JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2009, p. 734–737 Vol. 47, No. 3
0095-1137/09/$08.00+0 doi:10.1128/JCM.01395-08

Detection of Virulent *Rhodococcus equi* in Exhaled Air Samples from Naturally Infected Foals[▽]

G. Muscatello,† J. R. Gilkerson, and G. F. Browning*

Equine Infectious Disease Laboratory, School of Veterinary Science, The University of Melbourne, Parkville, Victoria 3010, Australia

Received 21 July 2008/Returned for modification 12 October 2008/Accepted 3 January 2009

Virulent *Rhodococcus equi* causes pyogranulomatous bronchopneumonia in foals. The route of infection of foals has been considered to be inhalation of aerosolized bacteria from soil that is contaminated with equine feces. Thus, disease caused by *R. equi* has been regarded as an opportunistic infection of environmental origin and not a contagious disease. In this study, we report the exhalation of virulent *R. equi* from the respiratory tract of naturally infected foals. A handheld air-monitoring system was used to recover virulent *R. equi* from the exhaled breath of foals, and the concentration of virulent *R. equi* organisms in exhaled air was compared to the concentration in environmental air samples taken from the holding pens and lane areas on farms. *R. equi* strains carrying the *vapA* gene of the virulence plasmid were detected by using colony blotting and DNA hybridization techniques in cultures of exhaled air from 67% (37/55) of foals tested. The concentration of virulent *R. equi* organisms in exhaled air from foals was significantly higher than that in environmental air ($P < 0.001$). There were no significant differences in the median concentrations of virulent *R. equi* bacteria exhaled by clinically healthy or diseased foals. The high concentrations of virulent *R. equi* bacteria in exhaled air suggested that aerosol transmission between foals is possible and may have a significant impact on the prevalence of *R. equi* pneumonia on farms. The air sampling technique described is potentially useful as a noninvasive method for the detection and quantification of virulent *R. equi* in the respiratory tract of foals.

Rhodococcus equi is a gram-positive coccobacillus found in the soil and the feces of grazing herbivores (3). Virulent strains of *R. equi* cause bronchopneumonia in horses, primarily affecting foals between 1 and 4 months of age (5, 10, 19, 29). Virulent *R. equi* contains an 85- to 90-kb plasmid that carries genes for virulence-associated proteins (Vaps) (24, 25). The virulence plasmid and these Vaps, particularly the VapA cell surface protein, are considered the factors that enable these strains to induce pneumonia in foals (6, 13). Recent *vap* mutagenesis studies have found that reversion to virulence occurred on the insertion of *vapA* into the virulence plasmid but not after insertion of the *vapC*, *-D*, *-E*, or *-F* gene (13). This finding highlights the importance of *vapA* in virulence and vindicates its use as a fundamental criterion for the identification of virulent *R. equi* in diagnostic assays in horses and epidemiological studies over the last decade (5, 15, 22, 23). The accepted pathogenesis of *R. equi* pneumonia is that virulent *R. equi* bacteria in the soil are aerosolized, inhaled, and phagocytosed by alveolar macrophages, in which they multiply and cause pneumonia. This hypothesis has been accepted by investigators because the organism is a soil saprophyte and is detectable as part of the healthy gastrointestinal flora of horses. Growth of *R. equi* has been shown to be enhanced (up to 10,000-fold) in the presence of volatile fatty acids common in equine feces (3, 11). Intestinal carriage has been demonstrated in adults, but foals up to 12 weeks of age and particularly those with clinical disease have been found to have considerably higher concentrations of *R. equi* organisms in their feces than adults. Thus, feces from foals are thought to be the main source of soil contamination (23, 26, 27). It has been suggested that as the concentration and proportion of the virulent organisms in the environmental *R. equi* population increase, there is an associated increased risk of inhalation of virulent *R. equi* from the soil (22), and thus, an increase in the prevalence of disease caused by *R. equi* infection (23, 27). Recent studies have also shown that higher concentrations of airborne virulent *R. equi* and high proportions of virulent *R. equi* among airborne *R. equi* populations are associated with an increased prevalence of *R. equi* pneumonia (15).

Areas frequented by foals and environmental conditions that favor multiplication in the soil and aerosolization of *R. equi* from the soil, such as poor pasture cover or dry or sandy soils, are considered higher risks for foals (15).

There are many reasons why *R. equi* pneumonia has not been considered a contagious respiratory disease. The intracellular habitat of the organism in the alveolar macrophages of infected foals, the sometimes problematic recovery of *R. equi* from tracheal lavage fluid samples from foals with clinical *R. equi* pneumonia, and the production of abscesses in the lungs of infected foals have suggested that direct animal-to-animal transmission is unlikely (2, 8, 9, 14). However, if foal-to-foal transmission by the aerosol route were possible, virulent *R. equi* might be acquired more readily within a closely confined group of foals than by the inhalation of aerosolized bacteria from soil. This could explain the variable efficacy of environmental management strategies, such as irrigation of pastures, pens, and laneways and collection of feces, in reducing the *R. equi* disease burden in herds.

