Interspecific differences between the DNA restriction profiles of canine adenoviruses[1]

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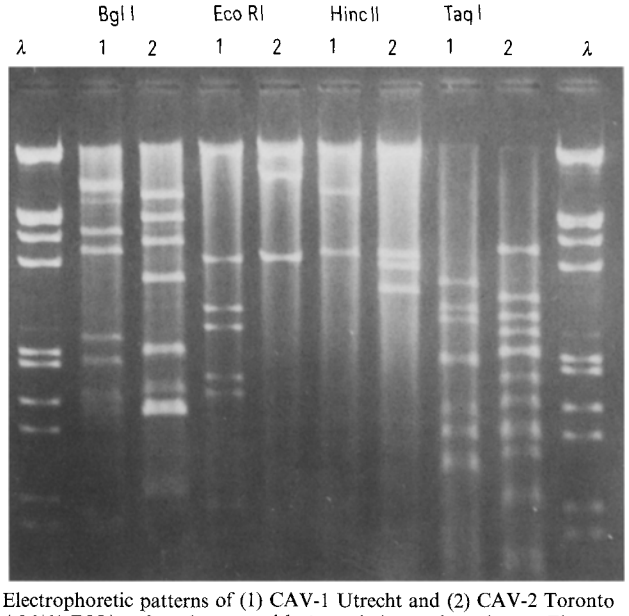
Summary. Identification of canine adenovirus-1 (CAV-1) and canine adenovirus-2 (CAV-2) strains was done by electrophoresis of restriction endonuclease-fragmented viral DNA. Results obtained with this sensitive and reproducible technique clearly show that CAV-2 is not a variant of CAV-1 but a distinct virus.

During the last 20 years, evidence has accumulated suggesting that the infectious canine hepatitis virus (CAV-1) and the infectious canine laryngotracheitis virus (CAV-2) are distinct adenoviruses[2,3]. The 2 CAVs differ in size and pathogenicity but share common antigenic properties, as shown by neutralization, hemagglutination-inhibition, and complement-fixation tests[4,5]. Because of this serological cross-reactivity, CAV-2 is still considered in the literature as a variant of CAV-1[6,7].

Restriction endonuclease analysis of viral DNA now appears to be a most useful addition to the previous methods for virus identification and classification[8-11]. A simplified version of this sensitive and reproducible technique was recently developed in our laboratories for the rapid identification of herpes simplex virus types 1 and 2 strains[12]. It therefore seemed interesting to determine the degree of genetic relationship between CAV-1 and CAV-2 by comparing their respective DNA cleavage patterns.

The CAV-1 (Utrecht) and CAV-2 (Toronto A26/61) prototype strains were propagated in primary dog kidney cells as described previously[13]. Viral DNA was extracted from the CAV-infected cells by the SDS-pronase-phenol method developed earlier for herpes simplex viruses[12]. Aliquots containing about 1 µg of DNA were digested for 3 h in the appropriate conditions with restriction enzymes (5–10 units) either prepared in our laboratories (*Bgl* I, *Eco*RI, *Hinc*II) or purchased from Boehringer Mannheim (*Hind*III, *Taq*I). Lambda phage DNA (Bethesda Research Labs) cleaved with *Hind*III plus *Eco*RI restriction endonucleases was used as size markers. Digestion products were resolved by electrophoresis for 6 h at 100 V in a 1% agarose gel as described previously[14]. DNA in the gel was stained with ethidium bromide (1 µg/ml) and photographed under ultraviolet light illumination.



Electrophoretic patterns of (1) CAV-1 Utrecht and (2) CAV-2 Toronto A26/61 DNAs after cleavage with 4 restriction endonucleases. Electrophoresis of DNA fragments was carried out in a 1% agarose slab gel at 100 V for 6 h. DNA in the gel was stained with ethidium bromide and photographed under ultraviolet light illumination. The *Hind* III plus *Eco* RI fragments of lambda phage DNA used as markers have a molecular weight of 14.3, 3.40, 3.30, 2.82, 2.30, 1.31, 1.25, 1.05, 0.90, 0.59, 0.55, 0.37, and 0.09 respectively.

Four endonucleases with distinct specificities for tetranucleotide (*Taq*I) or hexanucleotide- (*Bgl*I, *Eco*RI, *Hinc*II) sequences[15] were used to cleave the CAV DNAs. Large differences between the CAV-1 and the CAV-2 genomes were observed with each of these enzymes, as illustrated in the figure. Digestion of CAV-1 and CAV-2 DNAs with endonuclease *Bgl*I, for example, yielded 9 and 10 fragments, respectively. Only one of the *Bgl*I fragments of CAV-1 DNA with a molecular weight of approx. 0.9 M daltons had a counterpart fragment in CAV-2 DNA. Differences between the CAV genomes appeared most clearly in the *Taq*I profiles where a larger number of relatively small DNA fragments could be compared. Such a lack of homology between the CAV nucleotide sequences indicates clearly that CAV-2 is not a mere variant of CAV-1, but a distinct virus. This divergence between specified DNA sequences of CAV-1 and CAV-2, together with the structural, immunological and biological differences already found[2-5,13], should allow researchers to assume the distinctiveness of the canine laryngotracheitis virus (CAV-2) species.

Our results also confirm the usefulness of restriction endonuclease analysis in the identification and classification of DNA viruses. This sensitive and specific technique is now used in our diagnostic laboratories to identify as well as compare CAV-1 and CAV-2 isolates. With the appropriate restriction enzymes, this relatively simple procedure may greatly facilitate all the experimental and epidemiological studies required to develop efficient CAV vaccines.

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