Infection with *Corynebacterium pseudotuberculosis* in five alpacas

David E. Anderson, DVM, MS; D. Michael Rings, DVM, MS; Joseph Kowalski, DVM, PhD

Young alpacas are susceptible to infection with *Corynebacterium pseudotuberculosis* that results in lymphadenitis or subcutaneous abscesses. Excision appears to be effective in the treatment of alpacas with abscesses caused by *C. pseudotuberculosis*. Diagnosis of infection with *C. pseudotuberculosis* should be made on the basis of results of microbial culture of the lesion; however, a hemolysis inhibition test may be useful for serologic screening of herds of alpacas.

Five alpacas (aged 22 days to 14 months) that resided on a large commercial breeding farm were evaluated because of the development of swellings in the cranial part of the cervical area or the submandibular region, all of which were detected within a 40-day period during late summer and early autumn. The alpacas were bright, alert, active, and eating normally. For each alpaca, heart and respiratory rates and rectal temperature were within reference ranges at the time of physical examination.

The initial case was a 3-month-old female alpaca (alpaca 1) that was examined because 3 abscesses were detected in the left aspect of the cervical region (at the level of the thoracic inlet) by the owner and referring veterinarian. Two abscesses had opened prior to examination. The third abscess was surgically opened, and samples of abscess contents were obtained for microbial culture and antimicrobial susceptibility testing; the results of cultures indicated infection with *Corynebacterium pseudotuberculosis*. All lesions were debrided, cleansed with sterile saline (0.9% NaCl) solution, and left open to drain. Alpaca 1 and its dam (alpaca 3) that was examined because 3 abscesses were detected within a 40-day period during late summer and early autumn. The alpacas were bright, alert, active, and eating normally. For each alpaca, heart and respiratory rates and rectal temperature were within reference ranges at the time of physical examination.

Herdmates of alpaca 1 were examined, and any abscesses that were detected were closely inspected; a presumptive diagnosis of caseous lymphadenitis was made on the basis of the culture results obtained for the initial case, and treatment was initiated accordingly. During treatment of these abscesses, samples of abscess material were collected for microbial culture and antimicrobial susceptibility testing. Results indicated that 4 other alpacas were also infected with *C. pseudotuberculosis*, and antimicrobial treatment was modified on the basis of results of antibiotic susceptibility testing, if necessary (Table 1).

The other affected animals included a 2-month-old male alpaca (alpaca 2) that had an abscess adjacent to the right eye. A small laceration also was found dorsal to the orbit, but an association with the abscess was not identified. The abscess was treated similarly to those of alpaca 1. Despite antimicrobial treatment, the abscess in alpaca 2 failed to resolve and the swelling increased during the next 14 days. Therefore, alpaca 2 was anesthetized, and the abscess was excised and submitted for microbial culture and antimicrobial susceptibility testing. A 6.4-mm Penrose drain was placed through the surgical defect, and the skin was closed to allow dorsal and ventral exits for the drain. Penicillin G procaine (22,000 U/kg [10,000 U/lb], SC, q 24 h for 10 days) was administered, and the lesion resolved without complication and did not recur during the next 4 months.

Another 2-month-old male alpaca (alpaca 3) was examined because of a swelling in the ventral aspect of the midcervical region that had been observed by the owner. The structure ruptured during palpation; it contained purulent material and a small amount of matted hair. Abscess material was submitted for microbial culture and antimicrobial susceptibility testing, and the lesion was debrided and lavaged. Ampicillin (10 mg/kg, IM, q 12 h for 7 days) was administered to alpaca 3. The lesion appeared to have healed completely.

Table 1—Results of antimicrobial susceptibility testing of *Corynebacterium pseudotuberculosis* organisms isolated from 5 alpacas.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Isolate 1</th>
<th>Isolate 2</th>
<th>Isolate 3</th>
<th>Isolate 4</th>
<th>Isolate 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>ND</td>
<td>ND</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>ND</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Penicillin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>S</td>
<td>ND</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S</td>
<td>ND</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>ND</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>R</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>ND</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Neomycin</td>
<td>ND</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
</tbody>
</table>


From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210.
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Address correspondence to Dr. Anderson.

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Completely when the alpaca was examined 20 days after initial treatment, but the swelling returned approximately 28 days after treatment. At that time, alpaca 3 was anesthetized and the lesion was excised; material from the lesion was submitted for microbial culture and antimicrobial susceptibility testing. After excision of the lesion, amoxicillin trihydrate was administered (10 mg/kg, IM, q 12 h for 14 days). The lesion resolved and did not recur during the next 3.5 months.