The aim of this study was, first, to establish whether virulent

* Corresponding author. Mailing address: School of Veterinary Science, The University of Melbourne, Parkville, Victoria 3010, Australia. Phone: 61 3 8344 7342. Fax: 61 3 8344 7374. E-mail: glenfb@unimelb.edu.au.

† Present address: Faculty of Veterinary Science, The University of Sydney, Sydney, New South Wales 2006, Australia.
▽ Published ahead of print on 14 January 2009.

TABLE 1. Concentration of virulent *R. equi* in exhaled air from foals with or without clinical *R. equi* pneumonia

Disease status	No. of positive foals[a]/total	Concn of virulent *R. equi* (CFU/1,000 liters)[b]		
		Median	Range	IQR
R. equi pneumonia	31/48	8.0 (12.0)	0.0–160.0 (4.0–160.0)	0.0–16.0 (8.0–27.0)
Healthy	6/7	8.0 (8.0)	0.0–60.0 (8.0–60.0)	8.0–16.0 (8.0–24.0)
Total	37/55	8.0 (10.0)	0.0–160.0 (4.0–160.0)	0.0–16.0 (8.0–22.0)

[a] Proportion of foals in which virulent *R. equi* was detected in exhaled air.
[b] For values in parentheses, only positive samples were included. IQR, interquartile range (first to third quartile).

R. equi could be detected in the exhaled air of foals with or without *R. equi* pneumonia. If virulent *R. equi* was detected in exhaled air samples, then a second aim was to compare the concentrations of airborne virulent *R. equi* organisms in high-risk environmental areas with those detected in the exhaled air of foals. In this way, the possibility that virulent *R. equi* would be spread between foals and its significance in comparison with the currently accepted horse-environment cycle of infection could be evaluated.

MATERIALS AND METHODS

Collection and analysis of samples. Air samples were collected by using a portable air-monitoring system (M Air T; Millipore) onto ceftazidime-novobiocin agar modified by the addition of cycloheximide (40 μg/ml) as an antifungal agent (mCAZ-NB) (15, 16, 18). Cultures were blotted as described previously (16, 17), with virulent *R. equi* (isolate 7) and avirulent *R. equi* (isolate 128) organisms as controls and a negative control (*Bacillus fumarioli*) incorporated into each blot. The blots were probed with a ^{32}P-labeled PCR product amplified from the virulent *R. equi vapA* gene to identify virulent *R. equi* colonies, as described previously (16). Briefly, a single 500-liter environmental air sample was taken in the holding pens (small outdoor fenced areas used to confine horses prior to procedures such as breeding of mares, veterinary treatment, and farriery) and lanes along which horses were moved to and from pens and paddocks. Exhaled air samples from foals were collected by holding the air-monitoring device to the muzzle of a manually restrained foal. A single 100- or 250-liter sample was collected from each foal. The procedure took between 1 and 2 min, depending on the volume of air sampled. The sieve was disinfected with an isopropanol wipe (Isowipe; Kimberly-Clark, Milson's Point, NSW, Australia) before the collection of each air sample. The effectiveness of this disinfection method was tested prior to sampling. Ten microliters of broth-cultured *R. equi* ($\sim10^7$ colony-forming units [CFU]/ml) was used to inoculate four different areas of the sieve, and 1,000-liter air samples were collected onto mCAZ-NB agar. The sieve was disinfected, and the sampling was then repeated. The inoculation of the sieve, disinfection, and air sample collection were repeated another three times. The agar plates were incubated for 48 h at 37°C and examined for colonies of *R. equi*. *R. equi* was recovered from air samples on the regions of the agar plates that corresponded to the areas of the sieve that were inoculated but only from the samples collected prior to disinfection in each experiment. No *R. equi* was recovered from samples taken after disinfection with the isopropanol wipes, indicating that this method of disinfection was adequate for decontamination of the air sampler between collections of samples in the field.

Farms and foals. Exhaled air samples were collected from 55 foals on eight Thoroughbred farms in Victoria and New South Wales, Australia, during the 2000 and 2001 breeding seasons. Samples were collected from foals at a single point in time after diagnosis or at the time of ultrasonographic lung examination. Environmental air samples were collected monthly between November 2000 and February 2001 from the lanes and holding pens on six Thoroughbred farms. All six farms from which environmental air samples were collected reported cases of *R. equi* pneumonia during the sampling period. On three of these six farms, exhaled air samples were collected from foals with *R. equi* pneumonia.

There was no formal, randomized sampling frame used to determine which foals were sampled. Initially, only foals with *R. equi* pneumonia were selected to test the hypothesis that virulent *R. equi* could be detected in exhaled air. The foals from which exhaled air was collected ranged in age from 25 to 90 days. Of these, 35 foals (64%) were from two farms that had a prevalence of *R. equi* pneumonia of over 9% during the study period. Forty-five of the 55 foals were being treated for *R. equi* pneumonia when they were sampled. All 45 foals treated for *R. equi* pneumonia had clinical signs of pneumonia and at least one other diagnostic feature of *R. equi* pneumonia: specifically, ultrasonographically detectable pulmonary abscesses ($n = 36$), leukocytosis with neutrophilia and fibrinogenemia ($n = 37$), and/or an *R. equi*-positive culture from a tracheal lavage fluid sample ($n = 10$). Eleven foals on one farm were sampled while they were being restrained for routine thoracic ultrasonographic examination. Seven of these foals were considered healthy, with no ultrasonographically detectable lung lesions suggestive of *R. equi* pneumonia, while one had been diagnosed with *R. equi* pneumonia prior to sampling. The remaining three foals had ultrasonographically detectable lung abscesses and were diagnosed with *R. equi* pneumonia, despite the absence of overt signs of pneumonia.

