Journal of Veterinary Internal Medicine
Open Access
J Vet Intern Med 2015

Gallium Maltolate as an Alternative to Macrolides for Treatment of Presumed *Rhodococcus equi* Pneumonia in Foals

Noah D. Cohen, Nathan M. Slovis, Steeve Giguère, Samantha Baker, M. Keith Chaffin, and Lawrence R. Bernstein

Background: Macrolide-resistant isolates of *Rhodococcus equi* are emerging, prompting the search for clinically effective alternative antimicrobials.

Hypothesis: The proportion of foals with ultrasonographic evidence of pneumonia presumed to be caused by *R. equi* that had a successful outcome when administered gallium maltolate (GaM) PO would not be more than 10% inferior (ie, lower) than that of foals receiving standard treatment.

Animals: Fifty-four foals with subclinical pulmonary abscesses among 509 foals at 6 breeding farms in Kentucky.

Methods: Controlled, randomized, prospective noninferiority study. Foals with ultrasonographic lesions >1 cm in diameter (n = 54) were randomly allocated to receive per os either clarithromycin combined with rifampin (CLR+R) or GaM, and followed up for 28 days by daily physical inspections and weekly (n = 1 farm) or biweekly (n = 4 farms) thoracic ultrasound examinations by individuals unaware of treatment-group assignments. Treatment success was defined as resolution of ultrasonographically identified pulmonary abscesses within 28 days of initiating treatment. Noninferiority was defined as a 90% confidence interval for the observed difference in CLR+R minus GaM that was ≤10%.

Results: The proportion of GaM-treated foals that resolved (70%; 14/20) was similar to that of foals treated with CLR+R (74%; 25/34), but we failed to demonstrate noninferiority for GaM relative to CLR+R; however, GaM was noninferior to CLR+R treatment when results from a noncompliant farm were excluded.

Conclusions and Clinical Importance: Gallium maltolate is not inferior to macrolides for treating foals with subclinical pneumonia. Use of GaM might reduce pressure for macrolide-resistance in *R. equi*.

Key words: Antibiotic choices; Antimicrobial resistance; Antimicrobials; Equivalency.

Pneumonia caused by the facultative intracellular pathogen *Rhodococcus equi* is an important cause of disease and death in foals.[1] For approximately 30 years, the combination of a macrolide antibiotic and rifampin has been the treatment of choice on the basis of *in vitro* studies,[2–4] rodent models,[5] case series and other retrospective studies,[6,7] and expert opinion.[8] Recently, a placebo-controlled trial demonstrated that azithromycin (with or without rifampin) was significantly superior to placebo for treating foals with clinical signs of pneumonia and thoracic ultrasonographic findings attributed to *R. equi* infection.[9] Effective alternatives to macrolides for treating foals with *R. equi* pneumonia are exiguous.[1] Because a vaccine effective for protecting against *R. equi* pneumonia is not available, early detection and treatment of pulmonary abscesses or areas of consolidation presumed to be attributed to *R. equi* infection has become common practice at many large breeding farms.[1,9–12] The rationale for this practice is that earlier intervention will lead to reduced case fatality and decreased duration of treatment. Because the proportion of foals that will recover spontaneously can be high, this practice results in an increased prevalence of treated foals. Thus, the use of macrolides has increased at farms that use screening for earlier detection and treatment of *R. equi* pneumonia.[1,9–12]

Recent reports of isolates of *R. equi* from foals or their environment that are resistant to macrolides and rifampin have raised concerns regarding the use of macrolides to control *R. equi* pneumonia.[13,14] Although the epidemiology and ecology of macrolide-resistant *R. equi* remains ill-defined, evidence from one farm indicates that greater use of macrolides, as a result of a program of screening foals with thoracic ultrasonography and treating foals with lesions, contributed to the emergence of resistance.[13] The lack of effective alternatives to macrolides and the specter of emerging resistance to the macrolides and rifampin among *R. equi* isolates create a clinical imperative to identify novel antimicrobial approaches for foals with confirmed or presumed *R. equi* pneumonia.

Abbreviations:

CLR	clarithromycin
Ga	mgallium maltolate
HEMI	Hagyard Equine Medical Institute
PO	per os
R	rifampin

From the Department of Large Animal Clinical Sciences, Texas A&M University, College Station, TX (Cohen, Chaffin); the McGee Medicine Center, Hagyard Equine Medical Institute, Lexington, KY (Slovis, Baker); the Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA (Giguère); and Terrametrix, Menlo Park, CA (Bernstein).

This work was conducted at the Hagyard Equine Medical Institute, 6 farms in central Kentucky, and the Equine Infectious Disease Laboratory, Texas A&M University.

Corresponding author: Dr N.D. Cohen, Department of Large Animal Clinical Sciences, Texas A&M University, 4475 TAMU, College Station, TX 77843-4475; e-mail: ncohen@cvm.tamu.edu.

