Serologic and bacteriologic culture prevalence of Corynebacterium pseudotuberculosis infection in goats and sheep and use of Bayesian analysis to determine value of assay results for prediction of future infection

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Objective—To determine the serologic and bacteriologic culture prevalence of Corynebacterium pseudotuberculosis infection in sheep and goats and the value of such assays for prediction of future development of caseous lymphadenitis (CL).

Design—Observational study.

Animals—919 goats and sheep in 3 herds in southwest Texas.

Procedures—During an initial evaluation, serologic and bacteriologic culture status for CL was determined for all animals. Subsequently, animals were evaluated every 6 months for a 13-month period to detect external CL lesions. Affected animals in 2 herds were treated with tulathromycin or a control treatment; affected animals in 1 herd were culled. The value of assays for prediction of future development of CL lesions was determined.

Results—The serologic prevalence of CL in herds at the start of the study ranged from 7.52% to 69.54%. The bacteriologic culture prevalence of CL ranged from 0% to 6.12% at the start of the study and 0% to 9.56% at the end of the study. Synergistic hemolysin inhibition results were poor predictors of future development of CL lesions in animals during the study period; however, animals with positive bacteriologic culture results for CL were more likely to develop lesions in the future than were animals with negative bacteriologic culture results.

Conclusions and Clinical Relevance—Caseous lymphadenitis was detected in animals in this study despite prior management of affected animals in herds via culling. Use of a synergistic hemolysin inhibition test for management of CL may cause unnecessary culling of animals; treatment might allow retention of genetically valuable CL affected animals in a herd without substantially increasing the prevalence of CL. (J Am Vet Med Assoc 2013;242:997–1002)

Caseous lymphadenitis is an important disease of sheep and goats, and results of a study published in 1997 indicate this disease is the third most common cause of economic loss in the sheep industry. Results of another study in which culled sheep from 9 western US states were examined indicate the prevalence of CL in sheep is 42.41%. To the authors’ knowledge, few studies have been conducted to determine the prevalence of CL and the usefulness of the test for CL control programs; results of that study indicate the SHI test does not have high specificity for identification of animals with CL, which could result in inaccurate determination of the prevalence of CL and culling of genetically valuable seropositive animals that would not have developed active CL lesions (ie, lesions in which C. pseudotuberculosis is growing).

Various techniques have been used for management of CL in large herds or flocks of animals including testing and elimination of animals with positive assay results, vaccination, and culling animals with suspected external CL lesions (ie, externally detectable lesions likely attributable to C. pseudotuberculosis infection). Although these CL management techniques have been successful, methods that can be used to retain genetically valuable animals in herds or flocks are desirable. In another study, we compared outcomes of various treatment regi-
mings for sheep and goats with CL. Treatments of animals in that study included lancing, flushing, and draining of lesions and parenteral administration of penicillin G procaine or intralesional or parenteral administration of tulathromycin. Results of that study indicated no significant differences among treatment groups regarding proportions of lesions that had resolved by 1 month after initiation of treatment. We believe that the treatments used in that study do not affect future development of new lesions in affected animals. In addition, we believe that intralesional or parenteral administration of tulathromycin does not affect the odds of *C. pseudotuberculosis* infection for other animals in a herd without appropriate biosecurity measures. These opinions were determined on the basis of information reported in the literature and clinical experience.

Investigator interest in evidence-based medicine and the use of Bayesian statistics for medical applications is increasing. Bayesian statistical approaches assume that data are true values and mean values are derived. By use of Bayesian statistical techniques, credible value intervals can be determined that are centered near the mean values calculated for a sample. Frequentist statistical approaches use population-based inference and assume that a mean value for a population can only be estimated via analysis of data. In such analyses, confidence intervals are centered at calculated mean values. Bayesian statistical approaches may be advantageous for clinical research; by use of such methods, a credible interval can be determined that has a 95% probability of including the true mean value. For example, if results of a study indicate that the odds for subjects with serologically positive results to develop lesions in the future has a mean value of 3 and a credible interval of 1.5 to 6, the findings can be directly applied to evaluation of animals with clinical disease; such data would indicate that a patient with serologically positive results is 1.5 to 6 times as likely as a patient with negative results to develop lesions in the future. Furthermore, results of Bayesian analysis can be used to identify a specific probability for every value of the evaluated variables, whereas results of frequentist analyses can be used to accept or reject a null hypothesis. For example, for values of *P* < 0.05, results of frequentist analyses will typically indicate that the initial or fixed parameter value should be rejected, but results of such analyses do not apply to any other values of variables. Also, Bayesian estimates are influenced by prior beliefs and information determined with means of data for prognostic factors. Prior beliefs are useful in analysis if they are known with confidence and can therefore influence posterior likelihood (ie, evaluation of prognostic factors). When prior beliefs are considered vague or uninformative, the results are primarily influenced by the data and are therefore similar to results determined with frequentist statistical analyses.

