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# A new goat adenovirus isolate proposed as the prototype strain for goat adenovirus serotype 1

Brief Report

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**Summary.** A virus isolated from the brain of a 3-year-old goat with encephalitis was identified as an adenovirus based on morphological and physicochemical characteristics. Neutralization tests and restriction endonuclease analysis comparing the caprine adenovirus with the prototype ovine and bovine adenovirus serotypes indicated that the caprine isolate was antigenically different and produced a unique restriction pattern and may represent a new adenovirus species. A limited seroepidemiologic study using adult goat and sheep sera collected from around the Unites States indicated that approximately 60 and 80 percent, respectively, had specific antibody for this isolate.

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Members of the family *Adenoviridae* genus *Mastadenovirus* have been isolated from cattle, sheep and goats. Currently there are 10 species (serotypes) of adenoviruses in cattle (BAdV 1 to 10) and six species in sheep (OAdV 1 to 6) recognized by the International Committee on Taxonomy of Viruses [13]. Two probable caprine adenovirus (GAdV) serotypes [6] and an OAdV 5 [12] have been isolated from goats. Adenovirus-like particles have been identified by electron microscopy in the liver the of a goat that was a "poor doer" but virus isolation was not successful [16].

Brain tissue from a three year old goat with clinical signs of encephalitis was submitted to the National Veterinary Services Laboratories, Ames, Iowa for diagnostic assistance. Microscopic lesions were those of an acute nonsuppurative encephalitis presumably of viral etiology with caprine arthritis encephalitis being suspected [14]. Tests for caprine arthritis encephalitis virus were negative, however. A virus (NC90-7261) was isolated on ovine fetal cornea cell cultures

and tentatively identified as an adenovirus by electron microscopy. The objectives of this study were to characterize the adenovirus virus isolated (courtesy of A. L. Shafer, Diagnostic Virology Laboratory, NVSL, Ames, IA) using standard procedures and estimate the prevalence of this virus in the goat and sheep population.

The virus (NC90-7261) isolated on multiple attempts from brain was biologically cloned by three successive plaque purifications. Stock virus was propagated in ovine fetal turbinate cells, harvested after two freeze-thaw cycles and stored at $-80\,^{\circ}$C. The cells were grown in minimal essential medium supplemented with 10% fetal bovine serum and 100 μg gentamicin sulfate per ml. For negative-contrast and transmission electron microscopy, infected cultures were harvested by trysinization when viral induced cytopathic effect involved over 75% of the cells, the cell were pelleted by low speed centrifugation ($1\,000\times\mathbf{g}$), resuspended in either distilled water for negative-contrast electron microscopy or 2.5% glutaraldehyde for transmission electron microscopy, and prepared using standard procedures. The virus was tested for sensitivity to chloroform [5], acid [10] and heat [7]. Adenovirus group-specific antigen for NC90-7261 was determined using a cross-immunoperoxidase test. Conventional mastadenoviruses were represented by BAdV 2 (bovine subgroup 1) BAdV 7 (bovine subgroup 2) [1].

Hemagglutination ability of the virus was evaluated by mixing serial twofold dilutions of supernatant fluid harvested from viral infected cultures with a 0.25% suspension of freshly prepared red blood cells in 0.01 M phosphate buffered saline solution (pH7.4). Hemagglutination experiments were done in microtitration plates in triplicate at 4, 22, and 37 °C using bovine, ovine, caprine, guinea pig, rat, mouse and chicken red blood cells. Culture fluid prepared from non-inoculated cell cultures served as control.

The viral DNA was extracted from the infected cell monolayers by the method of Hirt [8] as modified by Shinagawa et al. [15]. Digestions with restriction enzymes *Bgl* II, *Hind* III, and *Pst* I were carried out according to the conditions recommended by the manufacturer and the restriction digests were electrophoresed at 100 V for 5 h in horizontal 1.0% agarose gels in tris-borate buffer. The gels were then stained with ethidium bromide and photographed over UV light. Genome size was estimated based on migration of fragments produced in restriction digests.