Submitted November 25, 2014; Revised March 9, 2015; Accepted March 17, 2015.

DOI: 10.1111/jvim.12595

Gallium maltolate (GaM) is a semimetal compound that has antimicrobial activity.[15] GaM has activity against *R. equi* in vitro, including the ability to kill *R. equi* within macrophages.[16–18] GaM can be administered safely to foals at doses of 25–30 mg/kg that achieve concentrations in blood that have been shown to result in reduced tissue concentrations of *R. equi* in experimentally infected mice.[15,19–21] On the basis of these results, we hypothesized that GaM administered PO to foals with ultrasonographic evidence of pneumonia presumed to be caused by *R. equi* infection would be no more than 10% less effective than standard treatment. The objective of this study was to provide an initial evaluation of the potential use of GaM as an alternative to macrolides for controlling *R. equi* pneumonia.

Materials and Methods

Study Population

The protocol for this clinical study was approved by Texas A&M University's Animal Care and Use Committee (2012-0242) and the institutional review board of the Hagyard Equine Medical Institute (HEMI). Farms were recruited by one of the authors (NMS) from Thoroughbred breeding farms in central Kentucky for which he provided consultation for diagnosis, treatment, and prevention of *R. equi* pneumonia. All foals at participating farms were inspected by experienced farm personnel at least twice daily, and foals that had clinical signs were examined by an equine veterinarian at least once daily. Eligible farms met the following criteria: (1) history of recurrent *R. equi* pneumonia with cumulative incidence of at least 5% during the preceding 5 years; (2) consent from the farm management to participate in the study; and, (3) routine use of sequential thoracic ultrasonography at intervals of no more than 3 weeks to screen foals for pulmonary abscess formation or consolidation attributed to *R. equi*, beginning within the first month of age. We estimated that we would need to identify 60 foals meeting the case definition for this clinical noninferiority trial (described below) to be randomly assigned to 1 of 2 groups (standard treatment with a macrolide and rifampin, or GaM). This sample size was calculated on the basis of the following assumptions: (1) the proportion of macrolide-treated and gallium-treated foals that would resolve successfully would be 95%; (2) GaM would be successful in at least 85% of foals (ie, a noninferiority limit of 10%); (3) an alpha (significance) level of 5% (0.05); and, (4) statistical power of 80%. To identify 60 foals, we estimated we would need to scan at least 400 foals on the basis of the assumption that approximately 20% of foals would have findings meeting the inclusion criteria and for which informed consent from the owner or owner's agent would be obtained, and that approximately 25% of foals would be lost to follow-up or withdrawn for reasons unrelated to *R. equi* pneumonia.

Treatment Assignment and Protocol

Foals at the 5 farms whose management or owners agreed to participate in the project were eligible to be included in the study provided that they were expected to reside at the farm through 16 weeks of age and that informed consent for participation was obtained. Transcutaneous thoracic ultrasonography to detect pulmonary abscesses or consolidations was performed sequentially beginning at approximately 1 month of age at the frequency that was routine for the farm: 4 farms screened foals at an interval of 2 weeks and 1 farm screened foals at an interval of 1 week. Briefly, both sides of each foal's thorax were examined from the 17th to the 4th intercostal spaces using a 5 mHz probe by the attending veterinarian for the farm. Veterinarians recorded the total number of lesions ≥10 mm and the maximal diameter of the largest lesion observed. The primary endpoint was the score of the largest lesion according to the Slovis scoring system.[10] All foals having scores ≥2 on the basis of the Slovis scoring system were eligible for inclusion in the study. The Slovis scoring system is based on the maximum diameter of the largest lesion detected and is scored as follows: 0 = no evidence of pulmonary abscesses or consolidation (lesions); includes pleural irregularities seen as vertical hyperechoic lines (commonly referred to as comet-tails); 1 = lesions <10 mm; 2 = lesions from 10 to 20 mm; 3 = lesions >20 mm to 30 mm; 4 = lesions >30 mm to 40 mm; 5 = lesions >40 to 50 mm; 6 = lesions >50 to 60 mm; 7 = lesions >60 to 70 mm; 8 = lesions >70 to 90 mm; 9 = lesions >90 to 110 mm; and, 10 = entire lung for the hemithorax is involved.