A 14-month-old female alpaca (alpaca 4) was examined because of a submandibular swelling and a swelling lateral to the right hemimandible that had been detected by the owner. The swelling on the right hemimandible had opened naturally. A 22-day-old male alpaca (alpaca 5) was examined because a left-sided submandibular swelling had been detected by the owner. Alpacas 4 and 5 were anesthetized, and the lesions were excised and submitted for microbial culture and antimicrobial susceptibility testing. A 6.4-mm Penrose drain was placed through the surgical defect in alpaca 4, and the skin was closed in a primary manner. Penicillin G procaine (22,000 U/kg, SC, q 24 h for 5 days) was administered to alpaca 4; the lesion resolved without complication and did not recur during the next 3 months. In alpaca 5, the abscess was completely excised and the surgical wound was sutured closed. Amoxicillin was administered (10 mg/kg, IM, q 12 h for 14 days, then q 24 h for 7 days) to alpaca 5, and the lesion resolved without complication and did not recur during the next 3 months.

All 5 affected alpacas and the dams of the crias that were not yet weaned were isolated from the remainder of the herd at the time that their lesions were identified until 90 days after treatment. Subsequent to identification of C. pseudotuberculosis infection, prospective and retrospective evaluations of the herd were performed. Blood samples were obtained from all affected alpacas, their dams (except for the dam of alpaca 4), and 129 unaffected herdmates. Sera were submitted for hemolysis inhibition testing for C. pseudotuberculosis. This serologic test detects the presence of toxin and is suggestive of current or recent infection with C. pseudotuberculosis. All affected alpacas were seropositive for C. pseudotuberculosis toxin, except for alpaca 3 that apparently had a dermoid cyst. All dams and unaffected herdmates were seronegative for C. pseudotuberculosis toxin.

The owners examined each member of the herd twice weekly for lesions, and the referring veterinarians examined the alpacas once weekly for 90 days after the first diagnosis was made. All alpacas in which a swelling was found were immediately isolated to the treatment barn. A total of 7 alpacas, including the 5 reported here, had abscesses. In the sixth affected alpaca, samples of an abscessed submandibular lymph node yielded negative results on microbial culture for C. pseudotuberculosis and results of hemolysis inhibition testing for the organism were negative. The seventh alpaca was treated prior to identification of caseous lymphadenitis in alpaca 1. It had an abscess located in the subcutaneous tissues along the ventral aspect of the neck, and samples of abscess material yielded negative results on culture for C. pseudotuberculosis. The alpaca had responded to treatment, which included insertion of a drain, lavage of the abscessed area and administration of antimicrobials. The only other livestock maintained on the farm were horses, which were separated from the camels by a distance of approximately 50 m. Previously, the property had been used as a commercial sheep farm, but had been vacant of all livestock for a period of 3 years prior to the purchase of the alpaca breeding herd. Health status of the previous resident sheep herd was unknown. All adult alpacas on the farm had been shorn during the early summer months. Wounds associated with shearing were not observed. The first affected alpaca was identified during the late summer and early autumn, but only the 14-month-old female alpaca (alpaca 4) had been shorn.

This herd was monitored for a period of 5 years after diagnosis of caseous lymphadenitis in the 5 alpacas, but no additional animals were affected. Two of the alpacas (3 and 4) that were seropositive for C. pseudotuberculosis at the initial testing remained seropositive after 5 years, as indicated by results of hemolysis inhibition tests. Thoracic radiographs were normal in both alpacas. One of those 2 alpacas (alpaca 3) was euthanatized 18 months after diagnosis of infection with C. pseudotuberculosis because of failure to achieve expected performance goals. At necropsy, no internal abscesses were observed but histologic examination of specimens of mediastinal lymph nodes revealed signs of chronic active inflammation. Results of microbial culture of specimens of mediastinal lymph nodes were negative. C. pseudotuberculosis was identified in the submandibular lymph nodes, but no additional animals were affected. Two offspring were reared by the dam without interference. One of these 3 crias (a male) was found to be seronegative for C. pseudotuberculosis at weaning (ie, at 6 months of age). This alpaca was euthanatized because of poor growth, but no lesions of caseous lymphadenitis were identified at necropsy. At the last report, the dam remained clinically normal and seropositive for C. pseudotuberculosis (as indicated via hemolysis inhibition tests).

