

Rotaviruses associated with neonatal lamb diarrhea in two Wyoming shed-lambing operations

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White scours was first recognized in 1927 as an important cause of newborn lamb loss in shed-lambing operations in the western United States.⁴ This disease was thought to be caused by bacteria, but no specific pathogen was identified. Lamb scours remains an important disease in the United States,³ and several bacteria are now considered etiologic agents, including *Bacteroides fragilis*⁵ and K99 piliated, enteropathogenic *Escherichia coli*.¹¹ Moreover, the coccidian parasite *Cryptosporidium* also has been associated with diarrhea in young lambs.¹ Viruses associated with neonatal lamb diarrhea were not reported in the United States until very recently.⁷ In this instance, group B rotavirus was detected during an outbreak of diarrhea in newborn confinement-reared lambs in Ohio.

Each spring during the past 5 years, several large shed-lambing operations in eastern Wyoming have experienced severe outbreaks of neonatal diarrhea associated with high morbidity and mortality. The purpose of this study was to determine if any potentially pathogenic enteric viruses could be detected by negative-stain electron microscopic (EM) examination of specimens collected from neonatal diarrheic lambs in 2 of these operations.

In early April 1994, specimens were collected from affected lambs in 2 eastern Wyoming shed-lambing operations situated about 40 km apart. Ranch A routinely shed-lambs over 1,300 lambs within a 30-day period commencing in early March. In some years the first scouring lambs were observed during the first week of shed-lambing, whereas in other years they occurred later. Clinical signs were observed when the lambs were 12-16 hours old and consisted of frothy salivation, inappetence, and scours. Some affected lambs died within 4-5 hours following the onset of signs; surviving lambs, however, often gained weight slowly and were prone to other infectious diseases. Once the outbreak was under way, morbidity approached 100%, and about 10% of the affected lambs died. Ranch B routinely shed-lambs between 3,000 and 5,000 lambs within a 30-day period starting in early March. Affected lambs were not ordinarily observed during the first week, but sometime afterward a lamb would become ill while still in the lambing pen. Within 4-5 days after the first case, many lambs were affected, and thereafter the majority of the lambs kept in the shed area became ill. Initial clinical signs

appeared 12-24 hours after birth and included excessive salivation and inappetence followed by diarrhea. Lambs that scoured during the first 24 hours of life might die soon thereafter, whereas others might live for several days before dying; those that developed scours after 24 hours of life usually survived but often were unthrifty. Neonatal lamb morbidity was about 50% at ranch B, and about 50% of the sick lambs died. Neither ranch experienced scouring outbreaks during range-lambing.

At ranch A specimens were collected from 8 neonatal lambs and included fecal specimens from 6 diarrheic lambs and intestinal contents from 2 dead lambs that had succumbed to diarrhea. At ranch B specimens were collected from 4 neonatal lambs and included fecal specimens from 2 diarrheic lambs and intestinal contents from 2 dead lambs that had died as a result of diarrhea. These specimens were kept cold and held at 4 C in the laboratory.

A filtrate was prepared from each specimen for EM examination as described previously.⁷ Briefly, about 1 ml of each specimen was diluted 6 fold in phosphate-buffered saline (pH 7.4), sonicated, and clarified of gross debris by low-speed centrifugation. Specimen supernatant fluids were then further clarified by passage through 0.8- and 0.45- μ m filters.^a Each filtrate was then subdivided, and portions were held at 4 C and -20 C before EM examination. Virus in 400- μ l aliquots of each filtrate was pelleted at about 178,000 x g for 15 minutes in an air-driven ultracentrifuge^b as previously described,⁸ resuspended in distilled water, and then repelleted by ultracentrifugation. These pellets were then each resuspended in 25 μ l distilled water, and 25 μ l of 3% phosphotungstic acid (pH 7.3) was added. Stained suspensions were then transferred to Formvar-coated, carbonized, 300, mesh copper grids^c and examined for virus with an electron microscope as previously described.⁸ Immune electron microscopic (IEM) examination of these filtrates for group B rotavirus was performed as previously described.⁷ Briefly, 75 μ l of each specimen filtrate was reacted overnight at 4 C with 375 μ l of hyperimmune gnotobiotic pig anti-porcine group B rotavirus serum¹⁰ diluted 1:400 in PBS. The reacted specimen filtrates were then ultracentrifuged, stained, and examined for virus as described above.