Participating foals with lesions of score ≥2 were randomly assigned to either standard treatment or GaM. The randomization mechanism was as follows. The standard treatment was assigned the number 1 and gallium was assigned the number 2. Using a computer running R statistical software,[a] a random sequence of length 100 of 1s or 2s was generated for each of the 5 farms. In an effort to generate equal numbers in the 2 treatment groups, if the first number in the list was a 1 then the first participating foal was assigned to standard treatment and the next (second) foal was assigned to the GaM group; if the first number was a 2 then the first foal was assigned to the GaM group and the second foal was assigned to standard treatment. If the second randomly generated number in the list was a 1, the next (third) foal was assigned to standard treatment and the fourth foal was assigned to GaM treatment; if the second randomly generated number was a 2 then the third foal was assigned to GaM and the fourth foal was assigned to standard treatment. This process was iterated to assign foals to treatment groups. The assignment list was maintained at the HEMI pharmacy from whence medications were dispensed. The standard treatment was compounded by the HEMI pharmacy and consisted of clarithromycin (CLR)[b] (7.5 mg/kg; per os [PO]; q 12 hours; compounded by the HEMI pharmacy) combined with rifampin[b] (5 mg/kg; PO; q 12 hours; compounded separately by the HEMI pharmacy). The dose of rifampin was 5 mg/kg administered PO q 12 hours. These medications were dispensed from the HEMI pharmacy according to their usual practice. The dose of GaM was 30 mg/kg PO q 24 hours, prepared as a paste formulation as previously described.[21] This formulation consisted of GaM at a concentration of 150 mg/mL in a paste consisting of methylcellulose gel blended with simple syrup, plus 1% benzoyl alcohol as a preservative. The formulation was prepared weekly at the HEMI pharmacy and was dispensed in tubs from which the appropriate dose could be aspirated into a syringe (eg, a 100-kg foal required 20 ml of GaM gel for its daily dose). No attempt was made to make the 2 treatments identical because this was beyond the scope of the study budget, and those administering treatment (usually farm technical staff under veterinary supervision) were aware of which treatment the foal was receiving. Ultrasonographic examinations were performed separately from treatment such that those performing the examination were unaware of the treatment, although there was no formal blinding of those assessing clinical outcomes.

At any time that the attending veterinarian for a participating farm felt that a foal's condition was deteriorating, that veterinarian was permitted to withdraw the foal from the trial and to initiate rescue treatment. The rescue treatment for this study was CLR (7.5 mg/kg; PO; q 12 hours) or azithromycin (10 mg/kg; PO; q 24 hours for 5–7 days, then 10 mg/kg; PO; q 48 hours) and rifampin (5 mg/kg; PO; q 12 hours); this rescue treatment was to be recommended even if the foal had already been receiving CLR

(7.5 mg/kg; PO; q 12 hours) in combination with rifampin (at 5 mg/kg; PO; q 12 hours).

Treatment success was defined as resolution of ultrasonographic evidence of pulmonary abscess formation or consolidation within 28 days of initiation of treatment (ie, Slovis score <1). Treatment failure was defined as failure to achieve resolution within 28 days, or an increase in score of ≥2 or more grades on the Slovis scale between ultrasound examinations separated by 2 weeks. Results of ultrasonographic examinations were recorded by a study technician in a computerized database that included the foal's identification, date of birth of the foal, dates of ultrasonographic examinations, ultrasonographic findings (as described above) at each examination, date treatment was initiated, date treatment was discontinued, whether any adverse event was observed, and whether the foal survived (ie, lived or died or was euthanized as a result of *R. equi* pneumonia).

Data Analysis

Data were analyzed using descriptive and inferential methods. For descriptive purposes, categorical data were summarized in contingency tables and continuous data were summarized as medians and ranges. Proportions were compared among groups using chi-squared or Fisher's exact tests; continuous variables were compared between treatment groups using the Wilcoxon rank-sum test.

The primary study outcome was the proportion of foals that resolved within the 28-day period of observation, with a hypothesis that GaM would be considered noninferior if the difference between standard treatment and GaM was ≤10% (ie, noninferiority limit of 10%), using an alpha level of 5% (0.05) and a 1-sided test (ie, the symmetrical 90% confidence interval for the difference in treatment proportions did not include 10%). Additionally, comparisons of treatments were made using both chi-squared or Fisher's exact tests and logistic regression analysis with the binary outcome variable of resolution at 28 days (yes or no), including multivariable logistic regression to adjust for effects of confounding by initial lesion size. Significance for analyses was $P < .05$, and they were performed using S-PLUS statistical software.[c]

Results

Five hundred and nine (509) foals from 6 farms were evaluated ultrasonographically to identify 58 foals with ultrasonography scores ≥2 using the scoring system proposed by Slovis et al[10] (Table 1); these represented all eligible foals at these farms. Four foals from 1 farm (Farm 2) that were assigned to the GaM group were excluded because they failed to follow the study treatment protocol. One excluded foal was switched from GaM treatment after its second dose (2 days) to treatment with a macrolide and rifampin because it developed diarrhea; this foal resolved within 28 days of treatment. Another foal treated with GaM was excluded because it had CLR and rifampin added to its treatment after 7 days. Two other foals were excluded because they were switched from GaM after 7 days of treatment on the basis of an increase in lesion size of <2 grades of the Slovis score, in violation of study protocols. Thus, there were a total of 54 study foals of which 34 were assigned to standard treatment and 20 were assigned to GaM treatment. The discrepancy in treatment was associated with Farm 2: 10 more foals were assigned to macrolides at this farm than GaM, whereas at other farms the proportion of foals assigned to macrolides was consistent with random assignment of 50% of foals in each group (Table 1). This imbalance occurred because of failure of one farm (Farm 2) to understand or comply with the study design.

