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Caprine Abscess Model of Tulathromycin Concentrations in Interstitial Fluid from Tissue Chambers Inoculated with *Corynebacterium pseudotuberculosis* following Subcutaneous or Intrachamber Administration

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Corynebacterium pseudotuberculosis causes chronic, suppurative, abscessing conditions in livestock and humans. We used an *in vivo* model to evaluate antimicrobial efficacy for focal abscesses caused by *C. pseudotuberculosis*. Tissue chambers were surgically implanted in the subcutaneous tissues of the right and left paralumbar fossa of 12 goats to serve as a model for isolated, focal abscesses. For each goat, one tissue chamber was inoculated with *C. pseudotuberculosis*, while the contralateral chamber served as an uninoculated control. Six goats were administered a single dose of tulathromycin at 2.5 mg/kg of body weight subcutaneously, while the other six received the same dose by injection directly into the inoculated chambers. Our objective was to compare the effects and tulathromycin concentrations in interstitial fluid (IF) samples collected from *C. pseudotuberculosis*-infected and control chambers following subcutaneous or intrachamber injection of tulathromycin. In addition, the effects of tulathromycin on the quantity of *C. pseudotuberculosis* reisolated from inoculated chambers were assessed over time. Tulathromycin IF concentrations from *C. pseudotuberculosis*-infected and control tissue chambers were similar to those in plasma following subcutaneous administration. Following intrachamber administration, tulathromycin IF concentrations in infected chambers were continuously above the MIC for the *C. pseudotuberculosis* isolate for 15 days. There were no significant differences for plasma area under the curve and elimination half-lives between subcutaneous and intrachamber administration. Six of the 12 infected chambers had no growth of *C. pseudotuberculosis* 15 days postadministration. Results of this study indicate that tulathromycin may be beneficial in the treatment of focal infections such as those caused by *C. pseudotuberculosis*.

Corynebacterium pseudotuberculosis causes chronic, suppurative, abscessing conditions in sheep, goats, horses, cattle, and humans. In humans, *C. pseudotuberculosis* most often manifests as suppurative, necrotizing, granulomatous lymphadenitis (1, 2). In livestock species, the characteristic lesions are described as caseous lymphadenitis (CL) and are single or multiple abscesses of the lymph nodes, skin, and viscera (3). 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 lymphatic system 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 (3). 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 (4). One report examining samples from culled sheep from regions representing 9 western U.S. states estimated the prevalence as 42.41% (5). More recently, our investigation of 3 large sheep and goat herds in West Texas that had CL control measures in place prior to our study revealed an initial culture-positive prevalence as high as 6.15% (6). The high prevalence of CL in small ruminant populations around the world and the production and animal losses associated with the disease have led to consideration of multiple modes of control and treatment. These studies have highlighted the potential usefulness of CL as a model for other

abscessing bacterial pathogens. Goats are an ideal animal model from the perspective of size, ease of husbandry in confinement, and availability. *C. pseudotuberculosis* is an ideal pathogen for the study of abscess formation, because it leads to the creation of thickly encapsulated sites of infection essentially walled off from the body and can reside in host cells, thereby creating an infection difficult to reach effectively by some antimicrobials (3).

In sheep and goats, attempts at treatment and control of CL have included lancing of peripheral abscesses and flushing the newly opened abscess with potentiated iodine solutions, systemic antimicrobial treatment, surgical removal of abscesses, intralesional injection of formalin, isolation of affected animals from other animals to prevent disease spread, and culling affected animals (3, 7). Although antibacterial protocols have been used for pharmacologic treatment of animals with CL (7), clinical resolution of lesions has not been consistently achieved. It is not clear if the lack of antimicrobial efficacy is related to ineffective bacterial
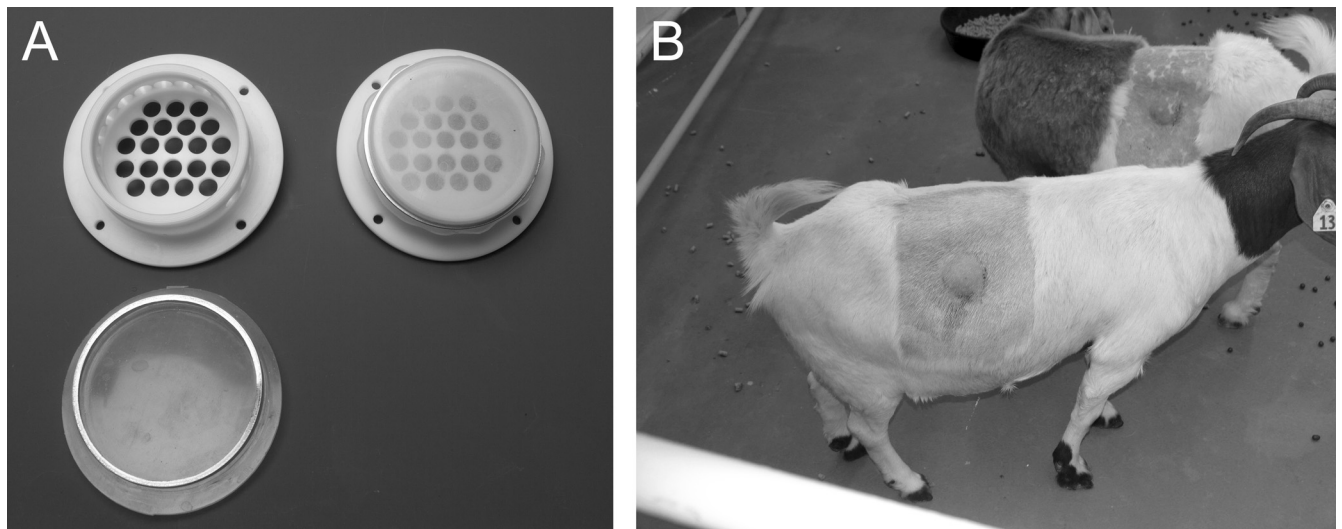
FIG 1 (A) Unassembled and assembled tissue chambers to be surgically implanted into the paralumbar fossa of goats; (B) surgically implanted tissue chambers in the right and left paralumbar fossa serving as an abscess model.

growth inhibition or killing, inactivation of drug in purulent material in abscesses, or bacterial virulence factors that protect the organism *in vivo*. Similar failures in antimicrobial treatment of abscesses in people have been noted. This is thought to be due to the development of isolated cores of infection unreachable by some antimicrobials (1, 8). Further, it has been proposed that phagocytic activity of macrophages and polymorphonuclear leukocytes within abscesses becomes impaired and may lead to ineffective resolution of infection (9).

