen in profile H2 but two new fragments with sizes of 1·69 and 1·43 kbp appear. A similar result was obtained with *Eco* RI; again two different profiles were seen (E1 and E2). Profile E1 shows five digestion fragments, all greater than 2000 bp in length. Profile E2 has an extra *Eco* RI recognition site in

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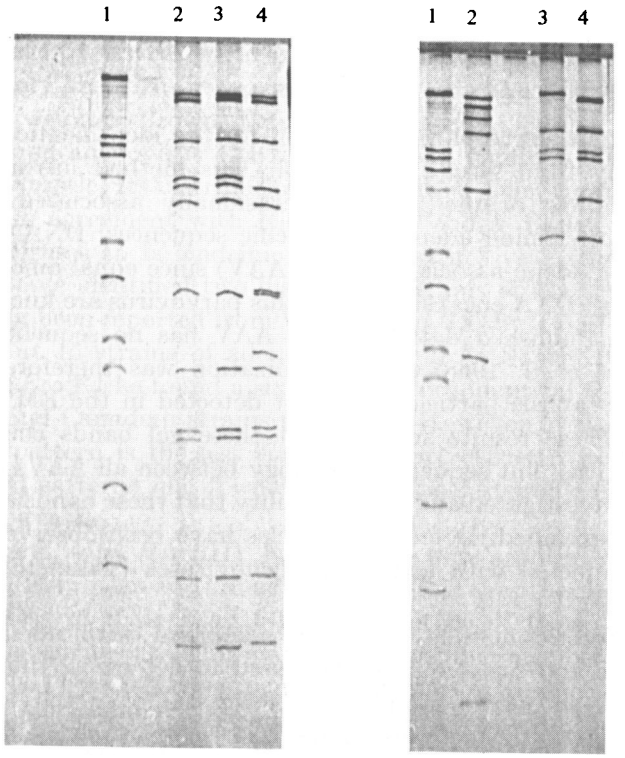

Fig. 4. *Hin* dIII (A) and *Eco* RI (B) digested adenovirus 41a (from faecal samples) electrophoresed in 7·5% polyacrylamide gel and silver-stained. Panel (A). Lane 1, DNA size markers (lambda, *Eco* RI *Hin* dIII digest); lanes 2 and 3, H1 restriction pattern; lane 4, H2 restriction pattern. Panel (B). Lane 1, DNA size markers (lambda, *Eco* RI *Hin* dIII digest); lane 2, lambda, *Sma I Nru* I digest; lane 3, EI restriction pattern; lane 4, E2 restriction pattern.

the largest fragment, which is reduced in size and a new fragment of 3·31 kbp appears (Fig. 4*b*).

Twenty-seven adenovirus 41a samples were tested by this method. Of these 21 showed *Hin* dIII profile H1 and *Eco* RIprofile E1. The remaining six samples showed *Hin* dIII profile H2 and *Eco* RI profile E2. In our samples changes in the *Hin* dIII digestion pattern were always accompanied by changes in the *Eco* RI profile.

Examination of samples from previous years

No adenovirus serotype 40 was detected during the 2-year survey period. This contrasts with published data which show an approximately equal distribution of the two serotypes (de Jong *et al.* 1983). To investigate this phenomenon further, samples likely to contain fastidious adenoviruses from earlier years (post 1976) were analysed. These samples were selected by positivity for adenovirus in the EM, and failure to yield cultivable viruses in tissue culture. Of the 23 samples which satisfied these criteria, and from which sufficient DNA could be recovered,

13 were found to be 41a and 10 were adenovirus 40. No adenovirus 41p was identified.

DISCUSSION

We have used an electrophoretic method for the identification of adenovirus serotypes directly from virus in the stool. This method has also shown that adenovirus infections of the gut are occasionally associated with subgenomic-sized DNA containing adenovirus-specific sequences. DNA at this position could derive from adeno-associated virus (AAV) since equal amounts of positive and negative sense DNA encapsidated by this parvovirus are known to hybridize when extracted. Published data suggests AAV has no sequence homology to adenovirus (Rose *et al.* 1968) and hybridization was therefore not expected. Furthermore parvovirus particles were not detected in the EM examination of these samples. These results suggest that these gel bands could derive from defective adenovirus, but sequence homology between all AAVs and adenovirus 41 has not been investigated and the possibility that these bands are derived from AAV cannot be excluded. Defective particles have been observed previously in tissue cultures infected with fastidious adenoviruses (Takiff, Straus & Garon, 1981).