Neither the age of the first ultrasound examination at which lesions were detected nor the maximal diameter of lesions differed significantly between study groups (Table 2). The lesion sizes were significantly larger at inclusion in the study for the standard treatment group than for the GaM group (Table 2). Most foals (70%; 38/54) had only a single lesion of grade ≥2; the remaining 16 foals were reported to have 2 lesions ≥2. There was no significant difference between treatment groups in the proportion of foals with 2 lesions (Table 2).

The proportion of GaM-treated foals that resolved (70%; 14/20; Table 2) was similar to that of foals treated with standard treatment (74%; 25/34) and did not differ significantly. The primary outcome of this project was to demonstrate that GaM would not be more than 10% less effective than standard treatment. Results indicate that we failed to exclude the possibility that GaM was inferior to standard treatment at a limit of 10%. The observed difference was 3.5% (ie, 73.5% for standard treatment minus 70% for GaM), and the noninferiority limit of 10% was exceeded. If we include the 4 foals excluded that were assigned to GaM (but were either switched to the combination of CLR and rifampin [n = 3] or had the combination added [n = 1] to

Table 1. Number of foals (%) examined ultrasonographically, number of examined foals (%) included in the study, and number of foals (%) assigned to each of the 2 treatments in an equivalency trial comparing oral administration of GaM and standard treatment (clarithromycin + rifampin) for foals with subclinical pneumonia presumed to be caused by *Rhodococcus equi*.

Farm	Foals Examined	Foals Included (% of total examined)	Foals Excluded (% of total examined)	Standard Treatment (% of included)	GaM (% of included)
1	36	13 (36)	0 (0)	7 (54)	6 (46)
2	268	24 (9)	4 (2)	17 (71)	7 (29)
3	18	2 (11)	0 (0)	1 (50)	1 (50)
4	27	5 (18	0 (0)	3 (60)	2 (40)
5	76	5 (7)	0 (0)	3 (60)	2 (40)
6	84	5 (6)	0 (0)	3 (60)	2 (40)
Total	509	54 (11)	4 (<1)	34 (63)	20 (37)

GaM, gallium maltolate.

Table 2. Comparisons of ultrasonographic and outcome parameters in a study of foals with subclinical pneumonia presumed to be caused by *Rhodococcus equi* treated with either GaM or standard treatment (clarithromycin + rifampin).

a. Continuous Variables

	Standard Treatment (n = 34)	GaM (n = 20)	
Variable	Median (range)	Median (range)	P^a
Age at 1st positive ultrasound (days)	47 (33–83)	48 (26–100)	.83
Maximal diameter (MD) at 1st positive ultrasound (mm)	34 (12–77)	24 (17–46)	.004
Total score 1st positive ultrasound	4 (2–12)	3 (2–6)	.007
Maximal MD (mm) at any age	31 (14–118)	25 (16–58)	.09

b. Categorical Variables

	Standard Treatment	GaM	
Variable	Number (%)	Number (%)	P^b
2 lesions (versus 1)	12/34 (35%)	20% (4/20)	.38
Died	3/34 (9%)	1/20 (5%)	1.0
Resolved	25/34 (74%)	14/20 (70%)	.97
Resolved among survivors	22/31 (71%)	13/19 (68%)	.90
Resolved lesions <27 mm	8/9 (89%)	12/14 (86%)	1.0
Resolved lesions >27	17/25 (68%)	2/6 (33%)	.17

GaM, gallium maltolate.
[a] *P* value from Wilcoxon-rank sum test.
[b] *P* value from chi-squared or Fisher's exact tests.

GaM) on the basis of intention to treat, the proportion of successes was similar for GaM-treated foals (75%; 18/24) and standard treatment (74%; 25/34). Even when we included the 3 excluded foals that were switched from GaM to standard treatment as failures for GaM, there was no significant difference ($P = .47$) in the proportion of successes for the GaM-treated group (61%; 14/23). Moreover, inclusion of any or all of these foals also did not change results regarding inability to conclude that GaM was noninferior to standard treatment.

