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COMPARISON OF THE GENOME OF OVINE ADENOVIRUS TYPES 1 THROUGH 5 BY RESTRICTION ENZYME ANALYSIS AND DNA HYBRIDISATION

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The DNA of the prototype strains of ovine adenovirus (OAdV) 1 through 5 was analysed by restriction enzyme (RE) digestion. The RE patterns generated by *Hin*dIII and *Pst*I enzymes were characteristic of the examined strains. OAdV-2 and 3 resembled each other the most, and their *Eco*RI and *Hin*dIII patterns seemed to be identical. Considering the number of comigrating fragments, serotypes OAdV-2, 3, 4 and 5 looked more closely related to each other than to OAdV-1. This finding was strengthened by Southern blot hybridisations probed with random *Hin*dIII clones of OAdV-3. The estimated genome size of the examined OAdV types ranged between 31.9 and 32.8 kilobase pairs. The results supported the new genus classification of OAdVs.

Key words: Ovine adenoviruses, restriction enzyme analysis, Southern hybridisation, taxonomy

Adenovirus isolation from sheep was first reported by McFerran et al. in 1969. To date, six ovine adenovirus (OAdV) serotypes are officially recognised (Benkő et al., 2000). Additional adenovirus strains found by serum neutralisation tests to be indistinguishable from, or closely related to, bovine adenovirus (BAdV) serotypes 2 and 7, respectively, were also recovered from sheep (Belák and Pálfi, 1974; Adair et al., 1982; Boyle et al., 1994). Adenovirus infection in sheep can be symptomless or associated with different pathological entities (Smyth et al., 1990), but is most often characterised by respiratory and/or enteric disorders (Belák, 1990). The pathogenic role of specific OAdV serotypes in different diseases is poorly studied. The elaboration of modern DNA-based diagnostic methods such as PCR or DNA hybridisation requires the characterisation of the viral genome.

As a first step, restriction enzyme (RE) analysis of the DNA of the prototype strains of the officially accepted OAdV types was carried out with the aim to obtain data on the eventual genetic similarities amongst OAdVs, and between OAdVs and BAdVs.

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Materials and methods

DNA RE analysis. A collection of the prototype OAdV strains was kindly provided by Professor Miklós Rusvai (Department of Microbiology and Infectious Diseases, Faculty of Veterinary Science, Szent István University, Budapest). The designation and origin of OAdV-1 through 6 are presented in Table 1, and were recently reviewed (Lehmkuhl and Cutlip, 1999). The strains were propagated on low-passage-number monolayer cultures of lamb kidney epithelial cells. When the cytopathic effect was complete, the viral DNA was extracted from the infected cells as described by Shinagawa et al. (1983). *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I enzymes were applied according to the manufacturer's instructions (MBI Fermentas). The electrophoresis was performed in 1% agarose slab gels. The approximate genome size of the OAdV strains was estimated by adding up the size of the fragments resulting from the different restriction enzyme digestions. The size of the individual fragments was determined graphically by relating their migration distance to that of the molecule mass standard.

Table 1

Designation and references of the prototype OAdV strains

OAdV strain	Designation	Reference
OAdV-1	S1	McFerran et al., 1969
OAdV-2	PX515	McFerran et al., 1969
OAdV-3	PX611	McFerran et al., 1969
OAdV-4	7769	Sharp et al., 1974
OAdV-5	SAV	Bauer et al., 1975
OAdV-6	WV419	Davies and Humphreys, 1977

*Molecular cloning and DNA hybridisation. Hin*dIII-cleaved fragments of OAdV-3 have been randomly cloned into pBluescript plasmid (Stratagene), and DH5α *E. coli* cells were transformed. A pool of different *Hin*dIII clones was labelled by Pharmacia Random Priming Kit, using [α-^{32}P]dATP. With this probe, Southern blots containing DNA of OAdV-1 to 5 and BAdV-1 serotypes were hybridised under stringent conditions (hybridisation at 68 °C, washing in 0.1 × SSC at 68 °C). Autoradiographs were made on Kodak X-ray films.