The objectives of the study reported here were to evaluate herds of sheep and goats in southwest Texas in which various CL control measures were used to determine the serologic prevalence of antibodies against *C. pseudotuberculosis*, the prevalence of active external CL lesions, and the odds for future development of CL lesions in animals with serologically positive assay results and those with active external CL lesions versus animals with serologically negative results and those without active CL lesions, respectively. Because of characteristics of data and populations of animals evaluated in the study reported here, we chose to use a Bayesian approach for statistical analyses. Our hypothesis was that animals with positive serologic results and those with external lesions attributable to CL would have higher odds for future development of CL lesions versus animals without such findings.

### Materials and Methods

**Animals and treatments**—Adult (≥ 2 years old; age range, 2 to 6 years) sheep and goats in 3 large herds in separate locations within a 100-mile radius in southwest Texas were used in the study. The animals were owned by Texas A&M Agrilife Research Stations, and the protocol for this study was approved by the Texas A&M University Institutional Animal Care and Use Committee. These herds were closed, with no animal purchases from outside sources. In 2 of the herds (herds 1 and 2), sheep and goats shared pastures and working facilities. The sheep in these 2 herds were Rambouillet and Dorper breeds, and the goats in these herds were Boer-Spanish crosses. The other herd consisted of Angora goats (herd 3). At the time of the initial examination of animals in this study, herd 1 included 245 adult goats and 163 adult sheep, herd 2 included 204 adult goats and 133 adult sheep, and herd 3 included 174 adult goats; therefore, 919 animals were included in the study. Until the start of the study, CL in these herds had been managed via removal of affected animals from the herd when suspected CL lesions were observed; these herds had been formed 7 to 10 years prior to the start of the study. Other management practices were similar among the herds regarding nutrition, vaccines administered, facilities, and husbandry. None of the animals in these herds had ever been vaccinated for CL. Animals in each of these herds were being used to evaluate genetic factors influencing forage utilization; therefore, these animals were valuable. At the start of the present study, different management strategies for control of CL were initiated for each of the 3 herds. Animals in the herds were evaluated during a 13-month period to determine whether serologic anti-*C. pseudotuberculosis* antibody status and presence of active CL lesions in the animals were predictors of the odds of development of CL lesions in the future.

During the initial examination, a blood sample (5 mL) was collected from each adult sheep and goat via jugular venipuncture for performance of an SHI test to detect antibodies against *C. pseudotuberculosis*. Animals were examined to detect suspected CL lesions (≥ 1 palpably enlarged mass consistent with CL); such animals were considered to be clinically affected with CL. Lesions were clipped free of hair and aseptically prepared with povidone-iodine scrub, and contents were aspirated with a 16-gauge, 1.5-inch needle. The aspirated material was placed on a culture swab and submitted for bacteriologic culture. Material from suspected CL lesions that were open and draining was manually expressed, placed on a swab, and submitted for bacterio-
logic culture. Affected goats and sheep were then assigned to 3 treatment groups (determined on the basis of herd); affected animals in each group received one of the treatment regimens that animals in another study had received or a control treatment. Animals in each of the 3 herds were examined every 6 months during a 13-month period for detection of external suspected CL lesions. A 6-month interval between examinations was used because animals in the herds were gathered at those times for shearing, weaning of kids and lambs, and sorting. For animals in herds 1 and 2 with suspected CL lesions, follow-up evaluations were conducted approximately 1 month following the 6-month interval examinations; animals in herd 3 were only evaluated at 6-month intervals. Performance of follow-up evaluations was feasible at those times because animals were in small pastures and accessible for examination. During the follow-up evaluations, each animal that had been treated for CL lesions was examined to determine CL lesion status (resolved vs unresolved). Animals with resolved lesions (no palpable lesion or a small scar) were returned to their herd. Animals with unresolved lesions (palpable lesions in the same locations as lesions detected previously or new lesions detected in different locations) were culled from the herd; some of these animals underwent necropsy.