Newly available, lipid-soluble, macrolide antimicrobial drugs have high volumes of distribution, suggesting good tissue penetration (10). Tulathromycin is a triamilide antimicrobial in the macrolide family and is currently approved at 2.5 mg/kg of body weight for cattle and swine respiratory disease. In cattle, tulathromycin maintains concentrations in lung tissue greater than the MIC ($MIC_{90} = 2.0$ µg/ml) for the primary respiratory pathogens for at least 7 days following a single injection (10). The long-lasting properties of this formulation of the drug and its high degree of lipid solubility may allow it to enter encapsulated abscesses and achieve adequate intracellular concentrations. In our laboratory, we demonstrated that systemic tulathromycin was as effective as traditional lancing and flushing in resolving CL lesions in sheep and goats (7), suggesting that tulathromycin could penetrate into abscesses to therapeutic concentrations.

However, we did not investigate concentrations of tulathromycin reached within active lesions in either of these studies. To date, none of the studies using systemic or intralesion antimicrobials for CL has described drug concentrations within lesions or demonstrated whether tulathromycin retains antimicrobial activity *in vivo* in abscessed purulent material. The pharmacokinetics of this drug in goats have been recently described (11, 12). The antimicrobial susceptibility of *C. pseudotuberculosis* to tulathromycin has not been reported, although we found that the MIC_{90} of 35 clinical case-derived isolates was 2 µg/ml, and 71% of these isolates had an MIC of <1 µg/ml (K. E. Washburn, M. Libal, unpublished data).

The objectives of this study were to quantify tulathromycin interstitial fluid (IF) concentrations in samples taken from infected and noninfected tissue chambers after systemic administration or direct injection of tulathromycin into the infected chambers, to document bacterial counts within the infected chambers following tulathromycin administration, and to evaluate whether tulathromycin maintained antimicrobial activity within infected chambers after direct injection into the tissue chambers. An additional objective of this study was to histologically evaluate the infected tissue chambers and environs to verify the appropriateness of the model for studying abscessing conditions.

MATERIALS AND METHODS

Animals. Twelve clinically healthy, adult, Spanish-crossbred, female goats obtained from a commercial source (mean weight = 85 kg) were enrolled. Although not tested serologically for antibodies to *C. pseudotuberculosis*, these goats were examined clinically and found to be free of any lesions suggestive of CL. The goats were housed in a large outdoor paddock prior to implantation with tissue chambers. After implantation, goats were housed indoors in a biosafety level 2-approved facility. Goats were fed free-choice Bermuda grass hay and a pelleted ration during the study. All goats were examined daily throughout the study for signs of illness and surgical site infections or dehiscence. This study was approved by the Texas A&M University Institutional Animal Care and Use Committee as well as the Institutional Biosafety Committee (AUP 2010-82).

Tissue chambers. Tissue chambers were assembled as previously described (13). Briefly, chambers were constructed of Delrin thermoplastic (Professional Plastics, Inc., Fullerton, CA) which measured 4.6 cm for the internal diameter, 5.2 cm for the outside diameter, and 1.5 cm in depth. Thirty-seven perforations measuring 0.6 cm in diameter were distributed over the base and walls of the chamber cup, leaving an area within the base free of perforations to allow a reservoir of fluid to accumulate in the chamber *in vivo*. This reservoir volume was 24.93 ml. A silicone rubber membrane (Professional Plastics, Inc., Fullerton, CA) covered the top of the chamber and was secured in place by a loop of stainless steel surgical wire around the rim of the cup (Fig. 1A). The assembled chambers were packaged individually and steam sterilized prior to implantation.

Surgical implantation of tissue cages. One tissue chamber was surgically implanted into each paralumbar fossa of the goats. The paralumbar fossa was clipped and cleaned, and the region was anesthetized with a

TABLE 1 Histopathology scoring system for subcutaneous tissues from adult goats experimentally infected with *C. pseudotuberculosis*

Score	No. of immature granulation tissue strata	No. of mature granulation tissue strata	Necrotic zone thickness	Inflammation	Exudate in cavitary lumen	No. of PMN per ×400-magnified field	No. of monocytes/macrophages per ×400-magnified field
0	Not present	Not present	Not present	Not present	Not present	<5	<20
1	<2	<2	Thin	Minimal	Minimal accumulation	6–50	21–50
2	3–5	3–20	Thick	Moderate	Moderate accumulation	51–100	51–100
3	6–10	21–50	Very thick	Severe	Severe accumulation	101–200	101–200
4	>10	>50	Massive	Massive	Massive accumulation	>200	>200

proximal paravertebral nerve block using 2% lidocaine. The skin was aseptically prepared for surgery using an iodine scrub. A vertical skin incision was made in the approximate center of the paralumbar fossa starting from approximately 3 cm ventral to the transverse processes of the lumbar vertebrae and extending ventrally approximately 10 cm. The skin cranial to the incision was undermined to create a pocket for the chamber. The chamber was placed into the pocket just under the skin and secured with 4 stay sutures to the subcutaneous tissue. The chamber was seated so that the areas without holes were situated ventrally to create an area for a fluid reservoir from which to sample. The skin incision was closed with a simple continuous suture pattern and allowed to heal for 2 weeks prior to challenge (Fig. 1B).

Preparation of inoculum and MIC determination. An isolate of *C. pseudotuberculosis* derived from a clinical case of caprine caseous lymphadenitis was used throughout. The MIC of tulathromycin for this isolate was determined using a commercially available panel of antimicrobial agents following the broth microdilution method of susceptibility testing established by the Clinical and Laboratory Standards Institute (CLSI) document M31-A3. The antimicrobial susceptibility was evaluated at 24 and 48 h following inoculation. The MIC was 2 μg/ml at both time points. Prior to inoculation of tissue chambers, the isolate was cultured on Trypticase soy agar supplemented with 5% sheep's blood (TSA) at 37°C in an atmosphere of air with 5% carbon dioxide (CO_2) for 48 h. Plates were routinely incubated for 48 h, because *C. pseudotuberculosis* grows slowly and colonies of this organism are typically pin point in size at 24 h. At 48 h, a characteristic colony was selected and used to inoculate brain heart infusion broth (BHIB; Becton, Dickinson and Company, Sparks, MD, USA). The broth was incubated overnight at 37°C on an orbital shaker at 250 rpm (Innova 40; New Brunswick Scientific, Enfield, CT, USA). The concentration of bacteria was determined spectrophotometrically (Genesys 10; Thermo Fisher Scientific, Pittsburgh, PA, USA) at an optical density of 600 nm. Bacteria were diluted to a concentration of 8×10^6 CFU/ml in BHIB. Sterile BHIB from the same lot that was used to culture *C. pseudotuberculosis* was used to inoculate control chambers.