Serum-virus neutralization tests for virus identification and tests for antibody to the NC-90-7261 virus determinations in goat and sheep sera were done in microtitration plates as previously described [11]. Stock pools of the prototype ovine and bovine adenoviruses (kindly provided by Dr. B. M. Adair, Veterinary Research Laboratory, Stormont, Belfast, Northern Ireland) were prepared following biological cloning. Antiserum to adenovirus strain NC90-7261 and antisera to prototype of OAdV 1 through 6 and prototype BAdV 1 through 8 and 10 prepared in rabbits were used in cross-neutralization tests for serotypes determination. Because repeated attempts to recover BAdV-9 from the provided stock were unsuccessful this serotype could not be included in the study. One hundred sera each from sheep and goats were used for prevalence estimation of the GAdV

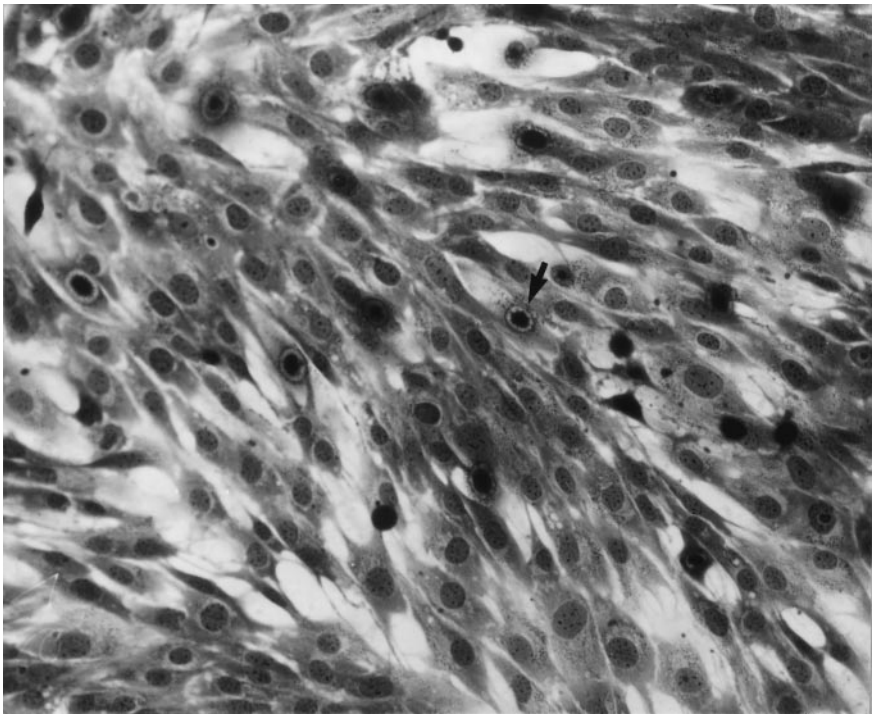

**Fig. 1.** Ovine fetal turbinate cell culture infected with virus strain NC90-7 261 showing distinct single central inclusion (arrow). A clear zone separates the central inclusion from the nuclear membrane. After 48 h postinfection cells were fixed with 100 % methanol and stained by the May-Grünwald Giemsa method. Original magnification × 200

strain NC90-7261. The sera were randomly selected from flocks throughout the United States using two to five per flock [3, 4].

