Isolation of an adenovirus and an adeno-associated virus from goat kids with enteritis


Abstract. A dairy goat operation in Minnesota experienced a sudden, markedly increased mortality among its neonatal goats. Approximately 60 of 130 kids (46%) died. The animals had diarrhea and dyspnea of 1–2 days duration before death. Necropsy of 4 goat kids revealed marked, acute, catarrhal enteritis and fibrinous pleuropneumonia. Mannheimia haemolytica was isolated from the lungs. Basophilic inclusion bodies filling the entire nucleus were present in enterocytes of the ileum of 3 goats. Adenoviral particles were detected in the feces by electron microscopy and adenovirus was subsequently isolated from the intestinal content together with a parvo-like virus (dependovirus). Morphology, physicochemical characteristics, and neutralization tests indicated that the adenovirus resembled ovine adenovirus-2 (OAdV-2). However, the PstI restriction endonuclease pattern produced by the goat adenovirus was distinct from that of OAdV-2. This is the first report of enteritis in goats with an adenovirus antigenically related to OAdV-2 and with a parvo-like dependovirus.

Four goat kids from a caprine dairy farm were submitted to the Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, Minnesota, for necropsy in March 2001. The farm had 850 milking does (Nubians, LaMancha, and Bohr crosses), but the approximately 100 preparturient does and kids were kept physically separate from the milking herd. Bred does on this farm are routinely transferred to a second farm until parturition. The 4 kids had acute watery, yellow diarrhea, and all died within 4–5 hours after onset of diarrhea. Approximately 130 goat kids were born on the premises. Sixty of these kids died with similar clinical signs over a 2.5-month period during the kidding season from January to April, 2001. Only suckling bottle-fed kids were affected, with an age range of 10–40 days and an average age of 27 days. There was no sex or breed predilection. All kids were routinely separated at birth from the dam and housed initially in groups of 3–4 animals. At 30 days of age, the pens were commingled into larger groups of up to 20 kids per pen for weaning. Young stock were fed heat-pasteurized goat colostrum for 2 days, followed by cow’s milk fed free choice in LamBar-type feeding units until weaning.

A postmortem examination was performed on 4 kids. For histology, samples of small and large intestines, lungs, liver, kidney, spleen, brain, and heart were collected. Sections cut at 4 μm were stained with hematoxylin and eosin (HE). Fresh samples of lung and liver from each animal were submitted for aerobic culture. The intestine was cultured under aerobic and anaerobic conditions by routine laboratory procedures. Feces were submitted for routine parasitological examination. Fecal samples were examined for viruses using direct negative-contrast transmission electron microscopy (TEM) as described previously.5 Samples of ileum were pooled, homogenized, and submitted for virus isolation on bovine turbinate cells (BT), primary lamb kidney cells (LK), and Madin–Darby bovine kidney (MDBK) cells.

At necropsy, all kids had marked acute catarrhal enteritis characterized by watery, yellow intestinal contents in the absence of formed feces. A moderate amount of curdled milk was present in the abomasum of all 4 kids. Each of the 4 kids had acute fibrino-hemorrhagic pleuropneumonia affecting up to 50% of the lung parenchyma.

On histopathology, numerous crypts of the ileum were dilated and filled with cellular debris in 3 of the 4 kids. In these kids, occasional enterocytes contained basophilic to amphophilic inclusion bodies that filled the nucleus (Fig. 1). Inclusion bodies were not observed in any other organs examined, including large intestine and lungs. There was moderate lymphoid depletion of Peyer’s patches. In addition, a severe fibrinohemorrhagic pneumonia was present, characterized by accumulation of fibrin, erythrocytes, neutrophils, streaming degenerate leukocytes (“oat cells”) in alveolar lumina, and large areas of coagulation necrosis of lung lobules. Numerous pulmonary blood vessels and lymphatics contained fibrinous thrombi.