Results

Agarose gels containing *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I cut DNA of OAdVs are presented in Figs 1 and 2a. The strain kept as OAdV-6 in this collection was obviously mistaken and showed identical RE patterns to those of BAdV-1 (Benkő and Harrach, 1990). Since our attempts to propagate authentic

OAdV-6 have not been successful yet, the RE patterns of OAdV-1 to 5 can only be presented. Cleavage with *Hin*dIII and *Pst*I enzymes resulted many DNA fragments, thus producing characteristic RE patterns. *Bam*HI and *Eco*RI enzymes, however, had too few recognition sites on the OAdV genomes. OAdV-2 and 3 had identical patterns after cleavage with *Eco*RI or *Hin*dIII enzymes, while their *Pst*I patterns apparently differed only in one extra recognition site on fragment A in OAdV-3. Several smaller fragments of seemingly identical size were present in every isolate.

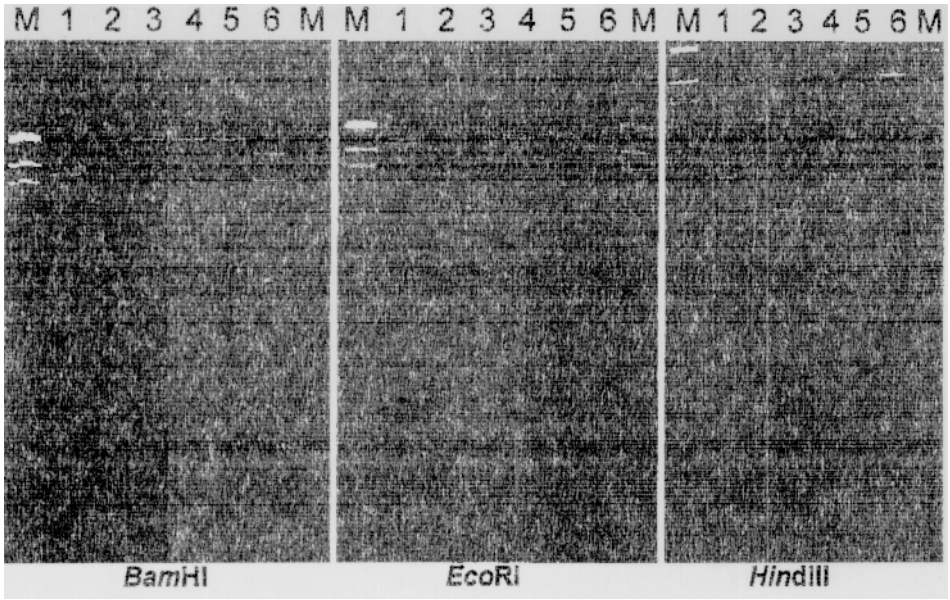

Fig. 1. Restriction enzyme patterns of the prototype OAdV strains. M: λ phage DNA cleaved with *Hin*dIII enzyme for molecule mass standard. In lanes 1 to 5, the DNA of OAdV types 1 to 5 were electrophoresed after cleavage with REs as marked. Lane 6 contains BAdV-1 DNA

The estimated complete genome size of each OAdV serotype examined was calculated on the basis of the *Eco*RI, *Hin*dIII, and *Pst*I cleavage patterns, and is given in kilobase pairs (kb) in Table 2. The genome size of OAdV types 1 through 5 ranged between 31.9 and 32.8 kb, and this range is closer to the genome size of BAdV-2 (32.5 kb; Salmon et al., 1993) than to those of other mastadenovirus BAdVs (Benkő et al., 1988).