Cytopathic effect produced by strain NC90-7261 was apparent 48 to 72 h after inoculation. In unstained preparation cytopathic effect was characterized by rounded refractile cells with few obvious intranuclear inclusions. When the titer of the inoculum was low, the cytopathic effect occurred first as foci and quickly became generalized over the entire monolayer with slowly progressive detachment of cells. Single, rarely multiple, intranuclear inclusions were seen in stained preparations (Fig. 1). Infectivity assays showed virus titers to be generally low, ranging from $10^3$ to $10^5$ median cell culture infective doses per ml. Electron microscopic examination of cell lysates showed typical adenovirus virions (Fig. 2). Hexagonal virions were seen to have equi-angular triangular faces with each triangle containing six subunits (capsomers) per side. The diameter of the virions was between 70 and 80 nm. In ultrathin sections of virus infected cells, crystalline arrays of virus were seen infrequently (Fig. 3). Further characterization studies showed NC90-7261 virus was resistant to chloroform and acid treatment and sensitive to heat treatment. In the cross-immunoperoxidase test for detection of group-specific antigen, the antiserum to BAdV 2 produced a weak reaction with NC90-7261 where as antiserum to NC90-7261 did not react with BAdV 2. There

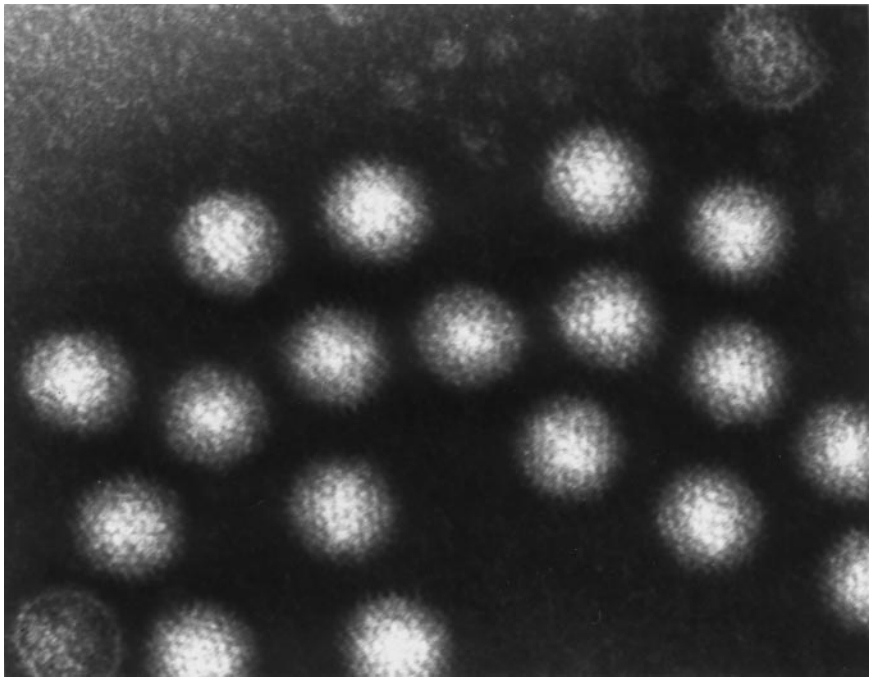

**Fig. 2.** Electron micrograph of virus strain NC90-7 261 negatively stained with potassium phosphotungstate. Original magnification × 220,000

