Comparison of three treatment regimens for sheep and goats with caseous lymphadenitis

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Objective—To compare the effectiveness of 3 treatment regimens for small ruminants with caseous lymphadenitis.

Design—Randomized clinical trial.

Animals—44 client-owned sheep and goats.

Procedures—Aspirates were obtained from 48 lesions of 44 enrolled animals and submitted for bacterial culture. Animals were randomly assigned to 1 of 3 treatment groups. Treatment for group A (n = 15 lesions) consisted of opening, draining, and flushing the lesions and SC administration of procaine penicillin G. Treatment for group B (n = 15 lesions) consisted of closed-system lavage and intraleSIONal administration of tulathromycin. Treatment for group C (n = 18 lesions) consisted of closed-system lavage and SC administration of tulathromycin. All animals were reexamined approximately 1 month after treatment, unless treatment failure was detected prior to that time.

Results—43 animals with lesions had positive results (Corynebacterium pseudotuberculosis) for bacterial culture. Proportions of lesions that had resolution of infection by 1 month after treatment did not differ significantly among the treatment groups (group A, 13/14 [92.9%]; 95% confidence interval [CI], 69.5% to 99.6%; group B, 10/12 [83.3%]; 95% CI, 54.9% to 97.1%; and group C, 14/17 [82.4%]; 95% CI, 59.1% to 95.3%).

Conclusions and Clinical Relevance—Acceptable alternatives to opening, draining, and flushing of lesions may exist for treatment of sheep and goats with caseous lymphadenitis. Use of tulathromycin and penicillin in this study constituted extralabel drug use, which would require extended withholding times before milk or meat of treated sheep and goats can be sold for human consumption. (J Am Vet Med Assoc 2009;234:1162–1166)

Caseous lymphadenitis is a chronic, suppurative disease caused by Corynebacterium pseudotuberculosis. Sheep, goats, horses, cattle, and humans can be affected by this disease. Caseous lymphadenitis is currently of most interest in small ruminants and is implicated as the third leading cause of economic loss to the sheep industry in the western United States.1 In 1 report2 in which investigators examined samples from culled sheep from regions representing 9 western states, the prevalence was estimated as 42.41%. Although prevalence studies for goats in the United States are lacking, there is an ever-increasing number of goats3 and caseous lymphadenitis should be an important health consideration.

The characteristic lesions of caseous lymphadenitis are single or multiple abscesses of the lymph nodes, skin, and viscera.4 The causative organism enters the body through broken or intact skin, via inhalation, or across mucous membranes. Inside the host, the organism spreads via the lymphatics to lymph nodes and internal organs, where abscesses develop during a period of 2 to 6 months. Bacteria are released into the environment through discharge from draining superficial abscesses or are aerosolized from ruptured abscesses in the lungs.5

Treatment and control modalities for caseous lymphadenitis include lancing of abscesses and flushing with potentiated iodine solutions, treatment with antimicrobials, culling of affected animals, surgical removal of abscesses, intralesional injection of formalin, and isolation from other animals to prevent disease spread.4 Lancing and flushing abscesses create a potential hazard for spread of purulent material to fomites and into the environment during the convalescent period. Although antibacterial protocols have been used for pharmacologic treatment of animals with caseous lymphadenitis,5,7 acceptable efficacy has not been determined because the abscesses typically are thickly encapsulated, which hinders penetration of antimicrobials.8 Additionally, because of the organism’s intracellular location, some antimicrobials may not reach efficacious intracellular concentrations. At best, clinicians can hope for a reduction in size of the abscess and nonrecovery of the condition. At worst, the abscesses can rupture and drain, which spreads organisms into the environment and could potentially infect others in the herd. Culling of genetically superior animals is often not a desirable or economically feasible option; howev-
er, retaining these animals in the herd greatly increases the risk of transmission. Although curative in the short term, surgical resection of abscesses does not address recurrence, requires local or general anesthesia, and is a more expensive option than the other reported treatment alternatives. Injection of formalin into the lesions reportedly is beneficial; however, a carcass containing formalin would be considered adulterated and would be unfit for human consumption. The potential for negative public perception related to this practice is also a problem.