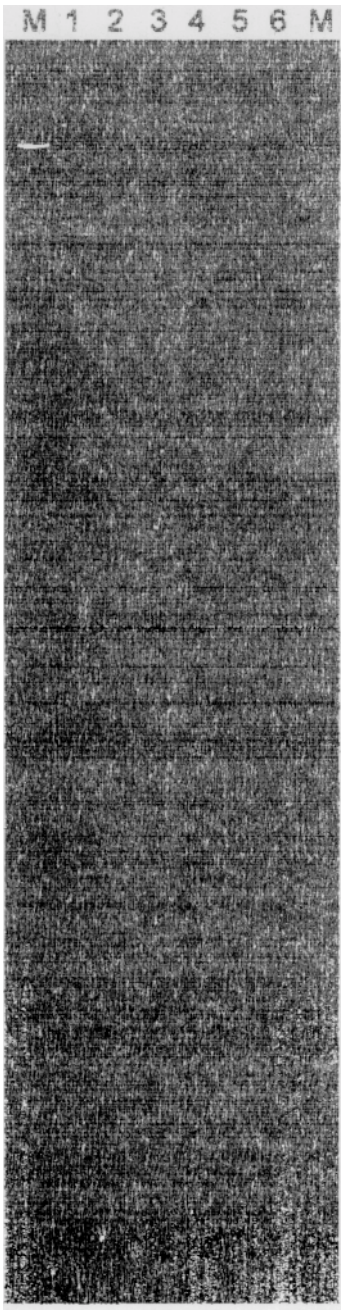

Fig. 2a. Restriction patterns of OAdV strains 1 through 5 (lanes 1 to 5) and BAdV-1 (in lane 6) generated by *Pst*I enzyme. *b.* Southern blot made of the gel shown in *a* after having been probed with random *Hin*dIII clones of OAdV-3. Molecule mass standard as in Fig. 1

Table 2

The estimated genome size of OAdV types 1 through 5

RE	Genome size of OAdV types (kb)				
	OAdV-1	OAdV-2	OAdV-3	OAdV-4	OAdV-5
*Eco*RI	32.8	33.1	33.1	32.6	33.8
*Hin*dIII	31.4	30.9	30.9	30.6	30.2
*Pst*I	31.5	32.9	32.9	33.7	31.3
Calculated mean	31.9	32.3	32.3	32.3	32.8

The results of the DNA hybridisation experiments showed that OAdV types 2 through 5 share high degree sequence homology, while OAdV-1 is genetically more distant. In Fig. 2b, the hybridisation of *Pst*I-cleaved OAdVs is presented. Similar result was obtained with a Southern blot containing *Hin*dIII-cut virus genomes, and positive hybridisation with the OAdV-1 DNA was detected after less stringent washing procedure only (data not shown).

Discussion

The DNA RE analysis proved to be a suitable method and has been used for type identification of isolates of different human, bovine, porcine, canine, and several fowl adenovirus serotypes. The aim of the present study was the RE characterisation of the officially accepted OAdV serotypes. The DNA cleavage patterns generated by *Hin*dIII and *Pst*I enzymes in general were characteristic of the OAdV types examined. Out of five OAdV prototype strains, OAdV-2 and 3 proved to be most closely related based on the number of comigrating fragments. This finding was strengthened by DNA hybridisation experiments, in which OAdV-1 showed the weakest signal with the probe prepared from OAdV-3 DNA.

We have also compared the RE patterns of OAdVs to those of BAdVs. On the basis of the calculated genome size and the *Eco*RI pattern, we concluded that OAdVs seemed to be most closely related to BAdV-2 (Belák et al., 1986; Benkő et al., 1988). This finding is not astonishing, since BAdV-2 and a subtype of it have repeatedly been isolated from diseased lambs (Belák et al., 1976), and partial cross neutralisation between BAdV-2 and OAdV-2 and 3 was previously reported (Adair et al., 1982). According to the result of a more recent survey, the incidence of BAdV-2 infection among lambs in Hungary is very high even nowadays (Rusvai and Fodor, 1998; Rusvai et al., 1999). BAdV-2 is a member of the formerly established subgroup 1 of BAdVs (Bartha, 1969), and should therefore resemble the other mastadenoviruses. Its relatively small genome size (compared to 35–36 kb of BAdV-1, 3 and 9) and the weak DNA hybridisation,

however, questioned its close relationship to these BAdVs. The present study supported our earlier hypothesis that BAdV-2, although it had been first isolated from cattle, should rather be considered as an OAdV type. This was recently confirmed by the phylogenetic comparison of the protease gene sequences of OAdV-3 and BAdV-2 (Barbezange et al., 2000).

