Association of pneumonia in foals caused by *Rhodococcus equi* with farm soil geochemistry

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Objective—To quantify and compare geochemical factors in surface soils from horse-breeding farms with horses with pneumonia caused by *Rhodococcus equi* (affected farms) and horse-breeding farms with no history of pneumonia caused by *R equi* (unaffected farms).

Sample Population—Soil from 24 *R equi*-affected farms and 21 unaffected farms.

Procedure—Equine veterinary practitioners throughout Texas submitted surface soil samples from areas most frequented by foals, on *R equi*-affected and unaffected horse-breeding farms in their practice. Soil samples were assayed for the following factors: pH, salinity, nitrate, phosphorus, potassium, calcium, magnesium, sodium, sulfur, zinc, iron, manganese, and copper. Median values for all factors were recorded, and differences between affected and unaffected farms were compared.

Results—Significant differences in soil factors were not detected between affected and unaffected farms; hence, there was no association between those factors and *R equi* disease status of the farms.

Conclusions and Clinical Relevance—The surface soil factors monitored in this study were not significant risk factors for pneumonia caused by *R equi*. As such, it is not possible to determine whether foals on a given farm are at increased risk of developing disease caused by *R equi* on the basis of these factors. Data do not support altering surface soil for factors examined, such as alkalinization by applying lime, as viable control strategies for *R equi*. (*Am J Vet Res* 2002;63:95–98)

Rhodococcus equi is a soil-borne gram-positive aerobic facultative-intracellular pathogenic actinomycete bacterium that shares many microbiologic characteristics with members of the genera *Mycobacterium* and *Corynebacterium*.[1,2] *Rhodococcus equi* is distributed worldwide and principally causes severe pyogranulomatous pneumonia in young foals and immunocompromised humans.[2,3] Although the lung is the most commonly affected organ, numerous other organ systems are often involved. During the past decade, it has been discovered that only *R equi* that possess an 85 to 90 kb virulence-associated plasmid are capable of causing disease in foals.[3] These plasmids and their 15 to 17 kDa **virulence-associated protein antigen (VapA)** products and encoding genes represent markers of virulence, but specific virulence factors have not yet been identified.[4]

Rhodococcus equi is a soil saprophyte, and virulent and avirulent strains have been isolated from soil of farms with or without horses and other herbivores and from areas not exposed to domestic animals.[2,3,5] *Rhodococcus equi* is most commonly isolated from surface soils and, as with most actinomycetes, the greatest numbers are obtained from soils with high concentrations of organic matter.[3,5,6] Inhalation of aerosolized *R equi* contaminated dust is thought to be the most common route of infection in foals.[2,7]

Numerous human and animal diseases caused by microorganisms have been associated with soil[8,9]; even when sterilized, soil possesses unidentified infection-potentiating factors.[10] Mechanisms that may be responsible for disease-potentiating effects include suppression of host defense factors; suppressed growth of competing, or enhanced growth, of complementary saprophytic bacteria, with concomitant increased pathogen growth; and aerosolization and inhalation of pathogens in soil.[8] Little is known, however, about the influence of soil geochemical characteristics on the distribution and virulence of most pathogens, including *R equi*.

Soil geochemistry, which includes mineralogic characteristics, pH, and elemental composition, has generated ever-increasing evidence that the virulence of certain pathogenic organisms is dependent on appropriate soil concentrations or bioavailability of specific cations and environmental conditions.[8,9,11-13] Soil pH can have a profound influence on the bioavailability of some nutrients to microorganisms.[8,9,13] *Rhodococcus equi* grows well over a wide pH range of 6.0 to 8.5, with an optimum pH of 7.5.[14] Expression of the VapA, however, is maximal at pH 6.5, and it is not produced at pH 8.0.[15] Although the micronutrient requirements for *R equi* are not known, actinomycetes generally require fairly precise concentrations of iron and zinc.[8] There may be other conditions or soil factors that have an impact on the ability of *R equi* to multiply and cause disease or on the ability of the host to com-

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bat infections.[8] In the case of a soil-borne pathogen such as *R equi*, these elements may be acquired directly from the soil or from the animal host.