Tulathromycin, a newly introduced triamidile antimicrobial, is a member of a subclass of the macrolide family labeled for treatment of cattle and swine with undifferentiated respiratory tract disease. It is highly lipid soluble and, in cattle, maintains concentrations in lung tissue greater than the minimum inhibitory concentration (2.0 µg/mL) for the primary respiratory pathogens for at least 7 days.³ The long-lasting properties and high degree of lipid solubility may allow this drug to enter encapsulated abscesses and achieve adequate intracellular concentrations.

The safety of tulathromycin used in an extralabel manner was investigated in another study¹¹ conducted by our laboratory group. In that study,¹¹ tulathromycin was administered at 25 mg/kg (11.4 mg/lb; 10 times the label dosage) to goats to investigate deleterious effects. No short-term adverse effects were detected in that study. To our knowledge, there have been no reported pharmacokinetic studies conducted in sheep or goats.

The objective of the study reported here was to compare the effectiveness of treatments for small ruminants with caseous lymphadenitis. Specifically, we evaluated the use of opening, flushing, and draining of lesions followed by penicillin administration, closed-system lavage and intralesional administration of tulathromycin, and closed-system lavage and parenteral administration of tulathromycin.

Materials and Methods

Sample population—Client-owned sheep and goats were used in the study. The criterion for enrollment in the study was that an animal had a solitary subcutaneous mass consistent with an abscess. The same animal could be enrolled more than once during the study period when a lesion resolved within 1 month after initial treatment and a new lesion developed in another location. A case was defined as an enrolled study subject whose lesion yielded positive results when cultured for *C pseudotuberculosis*. A study subject could also contribute > 1 case during the study period when a new lesion developed in another location and when that lesion also had positive results for culture of *C pseudotuberculosis*. Owners were required to sign a consent form prior to enrollment of their animals. The consent form and study were approved by the Texas A&M University Clinical Research Review Committee.

Data obtained for all enrolled animals included age, sex, breed, number of days the lesion was evident (ie, detected by the owners) prior to initial examination by the authors, caseous lymphadenitis vaccination status, history of caseous lymphadenitis on the farm, and recent antimicrobial treatments. Physical examinations were performed, and the lesions were described as firm or fluctuant, hair or no hair, and draining or not draining. All lesions were photographed, and the location of each lesion was recorded.

Isolation and identification of *C pseudotuberculosis*—For all sheep and goats enrolled in the study, each lesion was aseptically prepared by clipping the hair from around the lesion, scrubbing the area with betadine solution, and rinsing with isopropyl alcohol. Each lesion was then aspirated with a 16-gauge, 3-cm needle to obtain material for bacterial culture. Samples were inoculated onto 5% sheep blood agar² and MacConkey agar³ and into tryptose broth. The blood agar and tryptose broth were incubated at 37°C in 5% carbon dioxide for up to 72 hours. After incubation for 24 to 48 hours, tryptose broth was subcultured to 5% sheep blood agar and incubated at 37°C in 5% carbon dioxide for an additional 24 to 48 hours (total incubation of 72 hours). Plates containing MacConkey agar were incubated at 37°C in air for up to 72 hours. Suspect colonies were subcultured for purity and confirmed to be *C pseudotuberculosis* by use of a *Corynebacterium* identification system.

Serum hemolysin–inhibition test—Blood samples were obtained from all sheep and goats prior to treatment. Blood samples were collected via jugular venipuncture and used for serologic testing to determine serum hemolysin–inhibition titers. Briefly, the hemolysin inhibition titer was determined by the synergistic action of *Rhodococcus equi* and *C pseudotuberculosis* toxins. Establishing the point at which hemolysis was inhibited was performed on a blood agar plate. Serum samples were placed in 8 wells cut into the plate, which contained 8 different serial dilutions of hemolytic units.

