Isolation and Characterization of an
Equine Adenovirus

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Received for publication 7 November 1972

A viral agent was isolated from lung tissue obtained upon necropsy of an Arabian
foal which had exhibited clinical signs of pneumonia. The virus is 75 nm in diameter,
cubic in symmetry, and resistant to chloroform and low pH (3.0). It contains de-
oxoyribonucleic acid and has a buoyant density of 1.31 g/cm³ in cesium chloride.
These findings indicate that the virus is a member of the adenovirus group.

Respiratory disease is a common and often serious condition affecting equine foals. Numerous
agents have been associated with such conditions in the young equine (12, 16, 17). A pneumonia in
newborn and young Arabian foals characterized by the presence of intranuclear inclusion bodies
in the bronchial and bronchiolar epithelial cells has been previously described (8, 11, 20). Adeno-
viruses have been isolated from Arabian foals suffering from respiratory diseases (11, 20) as well
as from clinically normal foals (6, 14). This report concerns the isolation and characterization of an
adenovirus from pneumatic lung tissue of an Arabian foal in California.

MATERIALS AND METHODS

Cells and virus. An equine dermis cell line (ECID) provided by A. J. Hackett (Cell Culture
Div. of the Naval Biomedical Lab., Oakland, Calif.) was used to propagate the viral isolate.
Equine dermis cells were grown on Falcon plastic flasks in Eagle minimal essential medium containing
5% fetal calf serum, 2,000 U of penicillin per ml and 200 μg of streptomycin per ml. Other cell lines
used in this investigation were Madin Darby bovine kidney and pig kidney cells.

The viruses used in this study were infectious bovine rhinotracheitis virus (IBR), vesicular
stomatitis virus (VSV), New Jersey serotype, and the adenovirus of equine origin isolated in our
laboratory. The designation equine adenovirus (EAdV) is for convenience only, since its host
range has not been established.

Isolation of the equine adenovirus. Lung tissues taken upon necropsy of a 3-month-old
Arabian foal were homogenized with the aid of a mortar and pestle. A 10% (wt/vol) homogenate
was obtained by the addition of Eagle medium. The homogenate was centrifuged at 6,000 × g for
15 min and the resulting supernatant fluid was passed through a 0.45-μm membrane filter. The fil-
trate was inoculated onto 2-day-old ECID cells and incubated at 37 C for 7 days. Cells were then
scraped from the glass and frozen and thawed three times before being used to inoculate subsequent
passages. After several passages, cytopathic effect was consistently observable at 2 to 3 days post-
infection.

ECID cells from passages 20 to 30 were found to be the most sensitive to cytopathic effects, and
only cells of these passage numbers were used for all experiments. Likewise, the addition of arginine
at a concentration of 1.5 mM/ml enhanced cytopathic effects and was used throughout all exper-
iments.

Growth and assay of viral isolate. Confluent monolayers of ECID cells were washed once with
Eagle medium and the viral inoculum was allowed to adsorb for 1 h at 37 C before the addition of
Eagle medium containing 5% fetal calf serum, 60 μg of tyloxpin per ml, and 1.5 mM arginine
(EFTA medium). After an incubation period of 5 days at 37 C, infected cells were scraped into the
medium, frozen and thawed three times, and centrifuged at 6,000 × g for 15 min. The super-
natant fluid was stored at −70 C and passed three times before it was used as viral stock.

The titer of infectious virus was determined by the microtitration procedure (18). Briefly, serial
log₅ dilutions of the viral sample were made and 0.05 ml of each dilution was transferred to flat-
bottom wells constructed in plastic microplates (Model IS-FB-96, Belco Glass, Inc., Vineland,
N.J.). Eight replicates were made per dilution and 0.05 ml of ECID cells (8,000 to 10,000 cells/well)
suspended in EFTA medium was added to each well; the plates were covered with sealing tape and
incubated at 37 C. Microplate cultures were observed for evidence of cytopathic effect with the
aid of an inverted microscope. Infectivity titers, recorded after 8 days of incubation, were calcu-
lated by the method of Kärber (9).

Chloroform sensitivity. The procedure used
The titers were pooled and Schwartz/Mann mmol; viral stocks. Labeled min 90 C. 4 also was infected culture. Virus postinfection 5.0 of the plaque technique (3). Described previously hydrochloride, 0.001 in 0.01 M CsCl density gradient, (BUdR) 2'-deoxyuridine of stock virus. Untreated cultures at 5% (wt/wt) in TES buffer and centrifuged at 153,000 X g in a Spinco SW41 rotor at 4 C for 20 h. Fractions were collected from a hole punctured in the bottom of the tube and acid-precipitable radioactivity was determined by the method of Parks et al. (13). Samples were counted in a Mark II liquid scintillation spectrometer. Density gradient fractions were also examined by electron microscopy.