Mannheimia haemolytica and Streptococcus sp. were isolated from the lung and liver in mixed culture. There was an abundant growth of mixed nonhemolytic coliforms, mixed Streptococcus sp., and Proteus sp. in the intestines of all 4 kids. Clostridium perfringens was cultured from the intestine of 1 animal (kid 2).
Fecal samples were negative for parasite ova and oocysts by direct fecal examination. Icosahedral virus particles, approximately 70–80 nm in diameter, with morphologic characteristics of the virions of the Adenoviridae, such as angular nucleocapsids with 252 capsomeres and axis symmetry were detected in a pooled fecal sample by direct negative-contrast TEM.

Cytopathic effects were observed during the third passage in the inoculated primary LK and MDBK cell cultures. The virus culture was negative after 5 passages in BT cells. Electron microscopy of the cell culture supernatant revealed 2 types of viral particles. One type was identified as adenovirus (Fig. 2) and other icosahedral viral particles were identified as a parvovirus-like virus on the basis of viral particle size and shape (approximately 18–26 nm in diameter). Indirect immunofluorescence assays using bovine parvovirus and bovine adenovirus types 1 and 5 antisera were negative.

Serum–virus neutralization tests for adenovirus identification were done in microtiteration plates as described. Briefly, rabbit antiserum prepared from biological cloned stock pools of the prototype caprine (GAdV) and ovine adenovirus (OAdV) serotypes were used in neutralization tests. Ovine fetal turbinate cell cultures were used for virus replication. Serial 2-fold dilutions of reference antisera were made starting at 1:16. The goat adenovirus isolate (MN01-8548) was diluted to provide 100 tissue culture infective dose (50) per well. Control included back titration of the virus. Antiserum to OAdV-2 neutralized the goat adenovirus. Neutralization with the other GAdV and OAdV serotypes was not observed.

Goat adenovirus deoxyribonucleic acid (DNA) was extracted from infected cell monolayers as described. Pst I was used for DNA restriction enzyme analysis as described. The restriction endonuclease pattern produced by goat adenovirus MN01-8548 was different from that produced by OAdV-2.

Adenoviruses have been associated with clinical disease in goats. The virus tends to be transmitted by respiratory secretions or the fecal–oral route (or...
both) in domestic animals. The husbandry practices involving this subgroup of goats were inadequate from a biosecurity standpoint. There was a high degree of unmonitored movement of goats and people through the farm, and equipment (potential fomites) was shared between premises. New animals were repeatedly purchased despite the lack of quarantine stalls. Adenovirus may have been acquired through the introduction of new animals. The viruses isolated from the goats in this study had the morphologic characteristics of members of the Adenoviridae and Paroviridae families. Currently, there are 2 tentative goat adenovirus serotypes recognized by the International Committee on Taxonomy of Viruses. There are 4 reports of adenovirus isolations from goats. Adenoviruses, unrelated to the then recognized cattle and sheep adenovirus types, were isolated from goats affected with peste des petits ruminants in Nigeria. Two proposed new adenovirus serotypes have been isolated and characterized from goats in the United States. Finally, an adenovirus neutralized by antiserum to OAdV-5 was isolated from goats in Senegal with clinical signs of peste des petits ruminants. This is the first reported isolation of a goat adenovirus antigenically related to OAdV-2.

Small icosahedral (parvo-like) viruses were detected in the freeze-thaw of the cell cultures. The possibility that these parvo-like viruses represented a concurrent parvovirus infection of the goat was considered; however, the lack of the cross-reactivity with the bovine parvovirus antiserum led to the conclusion that these were adeno-associated viruses (Dependovirus). Members of the Parovirinae subfamily share group antigens among but not between the Parovirus and Dependovirus genera. In a review of the literature, no reports were found of parvovirus infections in goats. Alternatively, it may be speculated that these adeno-virus-associated viruses may have been dormant in the cell cultures. However, because the parvo-like virus particles were observed on EM examination of both LK and MDBK cells and because both cell cultures were inoculated with the original homogenized goat ileum independently of each other, it is highly likely that these viruses originated from the homogenized goat ileum. There were also no virus particles observed by EM examination of a freeze-thaw of the same lot of noninoculated LK cells. This represents the first reported isolation of a caprine adeno-associated virus. Adeno-associated virus isolation has been reported from a sheep infected with OAdV-2.