Four foals died. The cause of death was attributed to infection with *R. equi* in all these foals. One foal (a GaM-treated foal) was euthanized because it developed colic, underwent celiotomy, and was found to have a septic mesenteric lymphadenitis caused by *R. equi*, and a mesenteric rent with small intestinal entrapment; necropsy revealed chronic, focal granulomatous pneumonia and subacute, multifocal hepatitis attributed to *R equi* on the basis of microbiologic culture; this foal had been treated for 15 days. One of the CLR/rifampin-treated foals was anesthetized for an orthopedic surgical procedure; this foal died during anesthesia, and death was attributed to respiratory failure resulting from bilateral necrotizing granulomatous pneumonia caused by *R. equi* infection; this foal had been treated for 2 days. One of the CLR/rifampin-treated foals died after 21 days of treatment and necropsy revealed severe pyogranulomatous pneumonia caused by *R. equi* and peracute hemorrhagic enteritis. The third CLR/rifampin-treated foal that died was found dead in its stall 9 days after treatment was initiated; necropsy examination revealed severe, diffuse, multifocal pneumonia attributed to *R. equi* infection but for which microbiologic culture was not performed to confirm diagnosis. There was no significant difference in the proportion of foals that died between treatment groups (3 foals and 1 foal for the standard treatment and GaM treatment groups, respectively; Table 2). Excluding these foals, there was no significant difference between treatment groups in the proportion of foals that resolved (Table 2), but we could not reject the null hypothesis of GaM being ≤10% less effective. Consistent with previous reports,[19–21] no adverse events were reported among GaM-treated foals, except self-limiting diarrhea that began within 24 hours of treatment in 1 foal.

Because there was a significant difference between treatment groups in the size of lesions at baseline, we performed analyses to examine the impact of initial lesion size on time to resolution of lesions among surviving foals using 2 approaches. First, we stratified the contingency table analysis of treatment by outcome (resolution of signs) by a binary outcome of lesion size ≥27 mm or not; the rationale for the cut-point of 27 mm was that it was the median value for the study population of lesion size detected at the initial ultrasound examination. Among foals with smaller lesions (<27 mm), proportions resolving were similar for both groups (Table 2). Although the proportion resolving among the foals with larger lesions (ie, ≥27 mm) was lower for the GaM-treated foals than the CLR-treated foals, this difference was not significant (Table 2). Our second approach to account for differences in lesion size was to use logistic regression, where we modeled the outcome of resolution (yes or no) as a function of treatment, lesion size, and the interaction of treatment and lesion size, where lesion size was considered as a binary categorical variable (<27 mm or ≥27 mm) because it did

Table 3. Results of logistic regression analysis comparing whether foals had resolution of ultrasonographic lesions within 28 days following treatment with either GaM or standard treatment (clarithromycin + rifampin): effects of treatment group and pulmonary lesion size. There was no significant interaction between treatment group and lesion size.

Variable	Odds Ratio	95% Confidence Interval	*P* value
Treatment			
GaM	1	NA	NA
Standard	1.8	0.7–4.5	0.20
Lesion size			
<27 mm	1	NA	NA
≥27 mm	0.3	0.1–0.8	0.023

GaM, gallium maltolate.

Table 4. Comparison of the proportion of foals with resolution of ultrasonographic pulmonary lesions (Slovis score < 1) following 28 days with either standard treatment (clarithromycin + rifampin) or GaM, excluding results from 1 farm (Farm 2) that failed to reliably follow study protocols.

	Standard Treatment	GaM	
Variable	Number (%)	Number (%)	*P*[a]
Resolved	10/17 (59)	10/13 (77)	.44
Resolved lesions <27 mm	4/5 (80)	8/8 (100)	.39
Resolved lesions >27	6/12 (50)	2/5 (40	1.0

GaM, gallium maltolate.
[a]*P* value from Fisher's exact tests.

not appear to be linear in the logit. Using logistic regression, there was no significant effect of treatment but lesion size was positively and significantly associated with resolution (Table 3); there was no significant interaction between treatment and lesion size. The discrepancy in lesion size was associated with Farm 2: at this farm, 76% (13/17) of macrolide-treated foals had ultrasonographic lesions with maximal diameter ≥27 mm whereas only 14% (1/7) GaM foals had lesions ≥27 mm, and this difference was statistically significant ($P = .009$).

Because of the apparent discrepancy between Farm 2 and other farms regarding proportion of foals in each of the 2 treatment groups and lesion size, we performed post hoc analysis excluding Farm 2 using the same methods as for the full study population. Among the remaining 5 farms, the proportions of standard- and GaM-treated foals were 57% (n = 17) and 43% (n = 13), respectively, which did not differ significantly from a hypothetical distribution of 50% in each group (n = 15 per group). We observed that the proportion of foals that responded during the study period was greater for the GaM-treated foals (77%; 10/13) than for the CLR-treated foals (59%;10/17) but this difference was not significant ($P = .44$; Table 4). Moreover, there was neither statistically significant nor qualitative difference when stratifying foals by lesion size (Table 5). Among foals from these 5 farms, we found evidence that GaM was non-inferior to standard treatment: the 90% confidence interval for the difference in proportion of responses success between CLR and GaM did not include our 10% threshold for noninferiority.