Review of management practices used prior to identification of infection with C. pseudotuberculosis in the 5 alpacas indicated that alpacas 1 and 3 were housed in the same pasture; alpacas 1, 2, 3, and 5 had commingled with each other in the farm’s maternity barn at some time during a 4-month period; and alpaca 4 had been maintained on the same pasture with the dam of alpaca 2. Alpaca 4 had been purchased from a farm in Iowa and had been brought onto the farm 3 months prior to detection of the abscessed lymph nodes. During transport, alpaca 4 had been commingled with camels from farms in Colorado and Alaska.

To our knowledge, caseous lymphadenitis caused by C. pseudotuberculosis has not been reported in New...
World camelds in North America and only rarely has *C. pseudotuberculosis* been cultured from specimens obtained from alpacas in South America. Except for lesions in alpacas 2 and 3, the lesions observed in the alpacas of this report were similar to those described in sheep with caseous lymphadenitis (ie, unilateral, caseated abscesses associated with regional lymph nodes). In sheep, lesions are detected almost exclusively in adults, usually after the animals have been sheared or after a period of grazing on rough terrains. Transmission of *C. pseudotuberculosis* to susceptible individuals occurs most commonly via direct contact with exudates from infected animals; however, other routes of transmission such as inhalation of aerosols or the ingestion of organisms in milk and colostrum have been implicated. Abrasions to the skin are believed to facilitate colonization by the bacteria. After abscesses rupture, contamination of the environment may continue for more than 30 days. The 5 affected alpacas of this report were 22 days to 14 months of age, and only 1 had been shorn; the pastures on which they grazed were gently sloping, clean, and grass-covered and were separated by fences made of wooden boards and woven wire. The affected alpacas and their dams underwent a prolonged isolation period to prevent continued environmental contamination or direct transmission of *C. pseudotuberculosis*. Although 1 offspring of the persistently seropositive female alpaca was also seropositive for *C. pseudotuberculosis* 6 months after ingestion of colostrum, no localized source of infection could be identified in the offspring.

Caseous lymphadenitis has been reported in deer, cattle, horses, and humans, but the disease develops primarily in sheep and goats. In a survey of randomly selected sheep slaughtered at an abattoir in the western United States, the prevalence rate for caseous lymphadenitis lesions was 42%. In the affected sheep, lesions were detected most commonly in thoracic viscera (25%), followed by skeletal tissues including peripheral lymph nodes (23%) and abdominal viscera (12%). Visceral lesions of caseous lymphadenitis were not observed in the affected alpacas of this report, but thoracic radiography and abdominal ultrasonography were performed only in the alpacas with persistently positive results of the hemolysis inhibition assay. Although each of the 5 affected alpacas was maintained in isolation from the herd and examined twice weekly for 90 days, additional lesions did not develop after that period and clinical signs of visceral involvement were not observed.

*Corynebacterium pseudotuberculosis* is a nonspore-forming coccolid bacillus that may survive in a favorable environment for 55 days to 1 year or more. The bacteria may gain access to the body via skin lesions or possibly via inhalation. The bacteria then can spread via efferent lymphatic vessels and the bloodstream to distant areas of the body, including bones, viscera (eg, lungs and liver), and internal lymph nodes within the thoracic and abdominal cavities. Because *C. pseudotuberculosis* has a lipid capsule and is an intracellular organism, it is able to elude the body’s natural defense mechanisms and establish infections in the host. Although the *C. pseudotuberculosis*-associated morbidity rate in an affected sheep flock appears to be relatively low, the eventual mortality rate is believed to be high. Lesions observed in the alpacas of this report were localized to superficial lymph nodes. Signs of systemic infection were not detected, and deaths were not reported. The apparent morbidity rate of caseous lymphadenitis was low in this alpaca herd.