For each herd, 1 of 3 management strategies for control of CL was implemented. For herd 1, sheep and goats with suspected CL lesions were allocated via a randomization procedure to a treatment or control group. For animals in the herd 1 treatment group, a dose of tulathromycin (2.5 mg/kg [1.14 mg/lb]) was administered intraleonally and an additional dose of tulathromycin (2.5 mg/kg) was administered SC in the neck region. For herd 1 control animals, an equivalent volume (equal to the determined volume of a calculated dose of tulathromycin) of saline (0.9% NaCl) solution was administered intraleonally and an additional equivalent volume of saline solution was administered SC in the neck region. Herd 1 animals in treatment and control groups were housed together and isolated from the rest of the herd.

For herd 2, sheep and goats with suspected CL lesions were allocated via a randomization procedure to a treatment or control group. For herd 2 animals in the treatment group, tulathromycin (2.5 mg/kg) was administered SC in the neck region; herd 2 animals in the control group received an equivalent volume of saline solution SC in the neck region. Herd 2 animals in treatment and control groups were housed together and isolated from the rest of the herd.

Goats in herd 3 were examined at 6-month intervals. Goats in herd 3 with positive C pseudotuberculosis culture results for any of the 6-month interval evaluations were culled from the herd.

**Bacteriologic culture of suspected CL lesions**—Samples collected from suspected CL lesions at each 6-month interval evaluation of animals were inoculated onto 5% sheep blood agar and MacConkey agar plates and into tryptose broth. Blood agar plate and tryptose broth cultures were incubated at 37°C in air for up to 72 hours. Suspected *C pseudotuberculosis* colonies were subcultured to determine purity; growth of *C pseudotuberculosis* was confirmed with a *Corynebacterium* identification system. 

**Serum hemolysin inhibition test**—Blood samples collected from animals during the initial evaluation were analyzed via SHI testing to determine serum hemolysin-inhibition titers of antibodies against *C pseudotuberculosis*. Briefly, hemolysin-inhibition titers were determined via synergistic action of *Rhodococcus equi* and *C pseudotuberculosis* toxins. The titer at which hemolysis was inhibited was determined with blood agar plates. Goat and sheep serum samples were placed into 8 wells cut into the plate that contained 8 serial dilutions of hemolytic units in accordance with the standard methods of a diagnostic laboratory. A titer of ≥ 1:8 was considered positive.

**Statistical analysis**—The prevalence of external lesions consistent with CL in sheep and goats was determined during initial herd visits via identification of lesions with positive bacteriologic culture results for *C pseudotuberculosis*. The anti-*C pseudotuberculosis* antibody serologic status of goats and sheep was determined via evaluation of SHI test results. A logistic model was created; Y was the presence or absence of an active CL lesion and had a Bernoulli distribution, W was the serologic status (positive or negative results) or presence or absence of an active CL lesion, and ß was the log odds ratio of Y. The model was determined with a Bayesian method of inference, vague prior beliefs, and Markov Chain Monte Carlo implementation. The Markov Chain Monte Carlo implementation was performed with a readily available software package. The prior beliefs included a noninformative normal distribution for the intercept and the log OR of serologic status or presence or absence of current CL lesions with a mean of zero and a precision of 0.0001. Convergence was evaluated via visual examination of the history plots of the 2 chains and the Brooks, Gelman, and Rubin statistics. For parameter estimation, the initial 1,000 iterations were discarded to allow for convergence, then every 10th iteration was retained until 20,000 iterations had been recorded. Because of differences in management techniques for CL control at the herd level, this statistical approach was applied separately for each herd to decrease the potential for confounding. Credible intervals with a lower value of > 1 were considered to indicate an increased risk of animals for future development of external CL lesions.