Electron microscopy. Virus was concentrated by centrifugation at 78,400 X g for 90 min at 4 C and suspended in TES buffer. Samples from viral concentrate or from density gradient fractions were placed onto Formvar-coated copper grids and allowed to stand for 15 min. Excess fluid was removed with a piece of filter paper and the grids were stained with 1.5% potassium phosphotungstate (pH 7.4) for 2 min. Grids were examined with an AEI EM 6B electron microscope.

RESULTS

Morphology. Negatively stained EAdV particles exhibited cubic symmetry and did not possess an envelope (Fig. 1). The average diameter of EAdV (estimated by measuring 50 particles) was 75 nm, a value which is within the size range reported for adenoviruses (15).

Nucleic acid type. The replication of IBRV and EAdV was found to be inhibited by BUdR, whereas that of VSV was not impaired. Furthermore, BUdR inhibition of EAdV replication was reversed by the addition of thymidine. The results shown in Table 1 offer presumptive evidence indicating that the genome of EAdV is deoxyribo-nucleic acid (DNA) in nature. It is important to

Fig. 1. Morphology of an equine adenovirus showing cubic symmetry.
note that inhibition of replication by BUdR does not eliminate the possibility that EAdV is a ribonucleic acid (RNA) virus which depends upon DNA synthesis for the production of progeny virus. However, no cubically symmetrical, non-enveloped RNA virus has been shown to be inhibited by BUdR. The fact that substantial amounts of \(^{14}\)C-thymidine is incorporated into particles that have a density of 1.31 g/cm\(^3\) lends additional support to a contention that the nature of the nucleic acid is DNA.

**Chloroform sensitivity.** The effect of chloroform treatment on EAdV infectivity is illustrated in Table 2. The finding shows that this virus does not contain essential lipids.

**Resistance to low pH.** When the pH of the viral suspension was adjusted to 3.0 by the addition of sodium citrate-citric acid buffer and held at room temperature for 1 h, there was no appreciable loss in viral titer (Table 3), indicating that EAdV is acid stable.

**Buoyant density.** A representative density gradient profile is shown in Fig. 2. The peak of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EAdV (TCID(_{50}/)ml)</th>
<th>IBRV (PFU/ml)</th>
<th>VSV (PFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.0 ( \times ) 10(^4)</td>
<td>5.5 ( \times ) 10(^8)</td>
<td>3.5 ( \times ) 10(^8)</td>
</tr>
<tr>
<td>BUdR</td>
<td>5.0 ( \times ) 10(^3)</td>
<td>1.4 ( \times ) 10(^8)</td>
<td>3.2 ( \times ) 10(^8)</td>
</tr>
<tr>
<td>BUdR plus thymidine</td>
<td>4.2 ( \times ) 10(^4)</td>
<td>ND(^a)</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\) ND, Not done.

![Fig. 2. Distribution of \(^{14}\)C-thymidine radioactivity from fractions of a preformed cesium chloride density gradient.](http://iai.asm.org/)
radioactivity was found at a density of 1.31 g/cm³. Large numbers of adenovirus particles were observed when peak fractions were examined by electron microscopy. These particles appeared singly and were found to be free of membranous material.

**DISCUSSION**

A viral agent has been isolated from lung tissue taken upon necropsy of a 3-month-old Arabian foal which exhibited clinical signs of pneumonia. The isolate, propagated in equine dermis cells, was examined by electron microscopy and found to (i) be free of an envelope, (ii) possess cubic symmetry, and (iii) have a diameter of 75 nm. These morphological characteristics are distinctive to members of the adenovirus group (1, 15). Biological and physicochemical studies indicated that the isolate (i) was resistant to chloroform, (ii) contained DNA as its genetic material, and (iii) was resistant to inactivation at low pH (3.0). Such findings furnish additional evidence supporting the placement of our isolate into the adenovirus group.

The isolate was also shown to have a buoyant density of 1.31 g/cm³ in cesium chloride. Although this value is less than that of 1.34 g/cm³ reported by Green and Pina (5) for the type 2 human adenovirus, or that of 1.35 g/cm³ described by Carmichael (2) for infectious canine hepatitis virus, variation in the buoyant density of human adenovirus types has been demonstrated (5). This variability among individual types appears to be due to differences in their DNA content and base composition (5). These parameters were not determined for our isolate, but they may account for the lower buoyant density of the equine adenovirus isolated in our laboratory.

Adenoviruses have been isolated from numerous mammalian species. However, for many of these species only a few instances of disease have been attributable to natural adenovirus infection (1, 19). Adenovirus isolations have been made from various tissues of apparently normal animals (1, 7, 10), suggesting that they can be maintained as inapparent or subclinical infections. Due to the ubiquitous nature of adenoviruses, caution should be exercised when assessing their role in a particular disease. A possible epidemiological relationship between the viral isolate and the occurrence of pneumonia in Arabian foals is currently under investigation in our laboratory.

**ACKNOWLEDGMENT**

This investigation was supported by Public Health Service research grant FR 05457 from the Division of Research Facilities and Resources.

**LITERATURE CITED**


