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Experimental infection with a virulent pneumoenteric isolate of bovine coronavirus

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Bovine coronavirus (BCV) is 1 of the important causes of scours in young calves.[1] Although the role of BCV in respiratory tract infections has not been clearly defined, there is increasing evidence that it causes upper respiratory tract infection in addition to the intestinal disease.[5,6] Focal involvement of lungs was recently reported in 2 of 16 calves experimentally infected with BCV,[5] but the sequence of events including clinical signs has not been clearly described for pneumotropic BCV.

The present report describes a consistent, diffuse lung involvement in calves experimentally infected with a field isolate of BCV. The details of the experimental design have been described.[2] Briefly, newborn, colostrum-deprived, unvaccinated calves were infected orally at 5 days of age with either a virulent pneumoenteric isolate of BCV (Minnesota isolate, calves 2, 6, and 7) or with an attenuated strain of BCV (Mebus strain, calves 1 and 9). The virus suspension was fed slowly to the calves with a 20-ml syringe. Although direct inoculation of the virus into the nasal cavity was unlikely, it was not possible to control the carryover of virus to the nasal cavity via insertion of the tongue into the nostril. Four calves (calves 3, 4, 5, and 8), housed in separate rooms were included as uninfected controls. One of the uninfected controls (calf 3) developed a natural infection with BCV.

Nasal cells were collected by inserting cotton swabs[a] into the anterior nasal cavity of the calves. The swabs were rotated gently to dislodge the cells of the nasal cavity. Sufficient number of cells were collected without difficulty. The swabs were placed in Hanks' balanced salt solution (HBSS) and immediately transported to the laboratory where they were vortexed for 1 minute to separate nasal cells from the swab and the mucus. The swab was removed from the tube and the resulting suspension was centrifuged at 650 x g for 5 minutes. The pellet (approx. 0.05 ml packed cells) was mixed with equal volume of HBSS and vortexed again. The cell suspension was placed on 8-well slides with 30 µl/well and allowed to dry at room temperature.

The slides were washed with phosphate-buffered saline (PBS), pH 7.2, dried, and then fixed in acetone for 10 minutes. After drying again, the wells were covered with a drop of FITC-labeled bovine anti-BCV conjugate[b] and incubated in a humid chamber for 1 hour. The conjugate was found to be specific for bovine coronavirus and did not react with uninfected Madin-Darby bovine kidney cells or with uninfected tissues from control calves (gut and lungs). The slides were washed with PBS (pH 8.5) and counterstained with Evan's blue.

The calves were kept under strict quarantine and were observed closely for clinical signs of the disease at least twice daily. In the present report, only the signs and pathology associated with the pneumoenteric strain of BCV are presented. Calf 2 inoculated with the virulent pneumoenteric strain developed diarrhea within 1 day after infection but did not develop respiratory signs until 21 days postinfection (DPI). There was a severe loss of body weight. The sequence of appearance of clinical signs in calves 6 and 7 inoculated with the virulent isolate is shown in Table 1. Calves 4, 5, and 8 (uninfected controls) did not develop clinical signs. Of the calves inoculated with the attenuated virus, 1 calf (1) did not develop signs of illness and looked normal throughout the study, but the other calf (9) developed pasty, alkaline feces with excess mucus. However, this latter calf remained alert throughout the duration of the experiment.

On examination by direct fluorescence microscopy, less than 0.1% of the nasal cells from calf 7 were positive on 2 DPI. However, the number of nasal cells showing specific fluorescence increased to 5% on 3 DPI and to 20% on 7 DPI, with large syncytia of nasal cells showing fluorescence. This calf died on 7 DPI.

The calves were necropsied after they died or they were euthanized at the end of the experiment, and routine necropsy procedures were followed. No major gross lesions were noticed in calf 2 except for peritonitis and some fluid in the peritoneal cavity, possibly due to laparotomies, which were performed to collect intestinal contents for studies on intestinal immunity. Calf 6 had acute pneumonia, petechial hemorrhages in the heart, and straw-colored fluid in the abdominal cavity. Antero-ventral areas of the lungs including ventral aspects of the diaphragmatic lobe of calf 7 were dark red and firm in consistency, indicating pneumonia; trachea had blood clots but no hemorrhages; there was no peritonitis or adhesions; and no lesions were seen in the esophagus or the oral cavity. The probable cause of death in this calf was respiratory failure. No gross lesions were noticed in the intestines or lungs of calves 8 and 9.

On histopathology, calf 6 showed multifocal cryptitis with lumens of expanded crypts filled with neutrophils and necrotic debris. The crypt epithelium was flattened, ileal submucosal lymphocytic nodules were moderately depleted of lymphocytes, and the remnants of intestinal villi were shortened. The lungs showed interstitial pneumonia and emphysema. Calf 7 had acute pulmonary hemorrhage, pneumonia, and congestive changes. Large intestines had cryptitis and the jejunum showed depletion of lymphocytes in Peyer's patches with loss of surface epithelium. Spleen showed pyknotic nuclei and nuclear debris. There was depletion of lymphocytes in mesenteric lymph nodes. There were no abnormal findings in calf 8. Calf 9 inoculated with the attenuated virus had hyperplastic mesenteric lymph nodes, but the lungs, intestines, and Peyer's patches were normal.