**Results**

Approximately 200 sheep and goats were culled from the herds during the 13-month study period. Seven animals in herds 1 and 2 for which CL lesions did not resolve after treatment underwent necropsy during the 13-month study period. Of these animals, only 1 (a ewe) had internal abscesses with positive bacteriologic culture results for *C pseudotuberculosis*.
The prevalence of positive serologic and bacteriologic culture results for *C. pseudotuberculosis* in sheep and goats in herds 1, 2, and 3 was summarized (Table 1). The prevalence of positive serologic results for antibodies against *C. pseudotuberculosis* in sheep and goats in the herds at the time of the initial examination ranged from 7.52% to 69.54%; the prevalence of positive bacteriologic culture results for *C. pseudotuberculosis* in herds at the start of the study ranged from 0% to 9.56%. The prevalence of positive serologic results (Table 1) for sheep and goats in 3 herds in southwest Texas determined on the basis of results of an SHI test for antibodies against *Corynebacterium pseudotuberculosis* performed at the start of the study (serologic prevalence) and results of bacteriologic culture of lesions for *C. pseudotuberculosis* performed at the time of 3 examinations during the 13-month study period.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Serologic prevalence</th>
<th>Bacteriologic culture prevalence</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First examination</td>
</tr>
<tr>
<td>Herd 1 goats</td>
<td>124/245 (50.61)</td>
<td>15/245 (6.12)</td>
</tr>
<tr>
<td>Herd 1 sheep</td>
<td>33/163 (20.25)</td>
<td>9/163 (5.52)</td>
</tr>
<tr>
<td>Herd 2 goats</td>
<td>55/204 (26.96)</td>
<td>4/204 (1.96)</td>
</tr>
<tr>
<td>Herd 2 sheep</td>
<td>10/133 (7.52)</td>
<td>0/133 (0)</td>
</tr>
<tr>
<td>Herd 3 goats</td>
<td>121/174 (69.54)</td>
<td>3/174 (1.72)</td>
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Data are number of animals with positive results/total number of animals in the herd at the time (%).

Table 2—Results of Bayesian statistical analysis indicating the correlation between positive SHI test results for antibodies against *C. pseudotuberculosis* and odds of future development of CL lesions and between positive bacteriologic culture results for *C. pseudotuberculosis* and odds of future development of CL lesions for the sheep and goats in Table 1.

<table>
<thead>
<tr>
<th>Animals</th>
<th>SHI test</th>
<th>Bacteriologic culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First examination</td>
</tr>
<tr>
<td>Herd 1 sheep</td>
<td>1.96 (0.48–5.11)</td>
<td>44.39 (4.25–207.10)</td>
</tr>
<tr>
<td>Herd 1 goats</td>
<td>1.59 (0.54–4.73)</td>
<td>0.08 (1.34–0.54)</td>
</tr>
<tr>
<td>Herd 2 sheep</td>
<td>5.37 (1.05–17.02)</td>
<td>76.79 (1.41–335.70)</td>
</tr>
<tr>
<td>Herd 2 goats</td>
<td>1.86 (0.53–4.55)</td>
<td>460 (29.03–2,617.00)</td>
</tr>
<tr>
<td>Herd 3 goats</td>
<td>1.86 (0.19–46.18)</td>
<td>ND</td>
</tr>
<tr>
<td>All animals</td>
<td>1.19 (0.68–1.94)</td>
<td>2.68 (1.02–5.47)</td>
</tr>
</tbody>
</table>

Data are OR (95% credible interval). Values were calculated separately for each herd.

*Increased odds of future development of CL lesions as indicated by a 95% credible interval with a lower limit > 1.
**Not determined because animals with active lesions (ie, positive bacteriologic culture results for *C. pseudotuberculosis*) were culled from the herd.

The statistical analyses performed during the present study were used to determine the odds of future development of CL lesions in sheep and goats with positive serologic and bacteriologic culture results for *C. pseudotuberculosis*. Further analysis in which the effects of herd management techniques on future development of CL lesions were determined would have yielded additional information. However, such analysis was considered inappropriate because of factors beyond the control of the investigators that included the following: removal of animals from the herds, a lack of strict biosecurity of animals with active CL lesions (such as temporary movement of animals into pens or pastures shared with unaffected animals), an inability to examine each treated animal daily, transfer of animals between herds 1 and 2 during the study (and therefore a change in treatment protocol for such animals), and housing of animals in herd 1 and 2 treatment and control groups together (possibly allowing transmission of disease among animals). The prevalence of active external CL lesions in the 3 herds of sheep and goats in southwest Texas in the present study was lower than that determined in another study in which lesion samples obtained from culled sheep in 9 western US states were evaluated. However, only animals with external lesions were evaluated to detect CL in the present study; therefore, animals with internal abscesses may not have been identified. If animals with internal lesions had been identified in this study, the determined prevalence of CL might have been higher. Furthermore, we did not examine goats and sheep that were < 2 years old for detection of CL lesions; inclusion of animals < 2 years old may also have increased the prevalence of CL determined in the study. Animals < 2 years old were not tested for CL because a large proportion of these animals were sold at auction; therefore, the number of animals included in this study was representative of the number of animals selected for breeding purposes in the herds. Prior to the present study, the herds included in the study had been managed for control of CL via culling of affected animals. Estimates of the prevalence were not treated (control animals) because of the inability to separate them during the period between follow-up examinations. Therefore, efficacy of treatment with tulathromycin could not be determined.

