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Outbreak of *Streptococcus equi* ssp. *zooepidemicus* Polyserositis in an Alpaca Herd

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A 75 kg, 3-year-old male alpaca presented to the Kansas State University Veterinary Medical Teaching Hospital for complaint of recumbency and anorexia recognized 2 hours previously. The animal had been acquired 3 weeks earlier, along with 62 other male alpacas from a farm in Oregon, transported to Kansas, and housed on a farm where they were commingled with approximately 200 alpacas, originating from 2 farms.

Ten days earlier, the owner had presented 3 dead alpacas, all originally from the same farm in Oregon, for necropsy. Each had been found dead acutely over a 48-hour period. The first of these was a 1-year-old male alpaca, diagnosed with bacteremia, fibrinous peritonitis, and pleuritis. Histopathologically, the lungs, hepatic capsule, and small intestinal serosa showed a thin layer of fibrin and degenerate neutrophils. Intravascular coccoid bacterial organisms were present in the lungs, spleen, and small intestine, and *Streptococcus equi* ssp. *zooepidemicus* was cultured from the pleura, lung, peritoneal cavity, liver, spleen, and brain. The 2nd animal, also a 1-year-old male, was diagnosed with bacteremia, fibrinous peritonitis, and pleuritis. Intravascular coccoid bacterial organisms were present in the lungs, adrenal glands, spleen, heart, and kidney. *S. equi* ssp. *zooepidemicus* was cultured from the lung, liver, spleen, peritoneal cavity, and brain. The 3rd animal was a 6-year-old male alpaca that died of severe, diffuse, suppurative meningitis. Suppurative, multifocal perivasculitis was noted in the gray matter of the cerebrum and intravascular coccoid bacteria were seen. *S. equi* ssp. *zooepidemicus* was cultured from the lung, liver, spleen, and meninges.

On physical examination, the animal was hypothermic (32.8 °C; normal, 37.5–38.9 °C), tachycardic (120/minute; normal, 60–90/minute) and tachypneic at 40 shallow breaths per minute (normal, 10–30/minute). Body condition score was 5/10, no 1st-compartment contractions were noted, and the animal was mentally lethargic and recumbent. Neurologic examination revealed vertical nystagmus, left-sided facial nerve paralysis (evidenced by a droop of the left nostril) and intermittent seizure-like activity was noted. Scleral injection was present and capillary refill time was <2 seconds. Relevant hematologic findings were a leukocytosis (25.5×10^3/μL; reference range, 7.2–21.4×10^3/μL), neutrophilia with a regenerative left shift (segmented neutrophils 15.3×10^3/μL; reference range, 4.5–16.3×10^3/μL, band neutrophils 5.6×10^3/μL, metamyelocytes 0.3×10^3/μL), and a monocytosis (2.3×10^3/μL; reference range, $<0.1\times10^3$ K/μL). Fibrinogen concentration was increased (500 mg/dL; reference range, 100–400 mg/dL). Leukogram changes and hyperfibrinogenemia were attributed to an acute inflammatory response. Abnormalities in the serum biochemistry were hyperglycemia (256 mg/dL; reference range, 120–132 mg/dL), most likely caused by stress, mild hyperproteinemia (7.3 g/dL; reference range, 6.0–7.2 g/dL) characterized by mild hypoalbuminemia (3.4 g/dL; reference range, 3.8–4.3 g/dL) and hyperglobulinemia (3.9 g/dL; reference range, 2.0–3.0 g/dL), consistent with an inflammatory response.

Thoracic and abdominal ultrasonographic evaluations revealed no abnormalities. Cerebrospinal fluid (CSF) was obtained from the lumbosacral space and the fluid was grossly turbid and pink. Reference values for spinal fluid constituents were compared with published llama normals.[1] A neutrophilic pleocytosis due to a bacterial infection was diagnosed based on an increased protein concentration of > 200 mg/dL (reference range, 38.5–47.0 mg/dL), an increased total nucleated cell count of 53,700/μL (reference range, 0–3/μL), consisting of 95% degenerate neutrophils and 5% mononuclear cells. Many intracellular and extracellular bacterial cocci were seen (Fig 1). Culture of the CSF yielded *S. equi* ssp. *zooepidemicus*, but blood culture produced no growth.

Because of the declining neurologic state of the animal and poor prognosis associated with severe meningitis, the owner elected euthanasia. Necropsy examination confirmed diffuse, severe suppurative meningitis and *S. equi* ssp. *zooepidemicus* was cultured from the brain, CSF, and lung.