Treatment groups—Sheep and goats were randomly assigned to 1 of 3 treatment groups by use of a block design. For treatment group A, lesions were opened at their most ventral aspect by use of a No. 21 scalpel blade. An elliptic incision was made to remove skin and provide a sufficient opening for drainage. The cavity was drained and flushed thoroughly with diluted betadine solution. A single dose of procaine penicillin G (20,000 U/kg [9,091 U/lb], SC) was administered in the neck region. For treatment group B, lesions were pierced with a 16-gauge, 3-cm needle and filled with saline (0.9% NaCl) solution to break up purulent material. The abscess cavity was then treated with distention lavage, with saline solution used to remove purulent material. A single dose of tulathromycin (2.5 mg/kg [1.14 mg/lb]) was injected into the empty abscess cavity (ie, intralesional administration). For treatment group C, lesions were lavaged with saline solution (similar to the procedure for group B) and a single dose of tulathromycin (2.5 mg/kg, SC) was administered in the neck region. All sheep and goats were scheduled for a reexamination at 1 month after treatment.

Discharge instructions—Sheep and goats were discharged to their owners. Discharge instructions included information on the withholding period for animals because of the extralabel use of tulathromycin (45 days for milk and 36 days for meat) and penicillin (5 days for milk and 10 days for meat), as recommended by...
Follow-up telephone call—Approximately 1 month after initial treatment, owners were contacted by telephone by an interviewer who was not aware of treatment group assignment. Information gathered included whether the lesion had resolved and, if not, whether the lesion was larger, smaller, or had ruptured and drained. During the telephone conversation, the appointment was scheduled for the 1-month reexamination.

Follow-up examination—Approximately 1-month after initial treatment, all sheep and goats enrolled in the study were reexamined. At that time, a lesion was considered unresolved when it was still evident in the same location as that of the initial examination and was the same size, larger, or only slightly reduced in size (≥75% of the original size). Unresolved lesions were aseptically prepared, and material was aspirated for bacterial culture. Unresolved lesions were opened, drained, and flushed in accordance with the protocol established for group A animals. Although these lesions were considered unresolved for their original assigned treatment groups, another follow-up examination performed approximately 1 month after the second treatment was used to ensure resolution of lesions.

Statistical analysis—Data were summarized for the 3 treatment groups by use of descriptive statistics. The Kruskal-Wallis 1-way ANOVA was used to compare medians of quantitative data. Categoric variables were compared among the 3 treatment groups and on the basis of lesion characteristics by use of χ² tests and Fisher exact tests for pairwise comparisons. Treatment effects were evaluated by estimating risk ratios (comparing the proportion of treatment successes within each group) and their corresponding 95% CIs. Sensitivity and specificity of the serum hemolysin-inhibition test were estimated as the proportion of culture-positive and culture-negative animals, respectively, correctly identified by the serologic test. Confidence intervals were calculated for all proportions, and categoric analyses were performed with available software. Analyses of quantitative variables were performed with another program. All analyses were considered significant at values of P < 0.05.

Results

Sample population—During approximately 12 months, 44 animals (41 goats and 3 sheep) with 48 lesions were enrolled in the study. Two goats each represented 2 cases and 1 goat represented 3 cases because they developed lesions in other locations > 1 month apart. Eighteen farms from 2 states were represented; 12 farms each provided > 1 enrolled animal. Fifteen enrolled cases were assigned to group A, 13 were assigned to group B, and 18 were assigned to group C.

Animals ranged from 6 to 96 months of age. Ten were male and 34 were female. Goat breeds represented included Boer (n = 33 goats), La Mancha (3), Nubian (2), and mixed-breed goats (3). The 3 sheep were of the Suffolk breed. The number of days that the lesion was evident (detected by the owners) prior to initial examination by the authors ranged from 1 to 100. Seven goats had been vaccinated against caseous lymphadenitis; vaccinations were administered from 6 months to 1 year before initial examination by the authors. Thirty-five cases originated from farms with a history of caseous lymphadenitis. None of the animals had received antimicrobial treatment prior to enrollment in the study. Signalment and history of caseous lymphadenitis did not differ significantly among treatment groups (Table 1).