**Discussion**

In the present study, the herds included in the study had been managed for control of CL via culling of affected animals. The prevalence of active external CL lesions in the 3 herds of sheep and goats in southwest Texas in the present study was lower than that determined in another study in which lesion samples obtained from culled sheep in 9 western US states were evaluated. However, only animals with external lesions were evaluated to detect CL in the present study; therefore, animals with internal abscesses may not have been identified. Animals with internal lesions had been identified in this study, the determined prevalence of CL might have been higher. Furthermore, we did not examine goats and sheep that were < 2 years old for detection of CL lesions; inclusion of animals < 2 years old may also have increased the prevalence of CL determined in the study. Animals < 2 years old were not tested for CL because a large proportion of these animals were sold at auction; therefore, the number of animals included in this study was representative of the number of animals selected for breeding purposes in the herds. Prior to the present study, the herds included in the study had been managed for control of CL via culling of affected animals. Estimates of the prevalence were not treated (control animals) because of the inability to separate them during the period between follow-up examinations. Therefore, efficacy of treatment with tulathromycin could not be determined.
of CL in these herds at the beginning of the study were determined on the basis of information in herd records identifying numbers of animals culled because of external lesions consistent with CL. The estimated prevalence of external CL lesions in these herds ranged from 1% to 10% (10% for goats in herd 1). Culling of affected animals is theoretically the best method for eradication of CL in a herd. However, results of the present study indicated that CL was present in the herds (with substantial prevalence at the time of the initial examination) despite use of culling for control of the disease; the prevalence of CL determined via bacteriologic culture for herd 1 goats at the start of the study was 6.12%.

Many animals examined at the start of this study were removed from their herds by the end of the study. These animals were removed because they were sold (culled because of reproductive inefficiency, old age, udder disease, lameness, or CL), died of causes unrelated to CL, or died of predation.

The finding of the present study that sheep in herd 2 had a 0% prevalence of CL as determined via bacteriologic culture at the time of the initial examination and subsequently had a 1.64% prevalence of CL as determined via that method was surprising because a high number of suspected CL lesions were identified in these animals during the initial examination. Most of the suspected CL lesions in these animals were open and draining at the time of the initial examination because the animals had undergone shearing just prior to that examination. We suspected that overgrowth of other bacteria in cultures of lesion samples prevented growth of *C. pseudotuberculosis*. During subsequent examinations of sheep in herd 2, closed lesions were detected.

At the time of the initial examination in the present study, the serologic prevalence of positive results for *C. pseudotuberculosis* in the herds ranged from 7.32% to 69.94%. However, a positive SHI test result for an animal without clinically detectable lesions during the initial examination was a poor predictor for development of a positive bacteriologic culture result during the 13-month study period. Such positive SHI test results most likely indicated that animals had been previously exposed to *C. pseudotuberculosis*, had an internal *C. pseudotuberculosis* abscess, or had recovered from CL. Because of the characteristics of CL, we suspected that animals with SHI test titers consistent with recovery from CL would develop positive bacteriologic culture results for *C. pseudotuberculosis* during the study period because of recurrence of disease. Results of necropsies of 7 animals that did not respond to treatment in this study indicated that only one of those animals (a ewe) had internal abscesses with positive bacteriologic culture results for *C. pseudotuberculosis*; therefore, we considered it unlikely that positive serologic results in animals of this study would have been attributable to internal CL abscesses.