The electrophoretic method has been compared with adenovirus identification by culture and serology and then used to determine those viruses most commonly found in faecal samples in Newcastle over the last 2 years. A comparison of diagnostic methods shows that electron microscopy remains the most sensitive method, although no information on serotype is obtained. Furthermore the relevance of a single observed particle to disease may be questioned despite the insensitivity of EM.

PAGE is the second most efficient method. This approach works well with samples containing high particle numbers, and is thus most efficient in the detection of viruses from the high serotypes 40 and 41, which are shed in large numbers in stools during infection (Flewett, 1976). Four non-fastidious viruses were detected by PAGE out of 25 known to be present by virus culture techniques. Those found by PAGE (number found by PAGE/number found by cultures) comprised: 1/4 adenovirus type 1, 1/12 adenovirus type 2 and 1/2 adenovirus type 3. In addition one adenovirus type 31 was identified by PAGE but could not be typed by serology, even though it grew in culture, because a suitable antiserum was not available. Virus culture is the least efficient method but is best for detecting viruses belonging to lower numbered serotypes (1–12). Viruses of these serotypes tend to be shed in lower numbers and are thus harder to detect by the above methods. Fifty per cent of successful virus identifications by culture were made from the 24% of samples which contained very few particles. Cytopathic effect which failed to pass was observed in 43 cases of the 74 known to contain fastidious viruses by PAGE.

Our method provides an alternative to DNA hybridization methods (Hammond *et al.* 1987), which must be performed many times to identify virus serotypes. The method presented here is at least as sensitive as the dot-blot test and provides information on individual strain as well as indicating the serotype.

Low numbered serotype viruses were detected by culture in seven samples in

which a fastidious virus was the only virus detected by PAGE. DNA fragments from the lower serotype virus could not detected and this virus is therefore a minor component of the total virus particle population. This suggests that a virus grown is not necessarily the virus responsible for the disease, and confirms that dual infections are common (Brown, Petric & Middleton, 1984*b*).

We have found adenovirus 41a to be the most common adenovirus in faecal samples in Newcastle. Its distribution appears to be even throughout the 2 years of the study, in agreement with Richmond, Wood & Bailey (1988) who found enteric adenoviruses to be endemic throughout a 3-year study in Manchester, England. We have identified two distinct substrains of 41a. Profiles E2 and H1 have previously been reported from Washington, DC by Takiff *et al.* (1984). Kidd (1984) looked at 15 strains of adenovirus 41a from South Africa, Canada and Europe. Using *Eco* RI he found a six-band pattern identical to our pattern E2 in all European and Canadian strains examined. However in the samples we have analysed this pattern is the less common. South African samples showed four different *Eco* RI patterns, one of which was identical to profile E1, which was more common in Newcastle.

After digestion with *Hin* dIII Kidd found two profiles, one of which was exhibited by all European and Canadian strains and the other by all South African strains regardless of their *Eco* RI profile. Neither of these *Hin* dIII patterns exactly matches our results. However the major difference between the *Hin* dIII patterns observed by Kidd consists of the presence or absence of the fourth band which can contain extra *Hin* dIII sites. This is also true of the patterns H1 and H2 which we have observed although the positions of the extra *Hin* dIII sites within band 4, when present, appear to be different from those observed by Kidd.

We have attempted to document variations in stool adenoviruses over recent years. The sample size is too small to allow definite conclusions to be drawn about the changes in the prevalence of most adenovirus serotypes in the Newcastle area. However the decrease in cases of adenovirus 40 after July 1985 does seem to be a real phenomenon since a much more extensive survey of the next 2 years' samples by PAGE failed to reveal any examples of adenovirus 40 at all. The significance of this observation remains unclear and further work is required to investigate this finding.

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