Initial physical examination of lesions revealed that 26 were firm and 21 were fluctuant. Thirty-eight lesions were covered with hair, and 9 were considered to have no hair. Six lesions were draining, and 41 lesions were not draining. Physical examination information was not available for 1 lesion in a goat. Lavage of the firm (ie, nonfluctuant) lesions was not as rewarding for the removal of purulent material, compared with removal of purulent material in fluctuant lesions, and the typical volume of material aspirated and removed from the lesions varied widely on the basis of size and maturity of the lesions.

Culture results—Corynebacterium pseudotuberculosis was isolated from 43 lesions. Bacteria isolated from the remaining 5 lesions included Arcanobacterium pyogenes, coagulase-negative Staphylococcus spp, α-hemolytic Streptococcus spp, Pseudomonas spp, and Enterococcus spp.

Results for treatment groups—Of the 43 lesions that yielded C. pseudotuberculosis on bacterial culture, 14 were assigned to group A, 12 were assigned to group B, and 17 were assigned to group C. Examination findings of lesions for the 43 culture-positive cases prior to treatment did not differ significantly among treatment groups (Table 2). No adverse effects were recorded for any sheep or goat during the study period. Additionally, there were no treatment failures recorded by the owners. All 5 lesions that had negative results when cultured for C. pseudotuberculosis were considered resolved at the 1-month reexamination, whereas 37 of 43 (86%) culture-positive lesions were considered resolved. Of the 6 unresolved cases at the 1-month reexamination, 1 had been assigned to group A, 2 to group B, and 3 to group C. The proportions of resolution at the 1-month reexamination for the 43 C. pseudotuberculosis–positive lesions were 92.9% (95% CI, 69.5% to 99.6%), 83.3%...
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...and 82.4% (59.1% to 95.3%) for groups A, B, and C, respectively; these values did not differ significantly ($P = 0.668$) among treatment groups. Resolution of lesions did not differ significantly on the basis of lesion characteristics for firm (83%) versus fluctuant (89%); $P = 0.673$), hair (85%) versus no hair (89%; $P = 1.00$), or draining (100%) versus not draining (83%; $P = 0.569$).

Each of the 2 farms that enrolled the most animals had at least 1 animal that provided multiple cases. One buck from the farm with the highest number of animals enrolled was treated 3 times during a 10-month period (defined as 3 cases for that goat). Initially, this goat was assigned to group C, and the lesion was considered unresolved at the 1-month reexamination. At that time, the lesion was treated in accordance with the protocol for group A, and it subsequently resolved 1 month later. Five and 10 months after initial enrollment, the buck developed lesions in new locations. It was assigned to groups A and C, respectively, and for both of those treatments, lesions were considered resolved by the 1-month reexamination. The farm with the second highest number of enrolled animals had 2 does with lesions that were considered resolved at 1 month and that subsequently developed new lesions 8 months after initial treatment. Both of these does were assigned to group A for the original lesions, but both were assigned to group C for the lesions that subsequently developed in new locations.

**Serum hemolysin–inhibition test**—Antibody titers against *C. pseudotuberculosis* (as determined by use of the serum hemolysin–inhibition test) at initial examination ranged from 0 to 1:64. Of the 43 culture-positive lesions, 35 were in seropositive animals, with titers that ranged from 1:4 to 1:64, whereas 8 were in seronegative animals. Sensitivity for the serum hemolysin–inhibition test was 81% (95% CI, 68% to 91%). For the 5 culture-negative lesions, 3 were in seropositive animals and 2 were in seronegative animals. Specificity for the serum hemolysin–inhibition test was 40% (95% CI, 7% to 82%). Of the 7 animals vaccinated against caseous lymphadenitis, 6 were seropositive (titers ranged from 1:4 to 1:64); 2 of the 6 were culture-negative animals. The remaining vaccinate was a culture-positive animal that was seronegative.