Another aliquot of each specimen was processed for group A rotavirus isolation and passaged in roller tube MA104 rhesus monkey kidney cell cultures as described previously.⁷ All specimens were tested for *Cryptosporidium* oocysts by examination of acid-fast stained smears prepared using a commercial kit.^d Bacteriologic examination of the specimens was not done.

Characteristic rotavirus particles were detected by EM examination in 5 of 6 fecal specimens collected from ranch A and in 1 of 2 fecal specimens collected from ranch B (Fig. 1); rotavirus particles were not detected in the intestinal con-

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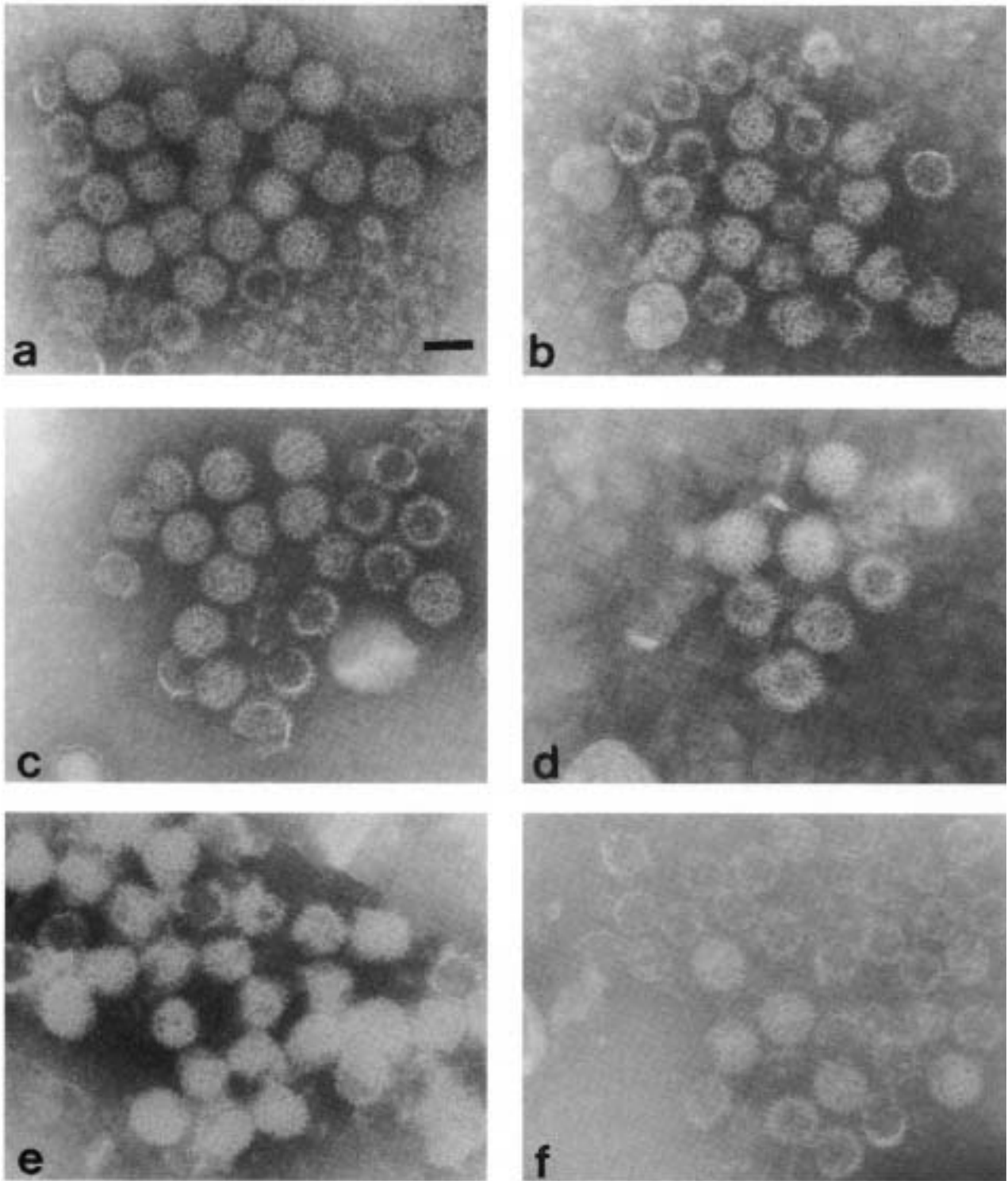