Table 5. Results of logistic regression comparing whether foals had resolution of ultrasonographic pulmonary lesions within 28 days with either standard treatment (clarithromycin + rifampin) or GaM, excluding results of one farm (Farm 2) that failed to reliably follow study protocol: effects of treatment group and lesion size. There was no significant interaction between treatment group and lesion size.

Variable	Odds Ratio	95% Confidence Interval	*P* Value
Treatment			
GaM	1	NA	NA
Standard	0.8	0.1 to 5.1	.80
Lesion size			
<27 mm	1	NA	NA
>27 mm	0.1	<0.1 to 0.8	.041

GaM, gallium maltolate.

Discussion

The objective of this study was to demonstrate noninferiority of GaM relative to standard treatment for foals with ultrasonographic evidence of subclinical pneumonia presumed to be attributable to *R. equi* pneumonia. Such noninferiority would provide veterinarians with a novel antimicrobial as an alternative to macrolides. Reducing the use of macrolides is considered desirable because of the emergence of resistance to macrolides in *R. equi* isolates from foals,[13,14] which might be driven by increased use of macrolides from increased use as a result of protocols for screening foals with thoracic ultrasonography to identify foals with pneumonia before the onset of clinical signs and treating these foals with macrolides.[13,14] Although the response to treatment was similar for the 2 treatments (74% for CLR and 70% for GaM), the 90% confidence interval for the observed difference between treatments exceeded our 10% noninferiority threshold specified *a priori*. When data from Farm 2 were excluded, however, we concluded that GaM was noninferior to standard treatment. We interpreted these results as indicating that Farm 2 biased results towards inferiority. This finding was somewhat surprising because Farm 2 appeared to treat more foals with macrolides that had larger lesions. The reasons for this discrepancy are unknown, but underscore the difficulties associated with successfully conducting multicenter, farm-based clinical trials and the importance of adherence to study protocols in multicenter trials because the bias introduced can alter the magnitude and direction of treatment

effects in an unpredictable manner. Irrespective of whether we excluded Farm 2, our data indicate that lesion size impacts the odds of resolution by 28 days of treatment. This finding is not surprising as 1 might expect larger lesions to take longer to resolve.

This study had a number of important limitations. The diagnosis of *R. equi* in study foals was presumptive: we did not perform microbiologic culture and cytologic evaluation of fluid obtained by tracheobronchial aspiration to determine whether *R. equi* could be isolated from the fluid and whether there was evidence of septic inflammation consistent with infectious pneumonia. Although it would have been ideal to have these microbiologic results, it is our experience that it is common practice to eschew tracheobronchial aspiration of foals suspected to have *R. equi* pneumonia at large breeding farms that have recurrent problems with this disease because the positive predictive value of a positive result is high.[10,22] Moreover, *R. equi* was identified as the cause of death for 3 of the 4 foals included in this study. The impact of misclassification would be most important if it were differential (ie, more likely to occur in one treatment group than the other). Differential misclassification was considered highly improbable because of the study design. Nevertheless, the absence of confirmatory testing is an important limitation of our study.

Despite a schedule for randomization, more foals were assigned to standard treatment than GaM. This appears to have been largely attributable to treatment assignment at Farm 2 (Table 1). It is unclear why this occurred, but it is possible that communications were more difficult at this largest of breeding farms included in our study. This finding suggests a bias (conscious or subconscious) for treatment assignment might have existed among personnel at this farm. This finding underscores the importance and impact of adherence to protocol in conducting multicenter clinical trials at equine breeding farms. This farm's veterinary staff also elected to perform weekly rather than biweekly thoracic ultrasound examinations. Treatment failure was defined as failure to achieve resolution within 28 days, or an increase in score of $\geq$2 or more grades on the Slovis scale between ultrasound examinations 2 weeks apart. None of the foals included in the study from this farm that were treatment failures did not meet these criteria. Although the effects of weekly examinations did not have an impact on the observed results, the possibility for bias from this deviation underscores the importance of application and compliance with standard protocols for multicenter trials.

Despite randomization of assignment to treatment independent of ultrasonographic findings, lesions in macrolide-treated foals at study entry were significantly larger than those of foals in the GaM group. This discrepancy also appeared to be attributable to Farm 2: at this farm, 76% (13/17) of macrolide-treated foals had ultrasonographic lesions with maximal diameter $\geq$27 mm whereas only 14% (1/7) GaM foals had larger lesions. In an effort to control for effects of lesion size, we conducted stratified analyses using contingency tables and multivariable logistic regression. After adjusting for effects of lesion size, there was still no significant difference in treatments; although the estimated odds ratio favored standard treatment for resolution of ultrasonographic lesions, this result should be interpreted cautiously because the small sample size of the study led to estimates being statistically unstable.