Association of soil constituents or conditions with a disease may identify risk factors for that disease. Disease control strategies may then be designed on the basis of those risk factors. Such is the case with Johne's disease of cattle, caused by *Mycobacterium paratuberculosis*. The prevalence of paratuberculosis-positive cattle is positively associated with acidic soil and increased soil iron content.[13] Herd-level control of Johne's disease can be achieved by alkalinizing pasture soils with lime, because iron, an essential element for most bacteria, has decreased bioavailability in alkaline soil.[13]

To our knowledge, the association of soil geochemical properties with disease caused by *R equi* has not been reported. Appropriately designed epidemiologic studies of soil geochemical characteristics from geographically diverse *R equi* disease-positive and disease-negative farms should provide information needed to evaluate associations between those characteristics and disease. Knowledge regarding such associations may be of value in identifying potential *R equi* virulence factors, risk factors for disease, and disease control strategies. The purpose of the study reported here was to evaluate the association of various geochemical variables of soil from horse-breeding farms throughout Texas with the presence of disease caused by *R equi* on those farms.

Materials and Methods

Farms—Veterinarians in Texas who were members of the American Association of Equine Practitioners were invited to participate in the study by submitting soil samples from actively operating horse-breeding farms in their practice. A horse-breeding farm was defined as a farm that produced at least 1 foal during the 1999 breeding season. Each veterinarian was requested to submit a sample from a farm that had at least 1 foal affected with *R equi* pneumonia during the 1999 breeding season (affected farm) and a farm that had no affected foals during that year or any known history of *R equi* infections (unaffected farm). A foal affected with *R equi* pneumonia was defined as a 3-week to 6-month-old foal with either clinical signs of pneumonia and from which *R equi* was cultured from a tracheobronchial fluid sample or lung sample obtained at postmortem, or clinical signs of pneumonia and at least 2 of the following: multifocal pulmonary opacities on thoracic radiography, ultrasonographically visible pulmonary abscesses, cytologically-visible gram-positive intracellular coccobacilli in tracheobronchial wash specimens, or a history of endemic *R equi* disease on the farm.

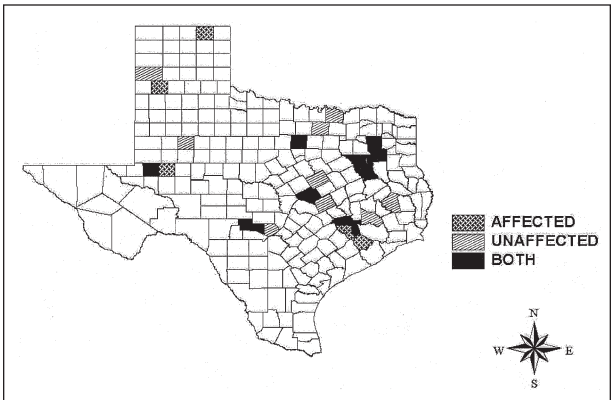

Figure 1—Geographic distribution of counties in Texas from which horse breeding-farm soil samples were assayed for the presence of *Rhodococcus equi*, with depiction as to whether samples from those counties were from farms that were affected, unaffected, or both.

Participants—Twenty-four veterinarians participated in the study by submitting soil samples from 21 affected and 24 unaffected farms. Participating farms were located in geographically diverse regions throughout Texas (**Fig 1**).

Soil collection—Approximately 225 g of surface soil were collected from 2 sites (no less than 10 m apart) most frequented by foals on each farm. The 2 samples were combined and submitted in a sterile plastic bag to a soil testing laboratory.[a]

Soil sample analysis—All soil samples were dried overnight at 70 C, pulverized through a soil grinder,[b] and passed through a 2-mm sieve. Samples were homogenized by mixing and assayed for the following variables and constituents: pH, salinity, nitrate, phosphorous, potassium, calcium, magnesium, sodium, sulfur, zinc, iron, manganese, and copper.

pH—Ten grams of soil were combined with 20 ml of deionized water. This suspension was allowed to equilibrate for 30 minutes, mixed, and pH was measured by use of a standard glass combination electrode.[16,c]