The present study also provided a new insight into the genetic relatedness of ovine adenoviruses. To date, the only full genomic sequence published from ovine adenoviruses is that of isolate OAV287 (Vrati et al., 1996). This virus strain has not been recognised as a new OAdV type because of its partial cross-reaction with BAdV-7 in serum neutralisation tests (Boyle et al., 1994). Phylogenetic analysis demonstrated that OAV287, along with BAdV-7 and other former subgroup 2 BAdVs, should be classified into a new genus within the family *Adenoviridae* (Harrach et al., 1997; Harrach and Benkő, 1998). The candidate members of this proposed genus *Atadenovirus* (Benkő and Harrach, 1998), besides the strikingly high (> 60%) AT content of their DNA, also share very unique genome organisation (Vrati et al., 1996) and several biological properties such as restricted growth. The reason why we were not able to propagate OAdV-6 in this study might be the use of ovine kidney cell culture. Like OAV287 and the former subgroup 2 BAdVs, OAdV-6 was described to require testicle cells for propagation (Adair et al., 1982), and might therefore also belong to the genus *Atadenovirus*. Nevertheless, it seems that sheep, like cattle and wild ruminant species (Sorden et al., 2000), can host adenoviruses of two distinct (*Mastadenovirus* and *Atadenovirus*) genera.

A new concept in the demarcation of the taxonomic category of the 'adenovirus species' has been introduced recently (Benkő et al., 2000). In the Seventh Report of the ICTV, the species *Ovine adenovirus A* comprises OAdV-2, 3, 4 and 5 together with BAdV-2. This classification is supported by partial DNA sequences (Rusvai et al., 2000). Further studies are needed, however, to confirm the species (or even genus) allocation of OAdV-1 and 6.

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References

Adair, B. M., McFerran, J. B. and McKillop, E. R. (1982): A sixth species of ovine adenovirus isolated from lambs in New Zealand. Arch. Virol. **74,** 269–275.

Barbezange, C., Benkő, M., Dán, Á. and Harrach, B. (2000): DNA sequencing and phylogenetic analysis of the protease gene of ovine adenovirus 3 suggest that adenoviruses of sheep belong to two different genera. Virus Res. **66,** 79–85.

Bartha, A. (1969): Proposal for subgrouping of bovine adenoviruses. Acta Vet. Acad. Sci. Hung. **19**, 319–321.

Bauer, K., Müller, H. and Gürtürk, S. (1975): Isolierung eines Virus von Schafen und seine Einordnung als neuer Serotyp oviner Adenoviren. Zbl. Vet. Med. B **22,** 556–565.

Belák, S. (1990): Ovine adenoviruses. In: Dinter, Z. and Morein, B. (eds) Virus Infections of Ruminants. Virus Infections of Vertebrates 3. Elsevier Science Publishers BV, Amsterdam, Chapter **17,** pp. 171–185.

Belák, S. and Pálfi, V. (1974): An adenovirus isolated from sheep and its relationship to type 2 bovine adenovirus. Arch. Ges. Virusforsch. **46**, 366–369.

Belák, S., Pálfi, V. and Palya, V. (1976): Adenovirus infection in lambs. 1. Epizootiology of the disease. Zbl. Vet. Med. B **23,** 320–330.

Belák, S., Virtanen, A., Zabielski, J., Rusvai, M., Berencsi, G. and Pettersson, U. (1986): Subtypes of bovine adenovirus type 2 exhibit major differences in region E3. Virology **153,** 262–271.

Benkő, M. and Harrach, B. (1990): Restriction site mapping of bovine adenovirus type 1. Acta Vet. Hung. **38,** 281–284.

Benkő, M. and Harrach, B. (1998): A proposal for a new (third) genus within the *Adenoviridae* family. Arch. Virol. **143,** 829–837.