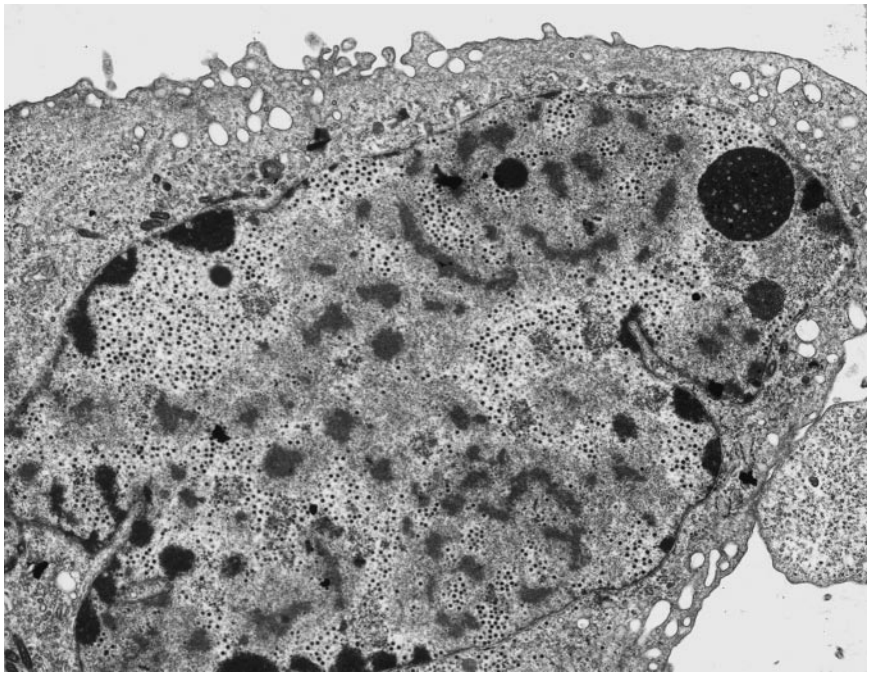

**Fig. 3.** Electron micrograph of ovine fetal turbinate cells infected with virus strain NC90-7 261. Note the margination of cellular chromatin, the type II inclusions of different densities, and the loose array of virus in the nucleus and cytoplasm of the cell. Original magnification × 6,500

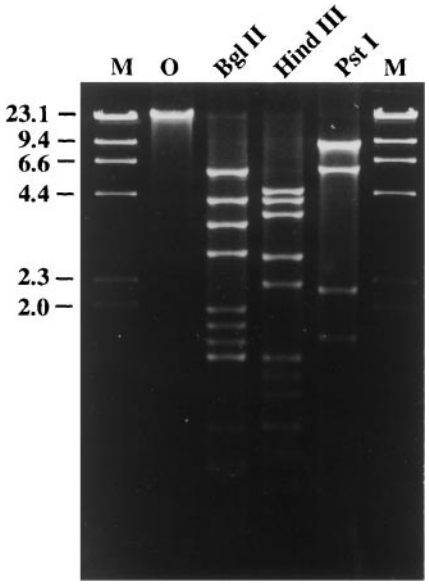


**Fig. 4.** Genomic DNA of virus strain NC90-7 261 digested with restriction enzymes as marked, separated in a 1% agarose gel, and visualized by ethidium bromide staining. *O* Undigested DNA; *M* molecular size standard bacteriophage lambda *Hind* III fragments

**Table 1.** Cross neutralization tests with prototype ovine adenoviruses and virus isolate NC90–7261

| | | Immune sera against ovine adenovirus prototype strains prepared in rabbits | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Serotype | Strain | OAdV-1 | OAdV-2 | OAdV-3 | OAdV-4 | OAdV-5 | OAdV-6 | NC90-7261 |
| OAdV-1 | S1 | 16 384 | – | – | – | – | – | – |
| OAdV-2 | P×515 | – | 2 048 | – | – | – | – | – |
| OAdV-3 | P×611 | – | – | 8 192 | – | – | – | – |
| OAdV-4 | 7 769 | – | – | – | 32 768 | – | – | – |
| OAdV-5 | SAV | – | – | – | – | 65 536 | – | – |
| OAdV-6 | WV419/75 | – | – | – | – | – | 8 192 | – |
| | NC90-7 261 | – | – | – | – | – | – | 32 768 |

–: Titer $\leq$ 16
Data are expressed as the reciprocals of serum dilutions

was a strong reaction both directions with BAdV7 and NC90-7261 indicating that NC90-7261 shares the group specific antigen with bovine subgroup 2 adenoviruses. Hemagglutination was not observed with any of the erythrocytes used. There was no cross-neutralization of GAdV strain NC90-7261 in tests with the prototype OAdV and BAdV (Table 1 and 2). Restriction endonuclease patterns generated for strain NC90-7261 are shown in Fig. 4. Genome size was estimated to be 28 kilobase pairs. Approximately 60% of the goats and 80% of the sheep had antibodies to GAdV strain NC90-7 261.