Inoculation of chambers and sample collection. Prior to inoculation and sample collection from the tissue chambers, the skin over the chamber was scrubbed with iodine and rinsed with alcohol. A 3-ml syringe with a 1-inch, 18-gauge needle was used to inject 1 ml of the *C. pseudotuberculosis* inoculum or sterile BHIB as a negative control into each of the predesignated chambers. The needle was inserted through the skin covering the chamber and was directed ventrally into the region corresponding with the reservoir for fluid. For IF sampling of chambers, the same-sized needle and syringe were used to aspirate 2 ml (approximately 8% of reservoir volume) IF from the chambers and into red-topped tubes. Plasma was collected by jugular venipuncture using a Vacutainer (Becton, Dickinson and Company, Sparks, MD, USA) needle directly into heparinized blood tubes for determination of drug concentrations.

Experimental design. Tissue cages were surgically implanted at least 14 days prior to inoculation of bacteria to allow healing of incision sites. Each goat had two chambers, one serving as an uninoculated control and the other inoculated with *C. pseudotuberculosis*, with the side of *C. pseudotuberculosis* inoculation being determined randomly. On day −6, the tissue chambers of each goat were injected with either BHIB (control) or

C. pseudotuberculosis (infected), according to assignments of control or *C. pseudotuberculosis* inoculated. Seven goats had the chambers on the right side inoculated with *C. pseudotuberculosis*, while the left-side chambers served as controls. Five goats were inoculated with *C. pseudotuberculosis* in the left-side chambers, with the right-side chambers serving as controls. On day 0, just prior to injection of tulathromycin, 2 ml of IF was collected from all chambers for culture and drug concentration determination to confirm that chambers were either truly "infected" or "noninfected" according to their designation and free of tulathromycin. As determined by coin flip, 6 of the 7 goats with right-side chamber infection had tulathromycin at 2.5 mg/kg administered directly into the *C. pseudotuberculosis*-infected chamber, while the remaining goat with right-side chamber infection and all 5 goats with left-side chamber infection were given tulathromycin at 2.5 mg/kg subcutaneously (s.c.) in the neck. In summary, there were 6 goats administered tulathromycin directly into the infected chamber (intrachamber [i.c.]), and 6 were administered tulathromycin s.c. At 24, 48, 72, 288, and 360 h after tulathromycin administration, IF was obtained from all chambers for culture and drug concentration analysis, and plasma was obtained from all goats. Goats were humanely euthanized immediately after collection of the 360-h-postadministration samples by first being anesthetizing with xylazine HCl at 0.1 mg/kg and ketamine at 3.0 mg/kg intravenously (i.v.) and then being euthanized with a supersaturated solution of magnesium sulfate i.v. to effect. The left and right subiliac lymph nodes from all goats were harvested for bacterial culture and histopathological examination. All chambers were removed to assess surrounding tissues by gross and histopathological examination to compare infected and control chamber implantation sites.

Culture of cage fluid samples and lymph nodes. At each sampling time point, quantified cultures were performed on collected IF by making serial dilutions in phosphate-buffered saline (PBS) and plating to TSA plates or using calibrated inoculating loops to inoculate TSA plates. Plates were incubated in an atmosphere of air with 5% carbon dioxide (CO_2) for 48 h, and colonies were enumerated by counting. In addition to quantified cultures, samples were directly plated to TSA plates using a swab. Swabs of lymph node tissue were used to inoculate TSA plates. These plates were incubated as described before. Results from lymph node tissue were recorded as positive or negative.

Assay for tulathromycin concentrations in plasma and tissue chamber interstitial fluid samples. Goat plasma and IF samples were analyzed for tulathromycin concentrations using an ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) assay that was originally established using bovine and porcine plasma samples (14) and has been recently described for goat plasma samples (15). Interstitial fluid was centrifuged prior to extraction, and several samples required extensive dilution to bring them within the standard curve range. Injections were made with a UPLC system (Waters, Milford, MA), and analytes were separated on an Ace C8, 2.1- by 50-mm, 3-μm column (MacMod, Chadds Ford, PA). A Thermo TSQ Quantum Discovery Max (Thermo Electron, West Palm Beach, FL) tandem quadruple mass spectrometer with a heated electrospray ionization source, operated in the positive ion mode, was used for detection. For IF, the limit of detection (LOD) was 2.9 ng/ml, and the limit of quantification (LOQ) was 9.7 ng/ml. For plasma, LOD was 0.2

TABLE 2 Histopathology scoring system for lymph nodes from adult goats

Score	State of activation	Marginal sinus thickness	No. of germinal centers per ×40-magnified field	Paracortex expansion	No. of PMN per ×400-magnified field
0	None	None	<5	No expansion	<5
1	Slight	Thin	6–10	Slight expansion (1–25%)	6–50
2	Moderate	Thick	11–15	Moderate expansion (25–50%)	51–100
3	Marked	Very thick	15–20	Marked expansion (50–75%)	101–200
4	Extensive	Extensive	>25	Extensive expansion (75–100%)	>200

ng/ml and LOQ was 1.6 ng/ml. Interassay variability was 5.4% for IF and 7.6% for plasma, as measured by relative standard deviation. Recovery was 100.5% and 99.5% for IF and plasma, respectively.

Pharmacokinetic analysis. Noncompartmental analysis of tulathromycin concentrations in plasma after s.c. or i.c. tulathromycin administration was performed, and two-tailed t tests were used to compare apparent elimination half-life and area under the concentration-time curve from 0 to infinity ($AUC_{0-\infty}$) by route of administration. Uninfected control tissue chamber IF concentrations of tulathromycin after both routes of administration were compared graphically.

Histopathology on lymph nodes and tissues surrounding tissue cages. Tissue from the surface of the tissue cage closest to the body wall and furthest from the skin was collected from each tissue chamber and fixed in freshly made 10% buffered formalin. Additionally, the draining lymph node from each side was collected. All tissues were processed by standard procedures for paraffin embedding, sectioned at 5-mm thickness, stained with hematoxylin and eosin, and examined by light micros-

copy by a veterinary pathologist who performed a blinded evaluation using the scoring systems in Tables 1 and 2. Subcutaneous tissues were evaluated for the thickness of the immature granulation tissue strata, mature granulation tissue strata, and necrotic zone. In addition, inflammation, presence of exudate in the cavitary lumen, and number of polymorphonuclear neutrophilic leukocytes per ×400-magnified field and number of monocytes and macrophages per ×400-magnified field were evaluated. Lymph nodes were evaluated for state of activation, thickness of the marginal sinus, number of germinal centers per ×40-magnified field, thickness of the paracortex, and number of polymorphonuclear leukocytes per ×400-magnified field.