There was concern that additional animals would become affected and efforts were made to reduce the incidence of disease in the at-risk alpacas that remained on the farm. The susceptibility profile from cultures obtained at necropsy indicated sufficient in vitro susceptibility to ceftiofur. Ceftiofur crystalline free acid[a] (6.6 mg/kg SC, neck), a long-acting ceftiofur preparation in cattle and swine, was administered to 153 alpacas, which included all animals from the Oregon farm of origin and any individuals exposed to or commingled with them. The owner was informed that this was an off-label, previously unreported use of this ceftiofur preparation in this species and consented to its use based on the large number of animals in the herd.

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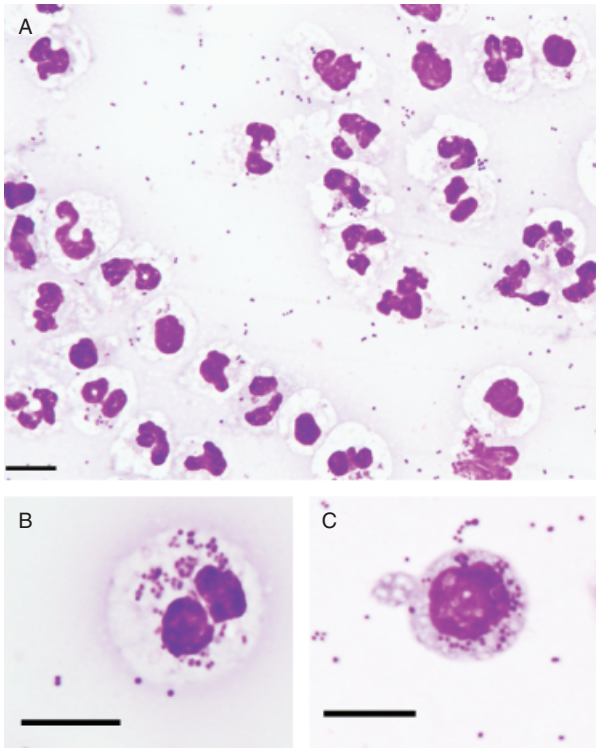

Fig 1. Direct preparation of cerebrospinal fluid from an alpaca. (**A**) The direct preparation consists of many degenerate neutrophils. Many intracellular and extracellular cocci are present. Wright stain. Scale bar = 10 μm. (**B**) A degenerate neutrophil that contains many intracellular cocci. Wright stain. Scale bar = 10 μm. (**C**) A large mononuclear cell that contains many intracellular cocci. Wright stain. Scale bar = 10 μm.

Twenty days after the herd prophylactic treatment, a 55 kg, 2-year-old male alpaca was presented with an 8-hour history of recumbency. The animal was febrile (39.4 °C), tachypneic (50 breaths/minute) and tachycardic (120 beats/min) and had a body condition score of 3/10. Severe adventitious pulmonary rales and pleural friction rubs were auscultated bilaterally, but were particularly audible over the right caudal lung field. The animal was mentally depressed and palpation over the thorax and abdomen elicited a pain response.

A CBC, serum biochemistry, and blood cultures were submitted. On CBC, the leukocyte count was normal (15.3×10^3/μL; reference range, 7.2–21.4×10^3/μL), segmented neutrophil count was normal (8.3×10^3/μL; reference range, 4.5–16.3×10^3/μL), and there was a regenerative left shift with band neutrophils (2.4×10^3/μL) and metamyelocytes (1.5×10^3/μL), and monocytosis (1.4×10^3/μL; reference range, $<0.1\times10^3$/μL). Mild toxic changes were noted on the peripheral blood smear and hyperfibrinogenemia (500 mg/dL; reference range, 100–400 mg/dL) was present. These changes were consistent with an acute inflammatory response. Serum biochemistry indicated azotemia (BUN 32 mg/dL; reference range, 20–31 mg/dL and serum creatinine concentration 3.0 mg/dL; reference range, 1.4–1.7 mg/dL), consistent with either pre-renal or renal azotemia (urine for a specific gravity was not obtained), hypoalbuminemia (3.1 g/dL; reference range, 3.8–4.3 g/dL), possibly due to 3rd-space loss, mild hypoglycemia (109 mg/dL; reference range, 120–132 mg/dL), thought to be due to increased tissue utilization or decreased production due to sepsis. Blood culture later yielded abundant growth of *S. equi* ssp. *zooepidemicus*.

Abdominal ultrasonographic examination showed normal gastrointestinal motility and normal peritoneal fluid volume. Thoracic ultrasonographic evaluation showed thickened, hyperechoic pleura, aerated lung, and normal pleural fluid volume. Bronchopneumonia and pleuropneumonia were diagnosed on radiographic examination of the thorax.

Initial therapy included IV fluid therapy (0.9% NaCl, 100 mL/kg/24 hours), ceftiofur sodium[b] (5 mg/kg IV q12h), and flunixin meglumine[c] (1 mg/kg IV q12h). Ceftiofur was administered for suspected septicemia, whereas flunixin was administered for its analgesic and antiendotoxic effects.