Farm 2 was different from the other farms regarding the association of treatment with lesion size: the fewer foals receiving GaM at this farm had significantly smaller pulmonary lesions identified sonographically than did the macrolide-treated foals at this farm. Consequently, we conducted post hoc analysis excluding data from Farm 2 to assess the impact of the potential bias of results from including data from Farm 2. Despite reducing sample size and this statistical power, excluding data from Farm 2 resulted in rejecting the null hypothesis of GaM being $\geq$10% less effective than standard treatment. Thus, we conclude that including data from Farm 2 biased our results.

The sample size was relatively small which limited both the statistical power and the statistical precision of the study. A larger-scale, more rigorously designed study will be difficult and expensive to conduct, but will be necessary to determine whether GaM might be used as an effective alternative to standard macrolide treatment.

The initial lesion sizes of thoracic ultrasonographic lesions were modest (median, 27 mm), and the foals did not have clinical signs at the time of inclusion. Thus, it is not possible to extrapolate results of this study to foals with lesions of larger size or with clinical signs.

The absence of formal blinding is a weakness of this study. Although treatment was assigned randomly and administered by an individual not engaged in evaluation or treatment of foals (viz., the HEMI pharmacist), those treating foals were not blinded to the treatment. Those who were performing thoracic ultrasonography of foals were not those administering treatments to those foals, but there was no formal blinding of evaluators to the foal's treatment. The results of this study demonstrating equivalency must therefore be interpreted with caution because of the potential for information/outcome bias. Ideally, follow-up would have been identical for all foals. To maximize participation, however, it was apparent during the design phase that each farm required the right to maintain their own schedules for screening.

Evidence from a farm in Germany indicates that many foals that have thoracic ultrasonographic evidence of pulmonary abscess formation or consolidation attributed to *R. equi* infection (with or without mild clinical signs) treated with placebo respond similarly as foals treated with antimicrobials including macrolides.[9,11,12] Thus, a limitation of the study reported here was the absence of a placebo control group. During the design phase of the study, consultation with veterinarians and farm managers by one of the authors (NMS) indicated that they would be unwilling to participate in a placebo-controlled study. Although evidence exists that treatment is superior to placebo

among foals with relatively large ultrasonographic lesions and clinical or hematological abnormalities,[9] a recent report from the United States demonstrated that many foals with thoracic ultrasonographic lesions attributed to *R. equi* at an endemic farm resolved without treatment.[22] Thus, we cannot establish whether the proportion of either treatment group was significantly different than what might be achieved using a placebo alone.

Despite the limitations of the study reported here, we believe our results provide evidence supporting the need for a larger-scale, more rigorously designed and conducted evaluation of GaM as an intervention for foals with ultrasonographic evidence of pneumonia attributed to *R. equi* infection. Emergence of resistance to macrolides[13,14] and the limited number of alternatives to macrolide antibiotics available to veterinarians underscore the importance of identifying novel antimicrobials for use in controlling equine infectious diseases such as *R. equi* pneumonia. Although we failed to demonstrate equivalency in the total population, we did demonstrate equivalency in the subgroup of foals in which farm veterinarians and staff were compliant with the study design. Our results indicate that a large proportion of foals with relatively small ultrasonographic lesions consistent with abscessing pneumonia can be treated successfully with GaM. By using GaM rather than macrolides to treat foals with ultrasonographic evidence of presumed *R. equi* pneumonia, it is plausible that selection pressure for macrolide resistance would be decreased. Evidence exists that the minimum inhibitory concentrations of GaM for macrolide-resistant isolates of *R. equi* are similar to those for macrolide-susceptible isolates,[17] such that this approach might be particularly important for farms at which foals have been infected with macrolide-resistant *R. equi*. The findings of this study provide evidence supporting the need for further evaluation of the clinical effectiveness of GaM for control of *R. equi* pneumonia at large breeding farms. Although GaM was safely used in foals of this study and other studies, further evaluation of the short- and long-term effectiveness and safety of GaM treatment are needed.

Footnotes

[a] Version 3.1.1, R Foundation for Statistical Computing, Vienna, Austria.
[b] Medisca, Inc, Plattsburgh, New York
[c] Version 8.2; TIBCO, Inc, Seattle, WA

Acknowledgments

This study was funded by the Grayson-Jockey Club Research Foundation, with additional support from the Link Equine Research Endowment at Texas A&M University. The authors thank Ms. Courtney Brake for technical assistance.