RESULTS

Tulathromycin concentrations, pharmacokinetic parameters, and tissue cage fluid and lymph node culture results. Concentrations of tulathromycin in plasma following s.c. or i.c. administra-
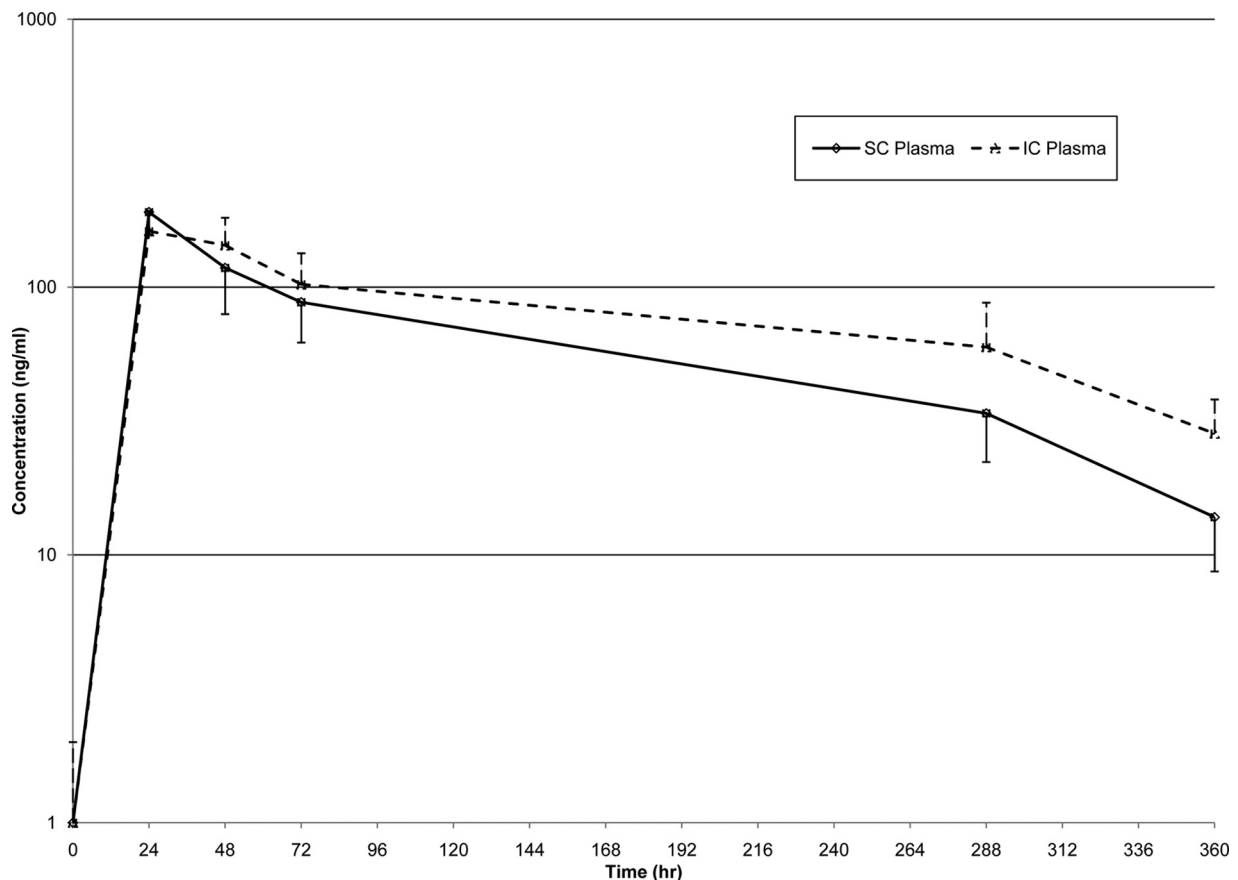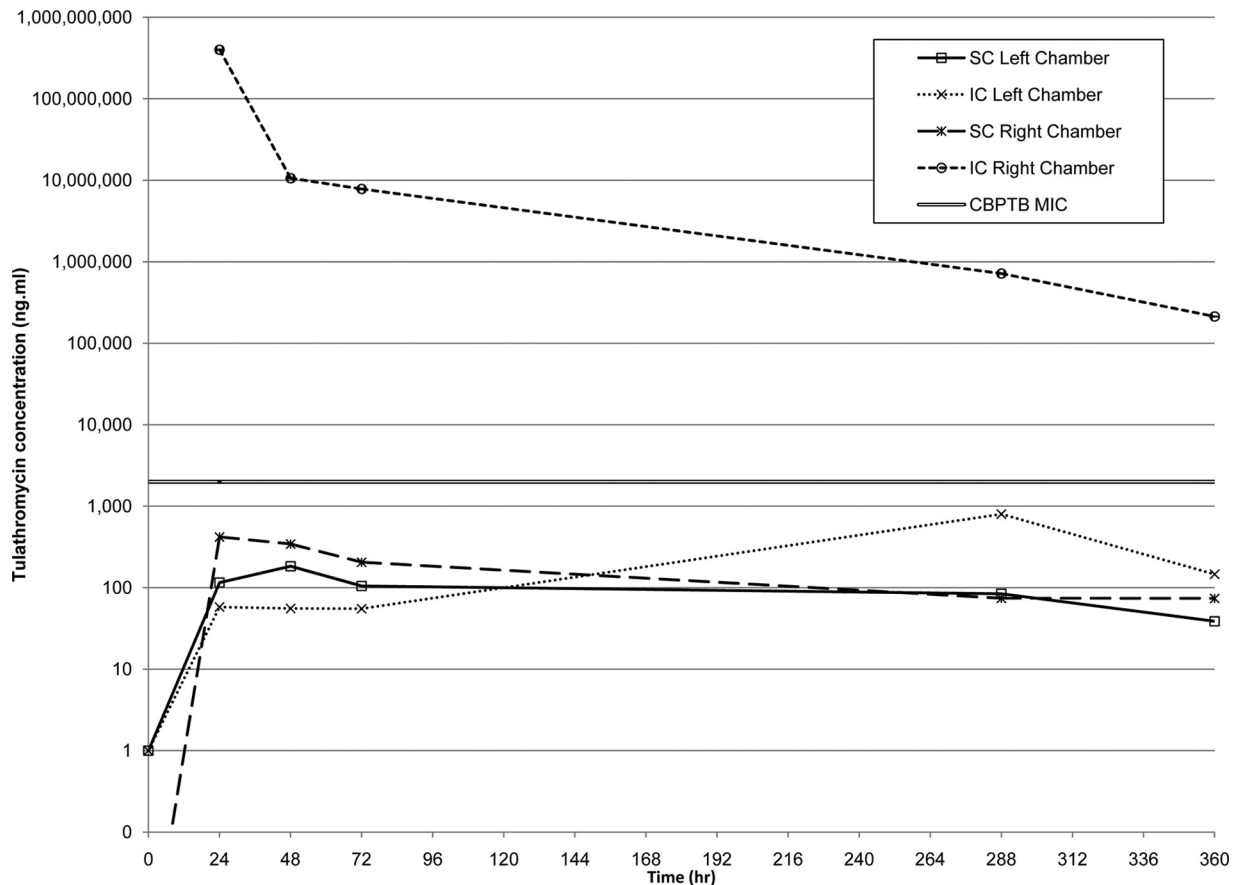