Lung tissue from calf 6 showed weak fluorescence with

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Table 1. Sequence of clinical events in 2 calves inoculated with pneumoenteric isolate of bovine coronavirus.

Days postinfection	Major clinical signs*	
	Calf 6	Calf 7
1	Alert	Dull, depressed
2	Dull, feces pasty	Normal
3	Pain, mucus	Soft feces
4	Bloody feces	Diarrhea, bloody feces, pain
5	Fluid feces	Respiratory distress
6	Dull, dehydrated	Labored breathing
7	Feces formed	Weak, died
8	Malabsorption	—
9	Respiratory distress	—
10	Died	—

* Severe loss of body weight was seen in both calves.

BCV, whereas the lung section from calf 7 showed strong fluorescence. No fluorescence was seen with other viral conjugates (Parainfluenza-3, bovine respiratory syncytial virus, and infectious bovine rhinotracheitis virus). The reason for weak fluorescence in the lung sections of calf 6 was probably because this calf died later in infection than did calf 7 (Table 1). The level of infection in the upper respiratory tract of calf 7, determined by the percent of fluorescing cells, had a direct positive correlation with the severity of the disease. The virus was demonstrated in calf 7 by electron microscopy after disrupting the nasal cells by sonication followed by negative staining. Most of the virus particles were devoid of their peplomers as has been reported previously.[1]

Bovine coronaviruses are generally not associated with severe lower respiratory disease.[1,3] The Minnesota isolate produced a severe, clearly defined lower respiratory disease (Kapil S, Pomeroy K, Goyal SM, et al.: 1990, Abstr 31st Ann Mtg North Central Conf Vet Lab Diagn) later in the infection (5-9 days postinfection) and was the cause of death in 1 calf. Blood was present in feces of these calves for l-2 days starting 4 days after infection. Focal emphysema has been described[5] in 2 of 16 calves experimentally infected with BCV. However, lung lesions were not considered as the cause of death.

Currently it is believed that respiratory and enteric coronaviruses belong to the same serotype despite their differences in sites of infection.[4] Additional information is needed to determine the importance of BCV in the respiratory syndrome of young calves.

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Sources and manufacturers

a. American Scientific Products, McGaw Park, IL.
b. National Veterinary Services Laboratories, Ames, IA.

References

1. Heckert RA, Saif LJ, Myers GW: 1989, Development of protein A-gold immunoelectron microscopy for detection of bovine coronavirus in calves: comparison with ELISA and direct immunofluorescence of nasal epithelial cells. Vet Microbiol 19:217-231.
2. Kapil S, Trent AM, Goyal SM: 1990, Excretion and persistence of bovine coronavirus in experimentally infected neonatal calves. Arch Virol (in press).
3. McNulty MS, Bryson DG, Allan GM, et al.: 1984, Coronavirus infection of the bovine respiratory tract. Vet Microbiol 9:425-434.
4. Reynolds DJ, Debney TG, Hall GA, et al.: 1985, Studies on the relationship between coronaviruses from the intestinal and respiratory tracts of calves. Arch Virol 8 5:71-83.
5. Saif LJ, Redman DR, Moorhead PD, et al.: 1986, Experimentally induced coronavirus infections in calves: Viral replication in respiratory and intestinal tracts. Am J Vet Res 47: 1426-1432.
6. Thomas LH, Gourlay RN, Scott EJ, et al.: 1982, A search for new microorganisms in calf pneumonia by the inoculation of gnotobiotic calves. Res Vet Sci 33: 170-182.

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Cholangiohepatitis associated with adenovirus-like particles in a pygmy goat kid

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There are only 2 reported cases of adenovirus infection in goats, both in association with outbreaks of peste de petits ruminants in Senegal[12] and Nigeria.[9] Disease associated with adenovirus has never been reported in goats in the US. The purpose of this paper is to describe the first case of adenovirus infection of a goat in the US.

An 11-day-old pygmy goat kid was submitted to the California Veterinary Diagnostic Laboratory System for necropsy. The doe kid came from a herd of 175 pygmy goats, 10 of which were neonates. The mother was 4 years old and had 1 other kid with no apparent problems. The owner had lost 1 other neonate. The doe kid was presented to the practicing veterinarian 1 week prior as a "poor doer." Physical examination revealed a swollen liver, pale mucous membranes, and hyperthermia (40 C). A CBC and liver panel were performed. The hemogram indicated leukopenia (2,400 cells/μl), anemia (PCV, 9.5%), and decreased platelets. Biochemical analysis revealed high glutamyl transferase activity

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