The next day, the animal was able to stand, ate some hay, and developed malodorous diarrhea. Fecal culture was negative for pathogenic bacteria (including *Salmonella* spp.), and quantitative and qualitative fecal examination were positive for strongyle-type eggs (360 eggs/g of feces) and *Capillaria* spp. (60 eggs/g of feces). Fenbendazole[d] (20 mg/kg PO) was administered q24h for 5 days.

Because of this additional case after herd antimicrobial prophylaxis, chlortetracycline was added to supplemental feed for the remainder of the herd. A ration was formulated to provide 2.2 mg/kg/day to each animal.

On day 2, myelocytes appeared in the peripheral blood film (0.2×10^3/μL). The animal became dyspneic with severe abdominal distension (determined to be free gas bloat) that was relieved by passage of an orogastric tube. Abdominal ultrasound examination revealed substantial free peritoneal fluid and fibrin strands. Furosemide[e] (1 mg/kg IV) was administered to treat the effusion. Butorphanol[f] (0.1 mg/kg SC) for analgesia and aminophylline[g] (5 mg/kg IV), in response to continued dyspnea after bloat relief, were administered. The IV fluid rate was decreased to a maintenance rate (50 mL/kg/day). Colloid therapy was considered due to the presence of body cavity effusion and decreased serum albumin concentration (1.9 g/dL, reference range, 3.8–4.3 g/dL). The effusion was considered to be inflammatory in nature, and colloids were not administered. Ampicillin sodium[h] (12 mg/kg q12h IV), was initiated and ceftiofur[b] continued. Ampicillin was added to the antimicrobial treatment regimen based on culture and sensitivity profiles from previous cases as well as the declining clinical status of the patient.

On day 3 of hospitalization, the animal developed grade IV/VI diastolic and IV/VI holosystolic heart murmurs with points of maximum intensity at the level of the pulmonic and tricuspid valves, respectively. Echocardiographic examination revealed aortic valve insufficiency and hyperechogenicity around the aortic valve, suggesting a vegetative lesion, whereas the pulmonic and tricuspid valves appeared normal.

Over the next 5 days, normal motility of the first compartment returned, the heart murmurs were reduced in intensity, and the pleural friction rubs became less apparent. Fluid therapy was discontinued and ceftiofur was continued for 10 days. Three months later, the animal continued to thrive and had an improved body condition score of 5/10. At that time, a physical examination was performed with no clinical abnormalities observed and routine castration was performed under injectable anesthesia with no adverse events or abnormalities noted.

S. equi ssp. *zooepidemicus* is a Lancefield group C *Streptococcus* that causes acute, subacute, and chronic disease in camelids. It is considered to be a commensal organism of alpacas in South America,[2] but not in North America.[3,4] It is the etiologic agent of "alpaca fever" in Peru, where it reportedly causes serositis of the thoracic and abdominal cavities and is associated with high mortality.[2] This organism also may be carried in clinically normal horses in the nasopharynx,[5] and also has been reported in a variety of other species, including dogs,[6] sheep,[7] and goats.[8] Peritonitis and pleuritis are common clinical manifestations of *S. equi* ssp. *zooepidemicus* bacteremia in South American camelids.[9,10] Camelids appear to have lesions restricted to the serosal surfaces, with parenchymal lesions occasionally occurring.[9] It is hypothesized that stressors, including transport, may result in subclinical carriers developing systemic infection or that infection may be acquired via the respiratory tract from camelids or other species.[10]

The source of this outbreak has not been determined. Possibilities include recrudescence of a carrier state in the newly acquired animals, transmission of the bacterium to the new animals via fomites (eg, trailer) during transport, or transmission of the bacterium from the resident herd to the naïve recently acquired animals. In both horses[11] and camelids,[9] transport or experimental inoculation generally results in acute disease within 24 hours. We suspect transmission from the resident herd to the new animals occurred, considering the 3-week delay from transport to disease. Genetic characterization of the isolates from these 5 cases could have clarified the mechanism of this outbreak of polyserositis. Unfortunately, genetic sequencing for *S. equi* ssp. *zooepidemicus* is not readily available, and this testing is not routinely performed at our institution. Clonal investigation would have allowed for individual and herd-based management by determining if a single clone was transmitted through the herd or if each affected animal experienced opportunistic invasion of individual clones. Such testing is a potentially important tool for categorizing disease outbreaks.