FIG 2 Tulathromycin concentrations in plasma samples drawn from adult goats following subcutaneous administration ($n = 6$; SC plasma) or intrachamber ($n = 6$; IC plasma) injections of 2.5 mg tulathromycin/kg body weight (error bars are standard deviations).

FIG 3 Tissue chamber interstitial fluid tulathromycin concentrations following subcutaneous (*n* = 6 goats; SC) or intrachamber (*n* = 6 goats; IC) administration of 2.5 mg/kg tulathromycin to adult goats. There were 6 animals sampled for all routes and sampling sites, with the exception of s.c. right chamber, for which one outlier was removed due to unexpectedly high concentrations. All but one animal was infected on the left side prior to s.c. drug administration, and all animals were infected on the right side prior to i.c. drug administration. CBPTB, *Corynebacterium pseudotuberculosis*. The horizontal line represents the MIC of the strain of *C. pseudotuberculosis* used to induce infection.

tion are depicted in Fig. 2. Figure 3 shows tulathromycin IF concentrations from *C. pseudotuberculosis*-infected and control tissue cage chambers. Following s.c. or i.c. administration, tulathromycin IF concentrations were detectable in samples from *C. pseudotuberculosis*-infected and control chambers throughout the study. Specifically, within 24 h following i.c. administration into *C. pseudotuberculosis*-infected chambers, tulathromycin IF concentrations were above the MIC for the *C. pseudotuberculosis* isolate used and remained so until the end of the study (Fig. 3).

The MIC for the isolate used in this study for tulathromycin was 2 μg/ml. Concentrations of tulathromycin in plasma and IF from control tissue cages following s.c. and i.c. administration are displayed in Fig. 4 and 5, respectively. Even though tulathromycin was injected into *C. pseudotuberculosis*-infected tissue cages on the right side, tulathromycin IF concentrations were detectable from left-side control tissue chambers. Pharmacokinetic parameters of tulathromycin administered s.c. or i.c. using a noncompartmental analysis were estimated (Table 3). Data from one chamber were not included in the final analysis because of lack of sufficient IF volume from the tissue cage at two time points. No significant difference was found between $AUC_{0-\infty}$ and apparent elimination half-life following s.c. or i.c. administration.

At time zero, just prior to administration of tulathromycin, all

IF samples from control chambers were negative for *C. pseudotuberculosis* growth, while all IF samples from *C. pseudotuberculosis*-inoculated chambers were culture positive and designated "infected." Interstitial fluid samples from uninfected control chambers remained negative for growth of *C. pseudotuberculosis* throughout the study. Figure 6 shows the change in *C. pseudotuberculosis* CFU over time following the administration of tulathromycin either s.c. or via i.c. injection. At the end of the study (time 360 h), 6 of the 12 initially infected tissue chambers were positive for *C. pseudotuberculosis* growth: 3 for each route of tulathromycin administration. Six of the 24 lymph nodes harvested were positive for *C. pseudotuberculosis* culture at time 360 h. Of these 6 lymph nodes, 5 were positive on the same side as the initially infected tissue chamber, while interestingly, 1 was on the side opposite of the initially infected chamber. Of further interest, 3 of these lymph nodes were culture positive despite having no remaining growth in the initially infected tissue chambers at this time point. Two of the three lymph nodes that were positive despite no growth in the tissue cages were from animals administered tulathromycin via i.c. injection.

Gross and histopathology examination of tissue cages and lymph nodes. Two weeks following chamber implantation, the incision sites of one chamber in three different goats partially de-
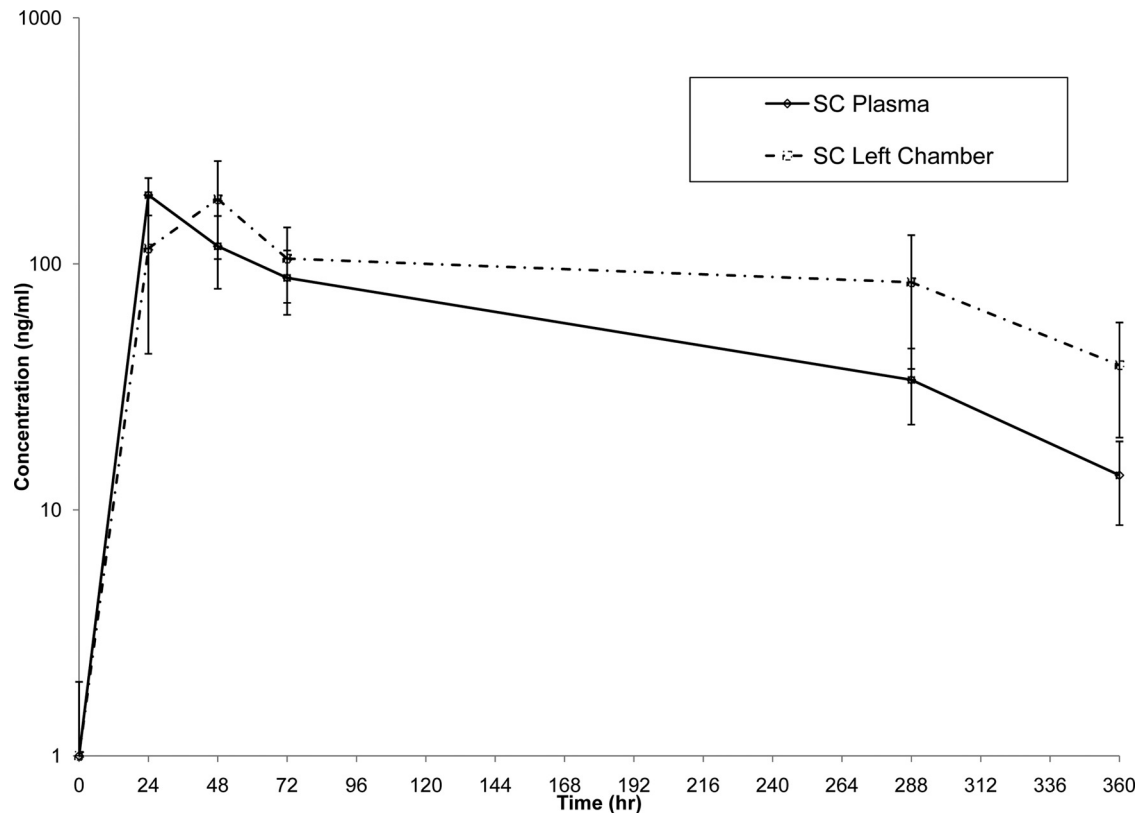
FIG 4 Concentrations of tulathromycin in plasma and interstitial fluid collected from uninfected tissue chambers after adult goats ($n = 6$) were injected with tulathromycin at a dose of 2.5 mg/kg subcutaneously (SC) (error bars are standard deviations).