Salinity—Water-soluble salts (dissolved inorganic solutes) in a 1:2 (soil:deionized water) solution used to measure pH were assayed with a conductivity electrode[c] and expressed as milligrams/grams of sodium chloride.[17]

Nitrate—Two grams of soil were combined with 20 ml of 1.0 *M* potassium chloride. The suspension was shaken for 10 minutes at 185 **cycles per minute (cpm)**, filtered through No. 2 filter paper,[d] and the filtrate was analyzed on an autoanalyzer fitted with a cadmium reduction column.[18,19,e] Results are expressed as milligrams/grams.

Phosphorous, potassium, calcium, magnesium, sodium, and sulfur—Briefly, 1.7 cm^3 of soil were combined with 20 ml of ammonium acetate-ethylenediaminetetraacetic acid extracting solution. The suspension was shaken for 1 hour at 185 cpm, filtered through No. 2 filter paper,[d] and the filtrate was transferred to a borosilicate test tube for analysis on an **inductively coupled plasma optical emission spectrophotometer (ICP-OES)**.[20,f] Results are reported as milligrams/grams.

Table 1—Results of soil sample analyses from 24 Texas farms with horses affected with pneumonia associated with *R equi* and from 21 Texas farms with horses that were not affected

Covariate	Affected farms Median (range)	Unaffected farms Median (range)	*P
pH	7.5 (5.4 – 9.3)	7.0 (5.3 – 8.6)	0.50
Salinity (µg/g)	561(104 – 2548)	445 (52 – 9,724)	0.18
Nitrate (µg/g)	78.5 (6 – 324)	41 (4 – 402)	0.22
Phosphorus (µg/g)	317.5 (9 – 1,302)	219 (17 – 1,095)	0.78
Potassium (µg/g)	499 (192 – 2,648)	469 (51 – 9,223)	0.71
Calcium (µg/g)	3,598 (812 – 30,018)	4,678 (570 – 30,015)	0.66
Magnesium (µg/g)	280 (100 – 1,338)	232 (22 – 1,462)	0.35
Sodium (µg/g)	197.5 (36 – 680)	257 (18 – 1,057)	0.25
Sulfur (µg/g)	91 (11 – 490)	76 (7 – 1,839)	0.88
Zinc (µg/g)	6.9 (1.2 – 34.6)	6.9 (2.4 – 55.1)	0.98
Iron (µg/g)	12.4 (2.5 – 61.2)	16.9 (4.2 – 86.7)	0.30
Manganese (µg/g)	12.4 (3.2 – 68.0)	16.9 (4.6 – 53.7)	0.14
Copper (µg/g)	0.6 (0.3 – 7.6)	0.8 (0.2 – 1.8)	0.84

*Value of *P* for Wilcoxon rank-sum test comparing distribution of affected and unaffected farms.

Zinc, iron, manganese, and copper—Twenty grams of soil were combined with 40 ml of extractant (0.005 *M* DTPA, 0.1 *M* triethanolamine, and 0.01 *M* calcium chloride, with a pH of 7.3) and mixed at 120 cpm for 2 hours.[21] The suspension was filtered through No. 2 filter paper,[d] and the filtrate was analyzed on an ICP-OES.[f] Results are reported as micrograms/grams.

Statistical analyses—Differences in soil covariates (pH, salinity, etc) between affected and unaffected farms were compared, using the Wilcoxon rank-sum test.[22] Values of $P \leq 0.05$ were considered significant.

Results

Soil analyses were performed on samples from 24 farms affected with *R equi* and 21 farms that were not affected. None of the soil covariates differed significantly between affected and unaffected farms (**Table 1**).

Discussion

This investigation compared the micronutrient composition and pH of soil samples from 24 horse-breeding farms affected with *R equi* pneumonia and 21 unaffected horse-breeding farms. There was no apparent association between these factors and the *R equi* disease status of those farms. It is important to know that risk analysis of a farm, for foals with pneumonia caused by *R equi*, cannot be based on the magnitude of these geochemical factors in surface soils obtained from pastures and paddocks.