Conflict of Interest Declaration: Dr Lawrence Bernstein is the founder of Terrametrix, a scientific consulting company headquartered in Menlo Park, California. Dr Bernstein holds a U.S. patent for medical uses of GaM in human and veterinary medicine. Dr Bernstein participated in the design of the study but did not play a role either in the conduct of the study or analysis of the data.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Giguère S, Cohen ND, Chaffin MK, et al. Diagnosis, treatment, control, and preveintion of *Rhodococcus equi* in foals. J Vet Intern Med 2011;25:1209–1220.

2. Prescott JF, Nicholson VM. The effects of combinations of selected antibiotics on the growth of *Corynebacterium equi*. J Vet Pharmacol Ther 1984;7:61–64.

3. Nordmann P, Ronco E. In-vitro antimicrobial susceptibility of *Rhodococcus equi*. J Antimicrob Chemother 1992;29:383–393.

4. Berghaus LJ, Giguère S, Guldbech K. Mutant prevention concentration and mutant selection window for 10 antimicrobial agents against *Rhodococcus equi*. Vet Microbiol 2013;166:670–675.

5. Nordmann P, Kerestedjian JJ, Ronco E. Therapy of *Rhodococcus equi* in disseminated infections in nude mice. Antimicrob Agents Chemother 1992;36:1244–1248.

6. Sweeney CR, Sweeney RW, Divers TJ. *Rhodococcus equi* pneumonia in 48 foals: Response to antimicrobial therapy. Vet Microbiol 1987;14:329–336.

7. Giguère S, Jacks S, Roberts GD, et al. Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with *Rhodococcus equi* pneumonia. J Vet Intern Med 2004;18:568–573.

8. Prescott JF, Sweeney CR. Treatment of *Corynebacterium equi* pneumonia of foals: A review. J Am Vet Med Assoc 1985;187:725–728.

9. Venner M, Credner N, Lämmer M, Giguère S. Comparison of tulathromycin, azithromycin and azithromycin-rifampin for the treatment of mild pneumonia associated with *Rhodococcus equi*. Vet Rec 2013;173:397.

10. Slovis NM, McCracken JL, Mundy G. How to use thoracic ultrasound to screen foals for *Rhodococcus equi* at affected farms. Proc Am Assoc Equine Pract 2005;51:274–278.

11. Venner M, Astheimer K, Lämmer M, Giguère S. Efficacy of mass antimicrobial treatment of foals with subclinical pulmonary abscesses associated with *Rhodococcus equi*. J Vet Intern Med 2013;27:171–176.

12. Venner M, Rödiger A, Laemmer M, Giguère S. Failure of antimicrobial therapy to accelerate spontaneous healing of subclinical pulmonary abscesses on a farm with endemic infections caused by *Rhodococcus equi*. Vet J 2012;192:293–298.

13. Burton AJ, Giguère S, Sturgill TL, et al. Macrolide- and rifampin-resistant *Rhodococcus equi* on a horse breeding farm, Kentucky, USA. Emerg Infect Dis 2013;45:507–511.

14. Giguère S, Lee E, Williams E, et al. Determination of the prevalence of antimicrobial resistance to macrolide antimicrobials or rifampin in *Rhodococcus equi* isolates and treatment outcome in foals infected with antimicrobial-resistant isolates of *R. equi*. J Am Vet Med Assoc 2010;237:74–81.

15. Bernstein LR. Mechanisms of therapeutic activity for gallium. Pharmacol Rev 1998;50:665–682.

16. Harrington JR, Martens RJ, Cohen ND, Bernstein LR. Antimicrobial activity of gallium against virulent *Rhodococcus equi* in vitro and in vivo. J Vet Pharmacol Ther 2006;29:121–127.

17. Coleman M, Kuskie K, Liu M, et al. In vitro antimicrobial activity of gallium maltolate against virulent *Rhodococcus equi*. Vet Microbiol 2010;146:175–178.

18. Martens RJ, Miller NA, Cohen ND, et al. Chemoprophylactic antimicrobial activity of gallium maltolate against intracellular *Rhodococcus equi*. J Equine Vet Sci 2007;27:341–345.

19. Martens RJ, Mealey K, Cohen ND, et al. Pharmacokinetics of gallium maltolate after intragastric administration in neonatal foals. Am J Vet Res 2007;68:1041–1044.

20. Martens RJ, Cohen ND, Fajt VR, et al. Gallium maltolate: Safety in neonatal foals following multiple enteral administrations. J Vet Pharmacol Ther 2010;33:208–212.

21. Chaffin MK, Fajt V, Martens RJ, et al. Pharmacokinetics of an orally administered methylcellulose formulation of gallium maltolate in neonatal foals. J Vet Pharmacol Ther 2010;33:376–382.

22. Chaffin MK, Cohen ND, Blodgett GP, Syndergaard M. Evaluation of ultrasonographic screening parameters for predicting subsequent onset of clinically apparent *Rhodococcus equi* pneumonia in foals. Proc Am Assoc Equine Pract 2013;59:268–269.