hisced. These were resutured under local anesthesia prior to the start of the study and prior to inoculation with either BHIB (control) or *C. pseudotuberculosis* (infected). One of these chambers was subsequently designated for *C. pseudotuberculosis* inoculation and became problematic from sample time 48 h until the end of the study, making sufficient sample volume collection difficult for drug analysis due to the thick purulent debris and fibrin proliferation within the chamber. However, samples from this chamber were available for the 360-h bacterial culture. The goat with this chamber received tulathromycin subcutaneously.

The infected tissue chambers were encapsulated by thick fibrous connective tissue and filled with thick purulent material upon gross postmortem examination. Gross appearance of the tissue surrounding the tissue chamber was similar to that which occurs in a naturally occurring CL abscess. Uninfected control chambers lacked purulent material, and the tissue underlying the tissue cage was less fibrous than that surrounding infected chambers, except in one goat that received i.c. administration of tulathromycin. In that goat, there was no apparent gross difference in the tissues underlying the infected and uninfected control chambers even though the control chamber was culture negative for *C. pseudotuberculosis*; however, culture of the tissues surrounding the control chamber revealed a polymicrobial infection that included both Gram-negative and Gram-positive organisms. Samples of the tissue surrounding the tissue chamber and the draining lymph nodes were collected from each paralumbar fossa and processed as described above. Histopathologic examination of the subcutaneous tissues medial to both infected and uninfected

chambers revealed the presence of mature granulation tissue and formation of chronic sinus tracts. Formation of foreign body giant cells was present in tissue surrounding both infected and uninfected tissue chambers in one animal, which is consistent with the presence of a foreign body (e.g., tissue cage). The scores for each feature evaluated in the scoring system were combined for a total score for the tissue and evaluated for statistical significance using Student's *t* test. Draining lymph nodes and tissues surrounding infected chambers had significantly higher combined histopathology scores than those associated with uninfected chambers both for goats treated with i.c. injection ($P < 0.05$) and those treated s.c. ($P < 0.01$) (Fig. 7). Moderate to severe inflammation was present in tissues surrounding infected chambers compared to the minimal to absent inflammation surrounding uninfected chambers. Exudate was minimal to moderate in tissue around infected chambers and absent in tissue surrounding all but two of the 12 uninfected chambers. Inflammatory cells, including polymorphonuclear cells (PMNs), monocytes, and macrophages, were largely absent in both the tissues and lymph nodes associated with uninfected chambers but were significantly increased in the tissues associated with infected chambers. Lymph nodes draining infected chambers exhibited moderate to marked activation and moderate to marked paracortex expansion, and the thickness of the marginal sinuses were thick to very thick compared to those of lymph nodes draining uninfected chambers, which exhibited only slight activation and slight paracortex expansion and had thin marginal sinuses.
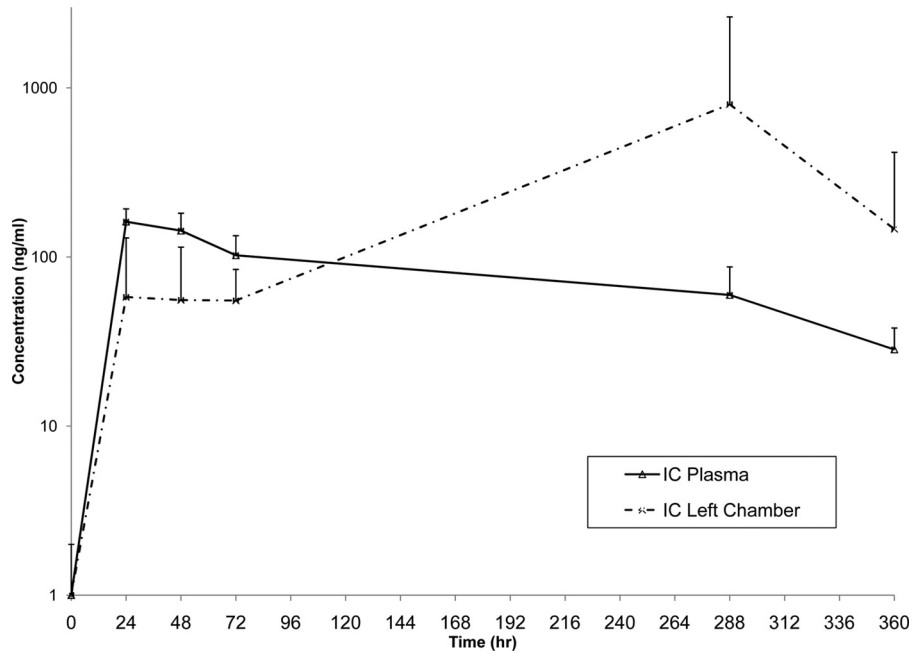
FIG 5 Concentrations of tulathromycin in plasma and interstitial fluid collected from uninfected tissue chambers from adult goats ($n = 6$) after direct injection of 2.5 mg/kg body weight of tulathromycin into surgically implanted infected tissue chambers in the paralumbar fossae of adult goats (error bars are standard deviations).