Diagnosis of caseous lymphadenitis depends on observation of the typical caseated (ie, onion skin) abscesses and identification of *C. pseudotuberculosis* via microbial culture. In the alpacas of this report, definitive identification of *C. pseudotuberculosis* was based on morphologic features, response to catalase, and the gram-stained microscopic appearance of the culture colonies. After 48 hours of incubation on sheep blood agar plates at 35°C in 5% carbon dioxide, colonies of *C. pseudotuberculosis* are somewhat larger than those of *Arcanobacterium pyogenes*. Additionally, *C. pseudotuberculosis* forms adherent colonies that crumble when touched with an inoculating loop, whereas colonies of *A. pyogenes* have a buttery consistency. Both organisms produce a narrow zone of beta (clear) hemolysis on sheep blood agar plates. Via catalase reaction testing, *C. pseudotuberculosis* yields a positive response, whereas *A. pyogenes* yields a negative response. On microscopic examination of gram-stained preparations, both organisms have a typical diphtheroid appearance. Furthermore, results of serologic testing by use of ELISA, immunodiffusion, or hemolysis inhibition tests may be useful in identifying alpacas affected by the visceral form of caseous lymphadenitis in which lesions are not apparent. In 1 study, 238 goats and 69 sheep were tested using the synergistic hemolysis inhibition test. Of 28 sheep and 52 goats that had abscesses that were caused by infection with *C. pseudotuberculosis*, 96% of sheep and 98% of goats yielded positive results by use of the synergistic hemolysis inhibition test. Those sheep and goats remained positive for *C. pseudotuberculosis* for at least 28 days after resolution of infection. In the same study, 41 sheep and 186 goats did not have abscesses, and of those animals, results of the synergistic hemolysis inhibition test indicated that 28% of the goats and 10% of the sheep were positive for *C. pseudotuberculosis*. The authors of that study concluded that the synergistic hemolysis inhibition test may lack specificity or that positive results of tests in the sheep and goats with no abscesses may have resulted from previous infections that had resolved. In the herd of this report, all affected alpacas yielded positive serologic results via hemolysis inhibition testing, except alpaca 2 that had an apparent dermoid cyst with secondary infection. Although specimens from alpaca 2 yielded growth of *C. pseudotuberculosis* on culture, humoral response to the infection may have been prevented if bacteria did not colonize surrounding tissues. Also, all clinically normal alpacas in the herd that were assessed via hemolysis inhibition testing for infection with *C. pseudotuberculosis* yielded negative results. Although the hemolysis inhibition test has not been validated for use in alpacas, the test is based on detection of bacterial toxin, not antibodies; thus, analysis of these
results seems to indicate that the hemolysis inhibition test may be useful for detection of infection caused by *C. pseudotuberculosis* in alpacas. Treatment of caseous lymphadenitis in individual sheep has not been considered practical because of the rapid dissemination of the infection to thoracic and abdominal viscera and because of economic constraints. However, results of antimicrobial susceptibility testing of *C. pseudotuberculosis* obtained from affected sheep have indicated that penicillin G procaine, ampicillin, and erythromycin should be effective. We chose a combination of surgical drainage or excision with administration of an appropriate antimicrobial for treatment of the alpacas of this report. Among the 3 alpacas treated with drainage and antimicrobials, 2 had abscesses that recurred, and subsequently, excision was performed. Thus, antimicrobial treatment success was deemed poor. Antimicrobial administration was continued after excision of lesions because of the clinician’s preference and the desire to prevent spread of any bacteria from the lymphatics surrounding the abscesses to regional lymph nodes. We recommend en bloc excision of affected tissue when infection with *C. pseudotuberculosis* is suspected to prevent further contamination of the environment. Interestingly, the analysis of results of antimicrobial susceptibility testing appeared to indicate that 2 or 3 distinct types of *C. pseudotuberculosis* were involved in the infections on this farm. Therefore, ideally, antimicrobial treatment should be determined on the basis of results of culture and antimicrobial susceptibility tests. However, we suggest that initial empirical selection of antimicrobials may be made on the basis of the antimicrobial susceptibilities of the isolates obtained from the alpacas of this report. As a result of natural infection with *C. pseudotuberculosis*, sheep develop an antibody response that is protective against subsequent inoculation. However, the prevention, control, and eradication of caseous lymphadenitis within a flock may be difficult. Measures for prevention of the disease should include maintenance of a closed herd, optimal nutrition and pasture management, use of good hygiene practices for equipment, and prevention of skin abrasions and wounds. Sheep may sustain wounds during shearing; in 1 group of lambs, use of a spray of iodine tincture on shearing-related wounds effectively decreased the *C. pseudotuberculosis* seroconversion rate. Although the adult alpacas in the herd of this report had been shorn within 2 to 4 months of the detection of the lesions, only 1 of the *C. pseudotuberculosis*-affected alpacas (alpaca 4) had been shorn. Control of the spread of caseous lymphadenitis in sheep may involve vaccination and isolation. Although commercial vaccines are available for use in sheep, we elected not to attempt vaccination of the alpaca herd because the likelihood of seroconversion or adverse reactions was unknown. Instead, we attempted to contain the infection by isolating affected alpacas, monitoring the herd, and performing a serologic survey. After diagnosis of infection with *C. pseudotuberculosis* in the fifth alpaca, additional alpacas did not develop infections. Hemolysis inhibition testing yielded negative results for all members of the herd from which samples were obtained, and additional lesions did not develop in the 5 *C. pseudotuberculosis*-affected alpacas.

