Prevalence and Virulence of *Rhodococcus equi* in Sick Foals and Soil of Horse-Breeding Farms in Texas

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*Rhodococcus equi* per se and *R. equi* containing a virulence-associated plasmid can be cultured with similar frequency from the soil of farms with and without a history of *R. equi* disease. This finding, in conjunction with variability in results between testing procedures and laboratories, indicates that identification of *R. equi* by culture from farm soils does not accurately predict the probability of disease on those farms. Authors’ addresses: College of Veterinary Medicine, Texas A&M University, College Station, TX 77843 (Martens, Cohen, Chaffin, and Liu); Kitasato University, Towada, Aomori 034, Japan (Takai); and Texas Veterinary Medical Diagnostic Laboratory, College Station, TX 77843 (Lingsweiler). © 1999 AAEP.

1. Introduction

Although many different organisms may cause pneumonia, *Rhodococcus equi* is considered the most common cause of severe pneumonia in foals.\(^1\) *R. equi* is a gram-positive, opportunistic, intracellular bacterium that can flourish and multiply in soil, and foals with *R. equi*-induced pneumonia are capable of shedding large numbers of *R. equi* organisms onto the soil.\(^1\) The disease is endemic on some farms, is sporadic on other farms and does not occur on most farms. Recent epidemiologic studies indicate that the difference in prevalence of the disease on farms is directly related not to the number of *R. equi* organisms in the soil, but rather to the prevalence of virulent *R. equi* in the soil.\(^1\)

There is a significant correlation between the presence of virulence markers in *R. equi* and virulence of the organism in horses.\(^1\) \(\beta\)-lactam antibiotic resistance has also been correlated with virulence in *R. equi* obtained from humans and a limited number of animals.\(^2\)

In an effort to determine whether a farm or a particular site on a farm is at risk of promoting infections with *R. equi*, veterinary practitioners may be requested to culture soil samples from the farm. This study was designed to simulate the conditions and capabilities that might routinely be available to practicing veterinarians through their diagnostic laboratories.

2. Materials and Methods

Isolates of *R. equi* were obtained in 1997 from foals that had spontaneous pneumonia. These bacteria were harvested from respiratory tract specimens submitted by veterinary practitioners throughout Texas. The isolates were examined for the presence...
of virulence-associated plasmids by two laboratories. One laboratory used polymerase chain reaction (PCR) to detect the presence of a plasmid, and the other laboratory used Western immunoblot analysis to detect presence of the plasmid-encoded protein.1

Soil samples were obtained as part of a prospective, matched case-control study of equine breeding farms. Veterinarians submitted soil samples from matched pairs of breeding farms. Each pair consisted of a farm that had at least 1 foal with R. equi-induced pneumonia in 1997 (affected farm) and 1 farm that had no known history of R. equi infections (control farm). Composite surface soil samples were collected from three sites frequented by foals on the farm. Soil samples, submitted in sterile plastic bags, were mixed, a saline suspension was prepared and serial dilutions were cultured for R. equi by the laboratories. The two laboratories used different types of selective growth media.3,4 Representative colonies of R. equi isolated from soil were assessed by the respective laboratories for the presence of virulence-associated plasmid DNA or virulence-associated proteins.

More than 100 R. equi isolates obtained from pneumonic foals and from the soil of affected and control farms were assessed for resistance to the β-lactam antibiotics penicillin G and cephalothin by comparing minimum inhibitory concentrations.

Proportions and paired categorical data were compared to determine statistical significance.

3. Results
Virulence-associated plasmids or their specific protein products were present in almost all R. equi organisms obtained from pneumonic foals. There was complete correlation between laboratories in detection of virulence-associated plasmids in the clinical isolates.

Selective R. equi culturing of soil samples by the two laboratories yielded significantly different results. One laboratory isolated R. equi from a significantly greater number of farms, both affected and control, than the other laboratory. In addition, there was poor agreement between laboratories on the isolation of R. equi from specific soil samples. Each laboratory recovered R. equi from samples that yielded negative test results at the other laboratory. However, if R. equi was isolated from the soil of a farm by either laboratory, that farm was considered culture-positive. When considering all matched pairs of farms, there was no significant difference between affected and control farms in the frequency with which R. equi was cultured from the soil.

When the two laboratories tested their respective R. equi soil isolates for the presence of virulence-associated plasmids, one laboratory detected plasmids significantly more often from both affected and control farms. In addition, there was not good correlation between laboratories in the detection of virulence-associated plasmids on specific farms. If either laboratory detected a virulence-associated plasmid in R. equi from a farm, that farm was considered plasmid-positive. Approximately two-thirds of the pairs of farms had R. equi cultured from soil of both affected and control premises. However, there was no significant difference between affected and control farms in the frequency with which virulence-associated plasmids were detected.

The frequency of virulence-associated plasmids in R. equi obtained from pneumonic foals was significantly greater than in R. equi isolated from soil on disease-positive and/or disease-negative farms. R. equi isolates, of which approximately one-half were virulence-associated plasmid-positive, were assayed for resistance to penicillin G and cephalothin. No significant correlation was found between β-lactam antibiotic resistance and presence of a virulence-associated plasmid, presence of disease or a farm’s disease status.

4. Discussion
The R. equi obtained in clinical cases were examined by both laboratories, using different methods to validate the correlation between PCR and immunoblot analysis for the detection of virulence-associated plasmids between the respective laboratories and to substantiate or refute the correlation between the presence of plasmids and disease.1 The excellent correlation between laboratory results validated the testing procedures between laboratories, and the high degree of association between virulence-associated plasmids and disease was corroborated.1

The disparity between laboratories in R. equi culture results was most likely attributable to different inhibitory effects of the two types of selective media. There is also the possibility that the samples were not adequately homogenized, thereby yielding different results. Because of the inability to culture R. equi consistently from a single composite soil sample, it may be preferable to culture samples from multiple sites on a farm. In addition, it may be advantageous to use more than one type of selective media when culturing soil for R. equi.

Previous studies indicate good correlation between the number of R. equi organisms, more specifically the quantity of virulent R. equi in the soil, and the R. equi disease status of the farms.1 When the matched pairs of farms on which R. equi was cultured from both farms (i.e., affected and control) are compared, there is no significant correlation between the presence of virulence-associated plasmids and disease status. When results from each laboratory are considered separately, there is still no association of culture-positive or virulence-associated plasmid-positive status with farm disease status.

One laboratory detected a significantly greater number of plasmid-positive isolates. However, there was not good correlation between laboratories in the detection of virulence-associated plasmids on specific farms. Because there was excellent correlation
between the laboratories in detection of virulence-associated plasmids in *R. equi* isolates from infected foals, the most probable explanation for divergent results in soil isolates is that the laboratories were identifying different isolates from the same soil samples.

When *R. equi* isolates were compared, there was no significant correlation between β-lactam antibiotic resistance and the presence of a virulence-associated plasmid. In addition, there was no significant correlation between β-lactam antibiotic resistance of *R. equi* obtained from a farm and the disease status of that farm. These results refute previous findings.²

Based on the results of this study, it appears as though specific variables have not yet been defined adequately to determine whether foals on a farm or at a specific site on a farm are at increased risk of developing disease caused by *R. equi*.

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References