Benkő, M., Bartha, A. and Wadell, G. (1988): DNA restriction enzyme analysis of bovine adenoviruses. Intervirology **29,** 346–350.

Benkő, M., Harrach, B. and Russell, W. C. (2000): Family *Adenoviridae*. In: Van Regenmortel, M. H. V., Fauquet, C. M., Bishop, D. H. L., Carstens, E., Estes, M., Lemon, S., Maniloff, J., Mayo, M. A., McGeoch, D., Pringle, C. and Wickner, R. (eds) Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, New York, San Diego, pp. 227–238.

Boyle, D. B., Pye, A. D., Kocherhans, R., Adair, B. A., Vrati, S. and Both, G. W. (1994): Characterisation of Australian ovine adenovirus isolates. Vet. Microbiol. **41,** 281–291.

Davies, D. H. and Humphreys, S. (1977): Characterization of two strains of adenovirus isolated from New Zealand sheep. Vet. Microbiol. **2,** 97–107.

Harrach, B. and Benkő, M. (1998): Phylogenetic analysis of adenovirus sequences; proof of the necessity of establishing a third genus in the *Adenoviridae* family. In: Wold, W. S. M. (ed.) Adenovirus Methods and Protocols. Methods in Molecular Medicine, Vol. 21. Humana Press, Totowa, NJ, USA, Chapter 25, pp. 309–339.

Harrach, B., Meehan, B., Benkő, M., Adair, B. M. and Todd, D. (1997): Close phylogenetic relationship between egg drop syndrome virus, bovine adenovirus serotype 7, and ovine adenovirus strain 287. Virology **229,** 302–306.

Lehmkuhl, H. D. and Cutlip, R. C. (1999): A new goat adenovirus isolate proposed as the prototype strain for goat adenovirus serotype 1. Arch. Virol. **144,** 1611–1618.

McFerran, J. B., Nelson, R., McCracken, J. M. and Ross, J. G. (1969): Viruses isolated from sheep. Nature **221,** 194–195.

Rusvai, M. and Fodor, L. (1998): Occurrence of some viruses and bacteria involved in respiratory diseases of ruminants in Hungary. Acta Vet. Hung. **46,** 405–414.

Rusvai, M., Harrach, B., Bánrévi, A., Evans, P. S. and Benkő, M. (2000): Identification and sequence analysis of the core protein genes of bovine adenovirus 2. Virus Res. (in press).

Rusvai, M., Izadpanah, R. and Fodor, L. (1999): Etiology of respiratory disease complex in some Hungarian ruminant livestocks (in Hungarian, with English abstract). Magyar Állatorvosok Lapja **121,** 255–259.

Salmon, K., Esford, L. and Haj-Ahmad, Y. (1993): Subcloning and restriction enzyme mapping of bovine adenovirus type 2. Intervirology **36,** 72–78.

Sharp, J. M., McFerran, J. B. and Rae, A. (1974): A new adenovirus from sheep. Res. Vet. Sci. **17,** 268–269.

Shinagawa, M., Matsuda, A., Ishiyama, T., Goto, H. and Sato, G. (1983): A rapid and simple method for preparation of adenovirus DNA from infected cells. Microbiol. Immunol. **27,** 817–822.

Smyth, J. A., McConnell, S., Cush, P. F. and Adair, B. M. (1990): Adenoviral infection of the renal interstitium of a lamb. Vet. Pathol. **27,** 290–292.

Sorden, S. D., Woods, L. W. and Lehmkuhl, H. D. (2000): Fatal pulmonary edema in white-tailed deer (*Odocoileus virginianus*) associated with adenovirus infection. J. Vet. Diagn. Invest. **12,** 378–380.

Vrati, S., Brookes, D. E., Strike, P., Khatri, A., Boyle, D. B. and Both, G. W. (1996): Unique genome arrangement of an ovine adenovirus: identification of new proteins and proteinase cleavage sites. Virology **220,** 186–199.