In the face of this outbreak, the owner initially desired a prophylactic treatment strategy that would avoid frequent handling or special feeding protocols. Cultures from each animal revealed susceptibility to several antimicrobials, including ceftiofur. In cattle and swine, ceftiofur crystalline-free acid[a] provides therapeutic antimicrobial concentrations for approximately 7 days in the bloodstream. Initial prophylactic treatment appeared successful due to the lack of additional clinical cases. However, when the final case presented, supplemental feeding with added chlortetracycline was incorporated into the prophylactic treatment plan. Recent transport was believed to contribute to this outbreak and, as such, all animals subsequently obtained from the Oregon farm were prophylactically treated with ceftiofur crystalline-free acid[a] before to shipment with no additional cases recognized on the Kansas farm in the next 4 months. The Oregon farm of origin has never experienced known illness in alpacas associated with *S. equi* ssp. *zooepidemicus*.

The decision to treat the herd with antimicrobials must be made with full consideration of the risks and benefits and in light of sensitivity profiles. This case presented an additional challenge in that all pharmaceutical and biological usage in camelids occurs in an extra-label manner. Herd treatment in this case presented the risks of establishing a microbial population resistant to ceftiofur and chlortetracycline, alteration of normal gastrointestinal flora and the possibility of an adverse event related to administration of the drug, including anaphylaxis. It is not known if *S. equi* ssp. *zooepidemicus* is harbored by carrier animals or if it functions as a contagion. Because genetic characterization of the various isolates was not performed in these cases, we cannot speculate on the mode of this outbreak. Even if genetic characterization had been performed, the results would not have been available in a reasonable time to have guided herd management. Herd prophylaxis was performed utilizing available sensitivity data from previous cases in an effort to decrease bacterial numbers in possible carrier animals and to limit transmission between animals.

Systemic infection with *S. equi* ssp. *zooepidemicus* has not been reported to occur as an outbreak in North America. In Peru, estimated morbidity is 5–10%.[2] Case reports of natural infection in North America confirm *S. equi* ssp. *zooepidemicus* as an etiologic agent of peritonitis in llamas.[10,12] *S. equi* ssp. *zooepidemicus* also has been reported as the etiologic agent of a case of mastitis in a llama[13] and septic orchitis in an alpaca.[14] The latter report hypothesizes that orchitis may have been a chronic sequela to systemic infection. The final animal in this report was castrated 3 months after discharge from the hospital and no gross abnormalities of either testicle or adhesions were noted.

This is the 1st report of *S. equi* ssp. *zooepidemicus* causing additional manifestations of meningitis and endocarditis. Meningitis and meningoencephalitis caused by this bacterium have been reported in humans[15–18] and veterinary species, including horses[19] and a goat.[8] In humans, infection is most commonly associated with consumption of unpasteurized dairy products or contact with horses.[15–17] The Oregon farm of origin in this report houses horses that are not in direct contact with the alpaca herd. Bacterial endocarditis is reported in humans with this agent,[18,20–22] with vegetative lesions commonly affecting the aortic valve,[20,21] as in the final case presented here. In a retrospective study of endocarditis in 10 alpacas,[23] a group D *Streptococcus* was isolated from a single case. All alpacas in the report had mural endocarditis of the right ventricle, with lesions also appearing in the left atrium and ventricle and left and right atrioventricular valves. No lesions were noted of

the aortic valve as observed in the final case presented here. All animals in the retrospective study were 3 years of age and younger and there was a 100% case fatality rate.

This report further demonstrates the high mortality associated with this syndrome and the range of ages that may be affected. Mortality reports from Peru range from 50 to 100%[2] and outbreaks typically are associated with stress.[24] A llama with fulminant infection in Canada died within 3 days after cessation and subsequent reinstatement of antimicrobial therapy.[10] In 1 trial of experimental infection in llamas,[9] 6 test animals were inoculated with *S. equi* ssp. *zooepidemicus*. All animals, including controls, were euthanized and at necropsy, 4 of 6 test animals had gross evidence of polyserositis. The remaining 2 test animals with no lesions also had negative tissue bacteriologic cultures throughout the study. Natural infection has been reported in a 7-month-old male llama,[10] and experimental infections were produced in animals ranging in age from 10 to19 months.[9] Animals in our report ranged in age from 1 to 6 years, with 4 of them aged 3 years or less. From these findings, it appears that young animals are predisposed to the systemic manifestation of the syndrome, although the disease should not be ruled out in mature animals.

Footnotes

[a] Excede, Pharmacia and Upjohn Company LLC, New York, NY
[b] Naxcel, Pharmacia and Upjohn Company LLC
[c] Banamine, Schering-Plough Animal Health, Union, NJ
[d] Panacur, DPT Laboratories, San Antonio, TX
[e] Furosemide, IVX Animal Health Inc, St Joseph, MO
[f] Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA
[g] Aminophylline, Hospira Inc, Lake Forest. IL
[h] Polyflex, Fort Dodge Animal Health

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