Figure 1. Electron microscopic detection of rotaviruses in diarrheic lamb feces. **a, b, c.** Rotavirus particles and core particles detected by negative-stain electron microscopy in diarrheic feces collected from 3 lambs at ranch A. **d.** Rotavirus particles detected by negative-stain electron microscopy in diarrheic feces collected from a lamb at ranch B. **e, f.** Rotavirus particles and core particles detected by immune electron microscopy for group B rotavirus in diarrheic lamb feces collected from 2 lambs at ranch A. These are the same specimens as in Figs. 1a and 1b. Bar = 60 nm.

tents specimens derived from the 2 dead lambs on ranch A or from the 2 dead lambs on ranch B. Rotaviruses were detected only in filtrates that were held at 4 C and not in those held at -20 C. Most rotaviruses were single-capsid particles, but 52-nm core particles lacking both capsid layers also were common. Rotavirus particles with double capsids were seldom detected (Fig. 1d). Although the rotavirus particles in these specimens were not abundant, they were readily detectable because they usually occurred in aggregates. Since these rotavirus particles tended to spontaneously aggregate, it was impossible to determine definitively if they had been clumped by the hyperimmune porcine group B rotavirus antiserum used in the IEM examination. Nonetheless, the rotavirus particles detected by IEM examination for group B rotavirus often appeared coated with antibody (Fig. 1e) or in some instances were mostly core particles (Fig. 1f), suggesting that the antibody may have caused virus degradation.

Viruses other than rotaviruses were not detected in these specimens by EM examination. Group A rotavirus was not isolated from any specimen, and *Cryptosporidium* oocysts were not detected in any specimen.

Rotaviruses are universally recognized as common etiologic agents of diarrhea in newborn domestic animals, and they have been identified as causing neonatal lamb diarrhea in the United Kingdom.^{2,6} Their role in causing this disease in the United States, however, is poorly understood. Our findings associating rotaviruses with neonatal diarrhea in 2 commercial shed-lambing operations constitute the second report of rotavirus infections of lambs in the United States. While it is possible that other etiologic agents could have induced the scouring observed in these shed-born lambs, the presence of rotavirus in 75% of the diarrheic fecal specimens collected on these 2 ranches suggests that the association is beyond coincidental. In fact, these 2 outbreaks are remarkably similar to outbreaks described in the United Kingdom in which nongroup A rotaviruses were considered to be the cause of acute fatal enteritis in lambs less than 1 day old.²

The rotaviruses detected in these 2 outbreaks were not group A rotaviruses but most likely group B rotaviruses. Attempts to isolate these viruses in cell cultures using procedures suitable for the propagation of group A rotaviruses were unsuccessful. Spontaneous aggregation of the rotavirus particles precluded determining by IEM examination if they would be clumped by group B rotavirus antibody; nevertheless, antibody interaction with the virus was apparent and consisted of its coating the surfaces of single-capsid particles. The paucity of rotavirus particles with double capsids in these specimens is similar to that noted with ovine non-group A rotaviruses detected in the United Kingdom.² Moreover, the common occurrence in the Wyoming specimens of core particles lacking both capsid layers resembles observations made on group B rotaviruses infecting other species.¹⁰ Insufficient quantities of specimens containing the rotaviruses precluded further characterization of these viruses.

Our observations raise several significant points relevant to the diagnosis of rotavirus infections in newborn lambs within the United States. First, the rotaviruses associated with neonatal lamb diarrhea in these 2 outbreaks and in a previous one involving a research station flock⁷ were not group A rotaviruses. Therefore, veterinary laboratories that

rely exclusively on commercial kits to diagnose rotavirus infections risk missing these infections because these kits detect only group A rotavirus. Next, the failure to detect rotavirus particles in specimen filtrates that were stored at -20 C suggests that the virus is fragile and most likely destroyed by freezing and thawing. Rotaviruses, other than those of group B, are stable and generally remain intact following freezing. For example, the group A and group D rotaviruses detected in our laboratory during previous investigations^{8,9} were readily demonstrable by electron microscopy in specimen filtrates that had been stored frozen. Finally, the lack of rotavirus particles in the intestinal contents of the dead lambs suggests that if this virus was indeed the cause of illness in these animals, then it was no longer present in demonstrable quantities at the later stages of illness. Consequently, successful diagnosis of these infections will require that specimens be selected and handled judiciously and that an appropriate diagnostic test be used.