**Discussion**

Animals enrolled in the study reported here were predominately Boer goats. This breed represents most of the goats examined at our hospital. Additionally, this study investigated only 1 form of caseous lymphadenitis. Diagnostics were not performed to determine whether...
any enrolled animals had visceral or internal lymph node involvement. However, those manifestations of caseous lymphadenitis are most often accompanied by a history of chronic weight loss, and none of the animals in this study had that history. Despite these limitations, it appears from our data that intralesional or parenteral treatment with tulathromycin after removal of purulent material via needle distention lavage may be an acceptable alternative to opening, draining, and flushing caseous lymphadenitis abscesses coupled with penicillin administration.

However, the small number of cases in each treatment group in this study did not provide the power necessary to make conclusive statements of efficacy. Additional studies are needed to accurately determine the benefit of various protocols for resolution of caseous lymphadenitis. Although not significantly different, lesions treated in accordance with the protocol for group A had a higher proportion of resolution than for groups B and C combined. Because of its spectrum of activity against gram-positive organisms, administration of penicillin to goats of group A could have contributed to the higher proportion of lesions that resolved. However, we believe that the benefits of not opening these lesions and avoiding spread of bacteria into the environment during the convalescent period outweighed the small differences in outcome among these groups. Thus, we believe that the higher proportion for resolution in group A is not clinically important.

Interestingly, the sensitivity of the serum hemolysin-inhibition test at initial examination was lower than that reported for animals with naturally developing infections, whereas the specificity was higher. False-negative results could have been attributable to the enrollment of animals with chronic, well-encapsulated abscesses that may have had low circulating antibody titers. False-positive results in this study could have been attributable to internal abscesses or exposure of animals to the organism, especially on farms that contributed multiple cases and that had limited biosecurity prior to participation in our study. Also, 2 animals were vaccinated and were seropositive, but their lesions did not yield *C. pseudotuberculosis* isolates.

Although no treatment failures were reported by the owners, it is possible that lesions opened and drained in such a manner that they were not noticed by the owners. Nevertheless, there was only 1 new animal that developed a lesion among all 18 farms represented during the approximately 12-month study period, which indicated that once affected animals within a herd were identified, enrolled, and treated, there was no widespread increase in disease incidence. This suggested that biosecurity measures on those farms were excellent or lesions that resolved did so in an innocuous manner. Similarities in outcome among treatment groups could have been attributable to many factors, most notably the number of days the lesion was evident before initial examination. Additionally, the stage of the lesion at initial treatment may have affected outcome. Lesions that were draining at the time of initial examination would have been more representative of animals in group A (without the betadine flush); therefore, draining lesions assigned to groups B (n = 2 cases) and C (2) may have resolved because they were open and not because of intralesional or parenteral administration of tulathromycin.

It is possible that the physical removal of purulent material via lesion distention lavage with saline solution, as performed in groups B and C, may have positively influenced outcome in these groups. A control group in which lesions were emptied by intralesional lavage but were not treated by administration of tulathromycin may have been an informative addition to the study; however, the willingness of clients to participate may have been compromised.

To our knowledge, minimum inhibitory concentrations of tulathromycin against *C. pseudotuberculosis* isolates in small ruminants are lacking, as are pharmacokinetic data for tulathromycin in goats. Such studies are warranted to determine more precisely the pharmacodynamics of tulathromycin when used to treat animals with caseous lymphadenitis. Other antimicrobials injected intralesionally may also be efficacious and should be examined in future studies.

Overall, it appears that intralesional or parenteral administration of tulathromycin may be an acceptable alternative to opening, draining, and flushing of caseous lymphadenitis lesions. More affected animals from diverse geographic areas and longer follow-up periods are needed in future studies to investigate the effects of tulathromycin treatment for animals with caseous lymphadenitis on the management of this disease in goat herds. Additionally, efficacy of this treatment for other manifestations of caseous lymphadenitis remains to be investigated.

References