The observed differences in the restriction endonuclease patterns between OAdV-2 and goat isolate MN01-8548 are not likely a result of the additional adeno-associated virus DNA. Members of the Dependovirus genus encapsidate single-stranded DNA of both polarities with equal frequency and form double-stranded DNA upon extraction. Different adenovirus isolates of the same serotype usually have restriction patterns typical of the prototype strain, although differences in restriction endonuclease patterns have been observed with BAdV-10. Further studies are needed to fully characterize the adenovirus and the adeno-associated virus isolates in this case. The clinicopathological significance of these viruses in goats remains to be established. Adeno-associated viruses have not been linked with disease in any of the hosts from which they have been isolated and in fact may actually be beneficial to the host through inhibition of the helper adenovirus replication.

The pleuropneumonia was caused by Mannheimia haemolytica and likely contributed to the cause of death in the goat kids. Adenoviral inclusion bodies were not detected in the lungs and it appears to be unlikely that the pneumonia was directly caused by the adenovirus; however, experimental infections of lambs inoculated with OAdV-6 and Mannheimia haemolytica resulted in respiratory disease of greater intensity and duration than those inoculated with either agent alone. These results suggest a potential synergy between the 2 pathogens. The Clostridium perfringens infection may have represented a secondary infection of the compromised small intestine.

Adenovirus infection may be considered as a differential diagnosis in cases of enteritis in goat kids, especially in cases of mass mortality. It may be important to isolate and characterize the adenoviruses so that specific prevention strategies may be put in place in a postdisease outbreak management plan.

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References


Eye infections due to Listeria monocytogenes in three cows and one horse

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Abstract. A retrospective study was conducted to determine case histories, microbiological characteristics, and molecular subtypes associated with Listeria monocytogenes infections of the eye in large animals. For selected cases, environmental L. monocytogenes contamination patterns on case farms were also evaluated to probe for potential sources and spread of listerial eye infections. Records of 170 L. monocytogenes isolates from animal infections were reviewed to determine the fraction of isolates associated with eye infections (conjunctivitis, keratitis, and uveitis) of animals and to gather information on the clinical history of these cases. Overall, 4 of 170 Listeria monocytogenes isolates were associated with eye infections; 3 of these had occurred in cows and 1 in a horse. Molecular subtyping (by EcoRI ribotyping) showed that 4 different L. monocytogenes subtypes were isolated from these 4 cases; the same ribotypes had previously been found among invasive animal listeriosis infections. Although a variety of L. monocytogenes subtypes were isolated from environmental sources, on 1 farm, the same ribotype associated with the eye infection was also isolated from a fecal sample of a healthy animal and from a soil sample. The data reported in this study further suggest that L. monocytogenes can be a cause of eye infections in several animal species. Listerial eye infections do not seem to require specific pathogen-related virulence characteristics but rather seem to be a function of environmental or host factors, such as direct exposure of the eyes of susceptible animals to high numbers of the pathogen. Although listerial eye infections are rarely diagnosed because of its ubiquitous nature, L. monocytogenes may have to be considered more commonly as a causative agent of eye infections in ruminants and horses.

Listeria monocytogenes is a ubiquitous gram-positive bacterial pathogen that causes disease in humans and a variety of animal species. In susceptible species, listerioses can present with signs of septicemia, encephalitis, and uterine infections, often resulting in abortion. In addition to these more common manifestations of listerial infection, eye infections caused by L. monocytogenes have been documented in sheep, cattle, fallow deer, and humans. Most ocular infections have common clinical signs, including swollen, hyperemic conjunctivae, epiphora, photophobia, clouding of the cornea, and scattered white corneal