This is the second report of ovine rotaviruses in the United States and the first associating rotaviruses with neonatal diarrhea in commercial shed-lambing operations.

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Sources and manufacturers

- a. Millipore Corporation, Bedford, MA.
- b. Airfuge, Beckman Instruments, Inc., Palo Alto, CA.
- c. Ted Pella, Inc., Tustin, CA.
- d. Volu-sol, Henderson, NV.

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Actinobacillus suis-like organisms associated with septicemia in neonatal calves

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Neonatal septicemia occurs commonly in calves and is often associated with a poor colostral antibody transfer.^{1,4} *Escherichia coli* has been the bacterium most commonly associated with calf septicemia.¹ Other bacteria, however, have been identified as the causative agent of calf septicemia, including *Salmonella* spp., *Pasteurella* spp., *Leptospira* spp., *Streptococcus* spp., and *Listeria monocytogenes*.¹⁴ This report describes 16 bovine neonates in which infection with *Actinobacillus suis*-like organisms was identified as the cause of septicemia and death.

Between January 1991 and April 1995, bacteria with phenotypic characteristics of *A. suis* were isolated from tissues of 16 calves submitted to the California Veterinary Diagnostic Laboratory System. Thirteen of the calves were Holstein, and 3 were beef calves. Nine calves were male, 6 were female, and the gender was not available for 1. The affected calves were from 9 different farms, with 7 of the calves from 1 farm (a large calf-rearing facility that acquired calves from many sources). At death, 1 calf was 7 days of age, 1 was 4 days, 1 was 3 days, 6 were 2 days, and 7 were 1 day of age. Eleven of the 16 calves were found dead with no clinical signs of disease observed, while a short course of dyspnea and recumbency prior to death was described in the remaining 5. Histories from 3 of the farms indicated that there were recent, unexpected deaths of additional neonatal calves with similar circumstances.

Gross lesions were generally minimal in the affected calves. Petechiation of serosal surfaces of thoracic and abdominal organs was observed in 6 calves. Five calves had fibrinosuppurative arthritis or hemorrhagic joint fluid involving carpal and tarsal joints. Two calves had fibrinosuppurative exudate

extending into the umbilicus, and 1 calf had fibrinous pericarditis. Suppurative bronchopneumonia with concurrent fibrinous pleuritis involving approximately one-half of the lung parenchyma was observed in 1 calf.

Bacterial evaluation was performed on selected tissues from calves, including lung (14/16), liver (14/16), and joint fluid (5/16). Samples were plated on 5% bovine or sheep blood agar and incubated aerobically with 7.5% CO₂ at 37 C. After 24 hours of incubation, colonies identified as *A. suis*-like organisms were 1-2 mm, convex, shiny, grey and opaque, and slightly agar-adherent. Organisms were gram-negative, pleomorphic bacilli that were nonmotile and produced oxidase, urease, and β -galactosidase. The isolates produced an acid reaction in the slant and butt of a triple-sugar iron agar tube without H₂S or gas production. All isolates hydrolyzed esculin and fermented trehalose but were indole-negative. All 16 isolates produced weak β -hemolysis on cow blood agar. Thirteen isolates were weakly β -hemolytic on sheep blood agar; the 3 isolates that were not β -hemolytic when grown on sheep blood agar were from calves from a single farm isolated over a 3-month period. *Actinobacillus suis*-like organisms were isolated from the liver and lung of 12 calves, joint of 5 calves, and pericardial fluid of 1 calf. In 1 calf that had concurrent *E. coli* septicemia, *A. suis*-like organisms were isolated from the joint only.

Histopathologic examinations were performed on tissues from all 16 calves. The calves had microscopic lesions compatible with gram-negative bacterial sepsis, with fibrin thrombi present in the vessels of many organs. Kidneys often were involved, with small, gram-negative coccobacilli readily apparent in the vessels of 8 calves. These bacteria often completely filled blood vessels, which were sometimes surrounded by small to moderate numbers of neutrophils. Glomerular capillaries often contained fibrin thrombi and organisms. Bacteria typically were observed in lung, liver, spleen, and heart. One calf had extensive hemorrhage and fibrin exudation with infiltrates of neutrophils in alveoli of the lung and also fibrinosuppurative pleuritis. Fibrinosuppurative

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