Retrospective and prospective evaluation of the herd failed to identify a specific source of the infection. The infection may have been introduced by an infected alpaca that was brought to the farm for breeding or may have originated with the purchased female alpaca (alpaca 4) that had been commingled with other camelids during shipment 3 months prior to the onset of the disease. The potential for inoculation of the young camelid stock with *C. pseudotuberculosis* from goat colostrum was considered because goat colostrum had been fed to supplement maternal antibody transfer in several of the crias. However, samples of the goat colostrum fed to the affected alpacas were not available for examination. The authors of this report have conducted microbial culture of samples of colostrum and milk obtained from 15 sheep and goats with active *C. pseudotuberculosis* infections and failed to isolate *C. pseudotuberculosis* organisms. At this time, we believe that ingestion of colostrum does not pose a high risk for transmission of *C. pseudotuberculosis*. The only common factor identified among the 3 affected alpacas of this report was that 4 of them had been housed in the maternity barn. Also, an outbreak of diarrhea attributed to *Cryptosporidium* sp had been identified in the birthing facility prior to the onset of disease attributable to *C. pseudotuberculosis*. This combination of factors may have caused sufficient immunosuppression in the crias to allow the development of caseous lymphadenitis. Additionally, compared with adult alpacas, the crias have very thin skin and may be more prone to skin injury during their interactions with their environment. In utero transmission was not thought to be involved in the development of infection in the alpacas of this report because the dams had not had the disease and were seronegative for *C. pseudotuberculosis* toxin on the basis of results of the hemolysin inhibition test. Species-specific strains of *C. pseudotuberculosis* may be identified on the basis of their nitrate-reducing capacity in culture; the equine strain usually reduces nitrate, whereas the ovine strain lacks nitrate-reducing capacity. The test to assess the nitrate-reducing capacity was not performed in our laboratory, so no inference can be made as to the possible origins of the infection.

Caseous lymphadenitis caused by *C. pseudotuberculosis* should be considered in the differential diagnosis of peripheral lymphadenopathy or abscesses in camelids. Abscesses in camelids are more commonly caused by *Streptococcus* spp, other *Corynebacterium* spp, and *A. pyogenes*, but because of the contagious and pathogenic nature of *C. pseudotuberculosis*, camelids with abnormal swellings should be isolated for an appropriate period of time and the routine use of microbial culture of appropriate specimens may be indicated, especially when affected camelids have contact with other livestock.

References


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Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Assessment of the effects of exogenous long-acting insulin on glucose tolerance in alpacas
Jaime Ueda et al

Objective—To evaluate the effects of long-acting insulin on glucose clearance in alpacas.
Animals—8 adult castrated alpacas.
Procedure—On 2 days, food was withheld from alpacas for 8 hours. Alpacas were randomly allocated to receive an SC injection of long-acting insulin (0.4 U/kg) or saline (0.9% NaCl) solution 1 hour before the first of 3 administrations of glucose (at 60, 480, and 1,200 minutes after treatment) on day 1 and the alternate treatment and procedure on day 2. Plasma glucose concentration was determined before and at 15, 45, 120, and 240 minutes after each glucose administration, and fractional turnover rates were calculated. The data were compared between alpacas with and without insulin administration and among the 3 glucose administrations for each day.
Results—Compared with sham-treated alpacas, insulin-treated alpacas had significantly lower blood glucose concentrations from 180 to 600 minutes after treatment; they also had glucose concentrations significantly below baseline values from 120 to 480 minutes, at which time the mean glucose concentration was in the hypoglycemic range. Also, mean fractional turnover of glucose was significantly higher in insulin-treated alpacas from 105 through 300 minutes.
Conclusions and Clinical Relevance—Compared with known effects of regular insulin in alpacas, the action of long-acting insulin was of slower onset but longer lasting; its administration may induce hypoglycemia, even in alpacas that receive glucose. To maintain the hypoglycemic effect, long-acting insulin may have to be administered more than once daily, and blood glucose concentration should be monitored to avoid hypoglycemic complications in alpacas. (Am J Vet Res 2004;65:1688–1691)