Virus strain NC90-7261 isolated from a goat brain had the cultural, morphological and physicochemical characteristics of members of the *Adenoviridae* family [13]. Serologic and restriction endonuclease analyses indicate that strain NC90-7261 is different from the currently recognized serotypes of ovine and

**Table 2.** Cross neutralization tests with prototpe bovine adenoviruses and virus isolate NC90-7291

| | | Immune sera against bovine adenovirus prototype prepared in rabbits | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serotpe | Strain | BAdv-1 | BAdv-2 | BAdv-3 | BAdv-4 | BAdv-5 | BAdv-6 | BAdv-7 | BAdv-8 | BAdv-9 | BAdv-10 | NC90-7 261 |
| BAdV-1 | B-10 | 8 192 | – | – | – | – | – | – | – | ND | – | – |
| BAdV-2 | B-19 | – | 8 192 | – | – | – | – | – | – | ND | – | – |
| BAdV-3 | WBR1 | – | – | 8 192 | – | – | – | – | – | ND | – | – |
| BAdV-4 | THT/62 | – | – | – | 8 192 | – | – | – | – | ND | – | – |
| BAdV-5 | B4/65 | – | – | – | – | 8 192 | – | – | – | ND | – | – |
| BAdV-6 | 6 71130 | – | – | – | – | – | 2 048 | – | – | ND | – | – |
| BAdV-7 | Fukuroi | – | – | – | – | – | – | 8 192 | – | ND | – | – |
| BAdV-8 | Misk/67 | – | – | – | – | – | – | – | 32 768 | ND | – | – |
| BAdV-9 | Sofia-4/6 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| BAdV-10 | 78–5 371 | – | – | – | – | – | – | – | – | ND | 4 096 | – |
| | NC90-7 261 | – | – | – | – | – | – | – | – | ND | – | 32 768 |

−: Titer $\leq$ 16

Data are expressed as the reciprocals of serum dilutions

*ND* Not determined. BadV-9 was not included in this study because repeated attempts to clone virus other than BAdV-1 from the provided stock were unsuccessful

bovine adenovirus. The reisolation of this virus from the brain tissue on multiple attempts, the lack of serologic cross-reactivity with currently recognized available adenovirus species found in sheep and cattle (Tables 1 and 2), and the occurrence of specific antibody for this virus in goats indicates that virus isolate NC90-7261 represents a goat adenovirus serotype. Unfortunately the two previously isolated serotypes of GAdV are no longer available for comparison studies (pers. comm., Dr. Paul Kitching, Pribright, Wording, Surrey, England). Because the GAdVs isolated by Gibbs and coworkers [6] no longer exist for comparative studies, we propose that strain NC90-7261 be designated GAdV 1. The utimate decision will depend on the adenovirus study group of the International Committee on Taxonomy of Viruses.

Comparison of the *Hind* III and *Pst* I restriction enzyme patterns produced by GAdV strain NC90-7261 to reference type strains of BAdV 1 through 4, 6 through 9 [1, 2], and OAdV 1 through 6 (Lehmkuhl et al., in preparation) and the Hind III pattern for BAdV 10 [9] established that the pattern produced by GAdV strain NC90-7261 was unique. Although digest patterns for BAdV 5 are not available for comparison, no neutralization occurred in cross-neutralization tests between strain NC90-7261 and BAdV 5. The estimated genome size of 28 Kb and presence of antigen in common with subgroup 2 bovine adenovirus indicates adenoviruses isolated from goats may also contain members of both antigenic subgroups [1]. Further studies will be needed for conformation.

Antibody to this GAdV appeared to be widespread in both goats and sheep in the United States. The presence of antibody to this serotype GAdV in sheep as well as goats indicates an antigenically similar if not identical virus exists in sheep. Neutralizing antibody to the Nig 75/1-435 strain of GAdV was found to be widespread in goats and sleep in Nigeria and England [6]. The isolation of OAdV-5 from goats [12] and the presence of neutralizing antibody to goat adenovirus strains Nig 75/1-435 and NC90-7261 support infection by at least some adenoviruses in closely related animal species [13].

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