DISCUSSION

To investigate the ability of tulathromycin to penetrate abscesses and subsequently inhibit bacterial growth, we used a subcutaneous tissue chamber model of *C. pseudotuberculosis* infection to mimic lymph node abscessation. The chambers allowed inoculation for a localized infection as well as sampling for bacterial and drug concentrations after systemic tulathromycin administration. While primarily used for pharmacokinetic studies examining various drugs in tissue, tissue cages have also been used extensively to mimic secluded infection sites in many species. In rodents and rabbits, tissue chambers have been used to model infection by difficult-to-treat abscess-forming bacteria (8, 16), and in calves they have been used to model the effect of focal inflammation on

drug pharmacokinetics (17). To answer the question of whether or not drug in an abscess would be effective, this model allowed direct injection of tulathromycin into infected tissue chambers, so the issue of drug penetration would be removed and the effectiveness of tulathromycin on bacterial growth within a localized infection could be evaluated.

Results of our study indicate that tulathromycin reaches and maintains concentrations within an isolated site of infection following a single intrachamber administration well above the MIC of *C. pseudotuberculosis*. These findings, along with no significant differences between s.c. and i.c. injection $AUC_{0-\infty}$ and apparent elimination half-life, indicate that tulathromycin is distributed to encapsulated areas and therefore quite possibly also into abscesses.

TABLE 3 Pharmacokinetic parameters based on noncompartmental analysis of time versus tulathromycin concentration data from goats that were administered tulathromycin at 2.5 mg/kg either s.c. ($n = 6$) or i.c. ($n = 6$)

Parameter[c]	Unit	s.c. plasma Average	SD	s.c. left side (no. 21 removed)[b] Average	SD	s.c. right side (no. 21 removed)[b] Average	SD	i.c. plasma Average	SD	i.c. right side[a] Average	SD
λ_z	/h	0.006	0.001	0.004	0.003	0.007	0.007	0.004	0.002	0.011	0.003
Half-life (λ_z)	h	115	25	417	511	244	218	198	102	68	19
T_{max}	h	24	0	34	13	29	11	28	10	32	12
C_{max}	ng/ml	191	33	169	33	12,231	26,189	176	35	4.02E8	9.37E8
AUC_{INF}	ng · h/ml	25,728	5,219	30,715	9,016	1.27E6	2.70E6	38,488	13,177	1.09E10	2.23E10
AUC_{INF} % extrapolated	%	9	4	34	25	26	26	22	12	1	1
$AUMC_{INF}$	ng · ng · h/ml	3.90E6	1.33E6	4.79E7	7.91E7	9.49E7	1.51E8	1.10E7	9.97E6	3.53E11	5.53E11
MRT_{INF}	h	149	31	556	668	327	294	258	122	64	20

[a] The only infected chambers injected were on the right side; therefore, the left-side chamber parameters are not depicted.
[b] No. 21 was eliminated from the calculation of parameters because two data time points were missing due to insufficient sample.
[c] λ_z, terminal elimination rate constant; T_{max}, time to maximum concentration of drug in serum; C_{max}, maximum concentration of drug in serum; AUC_{INF}, area under the concentration-time curve extrapolated to infinity; $AUMC_{INF}$, area under the moment curve extrapolated to infinity; MRT_{INF}, mean residence time extrapolated to infinity.
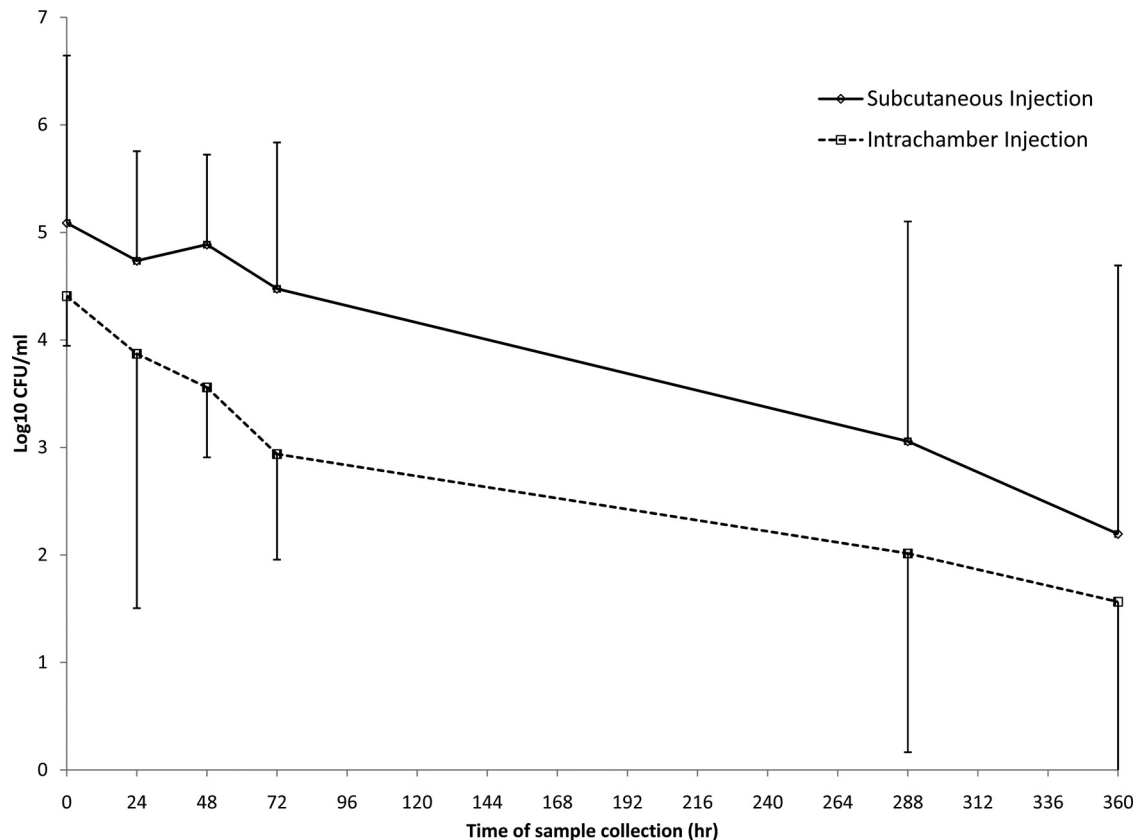
FIG 6 CFU of *Corynebacterium pseudotuberculosis* over time. Samples were drawn from surgically implanted infected tissue chambers following administration of 2.5 mg/kg tulathromycin subcutaneously ($n = 6$) or via intrachamber injection ($n = 6$) to adult goats (error bars are standard deviations).