Results of another study indicate that the SHI test may have low specificity for identification of animals with CL. In the herds of the present study, the low specificity of the SHI test may have resulted in inappropriate culling of genetically valuable animals. Although it would have been interesting to determine the performance of the SHI test for identification of animals with CL in these herds, the methods used in the present study precluded determination of the true sensitivity and specificity of the test. In these herds, animals with negative serologic results could not be confirmed to be free of CL, and CL could not be confirmed in animals with positive serologic results without performance of necropsies for all seropositive animals. We were unable to accurately assess the efficacy of treatment of animals in the present study despite the inclusion of animals that received control treatments because of several factors. Development or resolution of lesions in some animals may not have been detected because we were only able to examine all animals every 6 months during the study period. Although CL-affected animals were isolated after each examination from animals in the herd that were not affected, CL-affected animals could not be individually isolated. Therefore, control and treatment group animals were housed together during the study. Furthermore, the number of animals in each herd precluded daily examination of treated lesions; therefore, lesions may have opened, drained, and resolved before follow-up examinations and treatment of such lesions may have been inaccurately categorized as successful. The lack of strict biosecurity methods for the herds was problematic, but such methods could not be improved during the study because of herd sizes and established management procedures. However, management of herds in the present study was representative of the methods typically used to manage animals with CL in herds maintained on large areas of land. Not only did these factors preclude evaluation of treatment efficacy in this study, but they also prevented accurate determination of the true overall prevalence of CL in the herds. Determination of the true prevalence of CL for these herds would have required external (via physical examination) and internal (via necropsy) examination of all animals. Therefore, only the prevalence of external CL lesions in animals ≥ 2 years old was determined in this study.

The prevalence of externally detectable CL in almost 1,000 sheep and goats in 3 herds within a 100-mile radius in southwest Texas was determined in the present study; prior to the study, CL had been managed in these herds via culling animals with suspected lesions. Although culling of animals with suspected CL is considered the best method of herd management of the disease, goats in herd 2 had a 9.56% prevalence of CL as determined via bacteriologic culture of lesion samples obtained during the third examination; CL was detected in animals despite the fact that CL in this herd had been managed via removal of animals with suspected CL lesions for 7 to 10 years prior to the study. Prior to this study, estimates of the prevalence of CL in these herds (determined via evaluation of information in culling records) were as high as 10% (for goats in herd 1). Results of this study indicated that use of an SHI test for determination of the future odds of development of active CL lesions may result in unnecessary culling of genetically valuable animals. However, animals with active CL lesions detected during the initial examination in this study were 2.68 times as likely to develop CL lesions in the future as animals without active lesions at the time of the first examination. Although some animals with CL in herds 1 and 2 were managed via treatment in this study,
changes in prevalence of CL in these herds during the study could not be compared with changes in prevalence of CL in herd 3 because animals with CL in that herd were removed as soon as a diagnosis was determined. However, results of this study indicated that only goats in herd 2 and sheep in herd 1 had an increase in the prevalence of external CL lesions during the 13-month study period, whereas the prevalence of such lesions in other animals decreased or did not substantially change during that time. Although results for treatment and control group animals could not be compared in this study, that finding suggested management of CL via parenteral administration of antimicrobial drugs may be effective for herds in which it is desirable to maintain genetically valuable animals, without substantial increases in the prevalence of the disease.

References

Evaluation of plasma diazepam and nordiazepam concentrations following administration of diazepam intravenously or via suppository per rectum in dogs
Curtis W. Probst et al

Objective—To evaluate the pharmacokinetics of diazepam administered per rectum via compounded (ie, not commercially available) suppositories and determine whether a dose of 2 mg/kg in this formulation would result in plasma concentrations shown to be effective for control of status epilepticus or cluster seizures (ie, 150 to 300 ng/mL) in dogs within a clinically useful interval (10 to 15 minutes).

Animals—6 healthy mixed-breed dogs.

Procedures—Dogs were randomly assigned to 2 groups of 3 dogs each in a crossover-design study. Diazepam (2 mg/kg) was administered IV or via suppository per rectum, and blood samples were collected at predetermined time points. Following a 6- or 7-day washout period, each group received the alternate treatment. Plasma concentrations of diazepam and nordiazepam were analyzed via reversed phase high-performance liquid chromatography.

Results—Plasma concentrations of diazepam and nordiazepam exceeded the targeted range ≤3 minutes after IV administration in all dogs. After suppository administration, targeted concentrations of diazepam were not detected in any dogs, and targeted concentrations of nordiazepam were detected after 90 minutes (n = 2 dogs) or 120 minutes (3) or were not achieved (1).

Conclusions and Clinical Relevance—On the basis of these results, administration of 2 mg of diazepam/kg via the compounded suppositories used in the present study cannot be recommended for emergency treatment of seizures in dogs. (Am J Vet Res 2013;74:611–615)