Because there were no differences in soil factors monitored between affected and unaffected farms, results of this study do not support surface soil alteration of these factors as a disease control strategy for pneumonia caused by *R equi*. Alkalinization of soil by the addition of lime has proven to be of value in the control of Johne's disease.[13] On the basis of previous evidence, however, attempts to control *R equi* infections by alkalinizing soil may be counterproductive by encouraging growth of the organism and expressions of virulence.[3,14,15]

Respiratory disease is the most common cause of disease and death in foals in Texas.[23] Based on experiences of the authors, informal surveys of practicing veterinarians in Texas, and the worldwide literature, *R equi* is the most common cause of severe pneumonia in foals 3 weeks to 6 months of age.[2,3,7] A recent investigation in Texas indicated the widespread distribution of virulent and avirulent *R equi* in farm soils and in foals with pneumonia caused by *R equi*.[4] There appears to be a high prevalence of virulent and avirulent *R equi* in Texas farm soils and of *R equi*-induced disease on Texas horse-breeding farms. Perhaps it would have been more appropriate to compare soils from Texas with those from more divergent geographic regions (eg, other states or countries) that have a low incidence of the disease. In addition, the design of this study may have resulted in the farms being too similar with respect to geographic or geologic distribution, thereby diminishing the intended diversity.

Results of experimental and epidemiologic studies[24,25] indicate that foals are much more susceptible to infection with *R equi* during the first days to weeks of life and that most spontaneous infections are initiated during this period. Clinically normal foals, however, are highly resistant to infection by several weeks to months of age.[2,24] Bearing in mind that *R equi* is a soil saprophyte, it is conceivable that foals are exposed to the organism during the first hours to days of life when born and maintained in quarters with dirt floors. A recent report[26] suggested that foaling stalls with dirt floors pose a greater risk to foals of becoming infected with *R equi* than foaling stalls with synthetic floors. This same study indicated a positive association between horse density, particularly among foaling mares and foals, and increased risk of foals acquiring pneumonia associated with *R equi*. Foaling stalls and quarters where foals are maintained during the first few weeks of life are often used by numerous mares and foals over time. The horse density of those quarters relative to exposure of soil to *R equi* is often quite great. Consequently, it may have been more appropriate to sample soil from areas where the foals commonly reside during the first few days and weeks of life, rather than from paddocks and pastures.

Surface soils were analyzed in this study rather than homogenized samples from the top 6 inches of soil, as recommended for assessment of soil fertility.[a] There are several reasons why surface soils were selected for monitoring. The greatest concentrations of *R equi*, virulent and avirulent, are present in surface soils.[2,3,5,6] Surface soil in areas most commonly inhabited by horses generally contain the highest concentrations of manure, which provides essential volatile fatty acids for *R equi*, and may contain large numbers of *R equi*.[2,3,14] Surface soils are most readily aerosolized, and inhalation of aerosolized dust appears to be the most common route of infection with *R equi*.[2,3,7] Perhaps analysis of subsurface soils would have revealed an association between geochemical characteristics and *R equi* infections. Certain micronutrients can modulate host immune functions that may alter responses to infection, and those nutrients are indirectly obtained from the soil. It is important to bear in mind that trace elements in vegetal matter reflect the composition of the soils on which they grow, and those of the host reflect the composition of vegetal matter they consume.[27]

A variety of trace minerals and other micronutrients that may be obtained directly from the environment or host are required for survival and growth of bacteria, and some of them are also instrumental in regulating bacterial virulence. Because of these close associations, there is substantial justification for additional investigations on the impact of micronutrients on *R equi* disease pathogenesis.

[a]Texas Agricultural Extension Service-Soil Testing Laboratory, College Station, Tex.
[b]Dinocrush, Custom Laboratory Equipment, Orange City, Fla.
[c]Conductivity Meter, Model 220, Denver Instrument Co, Arvada, Colo.
[d]Fisher Scientific, Pittsburgh, Pa.
[e]Quik Chem 800, Lachat Instruments, Milwaukee, Wis.
[f]Autoanalyzer, Model FPT SA88D, Spectro Analytic, Fitchburg, Mass.

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