Tulathromycin injected directly into the infected chambers had antimicrobial properties resulting in no growth at 360 h postadministration in 3 of the 6 infected chambers. Subcutaneous administration also resulted in no growth at 360 h postadministration in 3 of the 6 infected chambers despite the fact that IF concentrations of tulathromycin did not reach the MIC of the isolate used in this study. While numbers of CFU following either subcutaneous or intrachamber administration appeared to decline over the course of the study, it is unknown why some chambers remained positive for growth even though IF concentrations of tulathromycin remained above the MIC for the isolate in the chambers injected directly. Achieving *in vivo* antimicrobial concentrations above the *in vitro* MIC does not guarantee clinical resolution, as a variety of other factors play a role in resolving infections, including host immune status, pathogen virulence factors, and the microenvironment of the site of infection. Even though IF tulathromycin concentrations were above the MIC, it is possible that the drug was not uniformly distributed in the chamber, as tulathromycin is known to concentrate in leukocytes. While this would likely result in enhanced bacterial killing over time, it is possible that release of tulathromycin from leukocytes resulted in concentrations of available tulathromycin that were periodically below the MIC or that a population of *C. pseudotuberculosis* remained extracellular or otherwise protected from exposure to the drug. Additionally, tulathromycin is a bacteriostatic drug, and although it impairs bacterial protein expression, extended exposure may be required for clinical resolution of lesions.

It is possible that chambers that remained positive for bacterial growth would have become negative for culture had the study continued beyond 360 h.

As stated previously, the authors have had some success treating naturally occurring CL with tulathromycin injected directly into abscesses or subcutaneously as a single dose. In the present study, IF concentrations of tulathromycin following intrachamber administration were above the MIC for most of the isolates of *C. pseudotuberculosis* that we encounter clinically, and the drug maintains antimicrobial activity, suggesting that our therapeutic approach may have clinical application. However, past experience with this therapy indicates relapses do occur in some patients, and some cases do not respond. The results of our study may partially explain these clinical failures, because some chambers maintained bacterial growth even when seemingly therapeutic drug concentrations were present. Reasons for this may include resistant subpopulations, reinfection, or possibly incomplete elimination of the organism from the body. In this study, 6 of the 24 subiliac lymph nodes harvested were positive for culture of *C. pseudotuberculosis* at time 360 h. Interestingly, 1 positive lymph node was on the contralateral side of the infected chamber, and 3 nodes were culture positive even though there was no growth in the infected chamber at time 360 h. This suggests that complete elimination of the organism from the animal may not always occur even though superficial lesions resolve. This would explain what we have observed clinically following therapy when lesions of CL recur in treated cases sometimes long after they
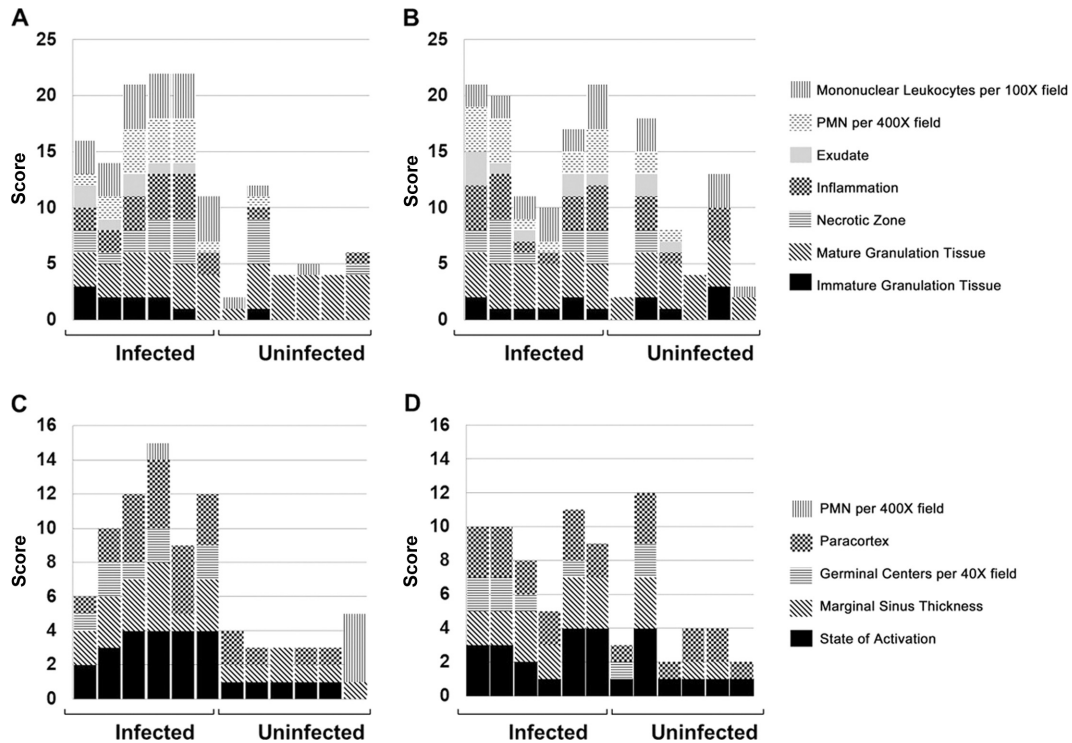
FIG 7 Summary of blinded histopathology scores for *Corynebacterium pseudotuberculosis*-inoculated and uninoculated control chamber-associated subcutaneous tissues and draining lymph nodes from adult goats. Pathological changes in the subcutaneous tissues (A and B) and draining lymph nodes (C and D) were determined 14 days after administration of 2.5 mg/kg tulathromycin subcutaneously (A and C) or via intrachamber injection (B and D). Total bar height represents the total score of an individual tissue.

have seemingly resolved. Even though we investigated only single-dose therapy, treating cases of CL with more than one injection at weekly intervals may improve results. It is also possible that treating cases of CL with both subcutaneous and intralesional tulathromycin would raise concentrations in the lesions above the MIC further. Although complete elimination of the organism from infected tissue chambers and draining lymph nodes did not occur in this study in all animals, there is evidence that treatment was beneficial in most by either eliminating bacterial growth inside the chambers or reducing numbers of CFU. Tulathromycin injected systemically penetrated tissue cages, suggesting this drug could be evaluated for other diseases characterized by abscessation and *C. pseudotuberculosis* in other species. Further studies are required to investigate the use of multiple doses of tulathromycin in cases of CL in goats, as this may lead to enhanced elimination of the organism and an improved clinical outcome. Additionally, this abscess model could be used to determine if other antimicrobials with a low MIC for *C. pseudotuberculosis* are capable of penetrating tissue cages in a similar manner.

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