Data and statistical analysis. Median concentrations of virulent *R. equi* organisms in the environmental and the exhaled air samples and the median concentrations of virulent *R. equi* bacteria exhaled by clinically healthy and diseased foals were compared using the Mann-Whitney test.

RESULTS

Concentrations of virulent *R. equi* organisms in exhaled air from foals. Virulent *R. equi* was detected in exhaled air samples from foals with and without detectable bronchopneumonia. Virulent *R. equi* was recovered from the breath samples of 37 of the 55 foals tested (67%). The median concentration of virulent *R. equi* organisms in exhaled air was 8 CFU/1,000 liters. There was no significant difference in the median concentrations of virulent *R. equi* exhaled by clinically healthy or that of diseased foals (Table 1).

Concentrations of virulent *R. equi* in environmental air. All but one farm had detectable airborne virulent *R. equi* in the lanes and pens during the sampling period. In total, 19/48 (40%) environmental air samples collected across the six farms sampled contained detectable concentrations of virulent *R. equi* organisms, with a median concentration of virulent *R. equi* in these positive samples of 2 CFU/1,000 liters. Farms C and F had the greatest number of positive samples (Table 2).

Comparison of the concentrations of virulent *R. equi* organisms in environmental and exhaled air samples. The frequency of detection of virulent *R. equi* in environmental air samples was almost half that of the frequency of detection in exhaled air samples from foals, even though the volume of air sampled from the environment was more than twice that sampled from foals. The median concentration of virulent *R. equi* organisms in positive samples of exhaled air (10 CFU/1,000 liters) was fivefold higher than that in environmental air samples (2 CFU/1,000 liters). The median concentration of virulent *R. equi* organisms in all exhaled air samples was significantly higher than the concentration in all environmental air samples ($P < 0.001$) and was also significantly higher when only positive samples were considered ($P < 0.001$).

TABLE 2. Concentration of airborne virulent *R. equi* in the pens and lanes on 6 farms during the 2000 foaling season

Farm	No. of positive samples[a]/total	Concn of virulent *R. equi* (CFU/1,000 liters)[b]		
		Median	Range	IQR
A	2/8	0.0	0.0–2.0	0.0–1.5
B	0/8	0.0	0.0–0.0	0.0–0.0
C	5/8	2.0	0.0–24.0	0.0–9.5
D	4/8	1.0	0.0–8.0	0.0–2.0
E	3/8	0.0	0.0–6.0	0.0–3.5
F	5/8	2.0	0.0–2.0	0.0–2.0
Total	19/48	0.0 (2.0)	0.0–24.0 (2.0–24.0)	0.0–2.0 (2.0–4.0)

[a] Proportion of samples in which virulent *R. equi* was detected.
[b] For values in parentheses, only positive samples were included. IQR, interquartile range (first to third quartile).

DISCUSSION

Despite being an intracellular pathogen of the equine lower respiratory tract, virulent *R. equi* was detected in air samples collected near the muzzles of foals. The median concentration of virulent *R. equi* organisms in breath samples was significantly higher than the median concentration in environmental air samples. These environmental air samples were collected from areas of the farm that have been shown previously to be the most highly contaminated with airborne virulent *R. equi* (15). This suggested that exhaled air from foals may be a significant source of virulent *R. equi* for other foals. Studies of experimentally infected foals suggest that the minimum infectious dose of virulent *R. equi* required to cause pneumonia is approximately 10^4 CFU/foal, although not all foals inoculated with this dose subsequently developed clinical disease (28). Based on the concentrations of airborne virulent *R. equi* detected in this study, a foal would require a substantially shorter period of exposure to a contaminated airborne source to acquire this minimum infectious dose when in contact with another foal exhaling high concentrations of virulent *R. equi* than when only standing and inhaling contaminated dust in a contaminated laneway or holding pen. Foals are unlikely to spend long periods of time in lanes and pens (tens of hours or days) but would routinely spend shorter periods of time (up to several hours) in crowded pens during the breeding season on large horse breeding farms while awaiting veterinary attention or farriery.

Virulent *R. equi* was detected in exhaled air from 31/48 (65%) of the foals with *R. equi* pneumonia. In a previous study, Hillidge (8) recovered *R. equi* from lesions from 7/11 (67%) foals with *R. equi* pneumonia at necropsy and from 57/89 (64%) tracheal lavage fluid. In a more recent study, *R. equi* was recovered from 14/21 (67%) transtracheal and 59/96 (61%) nasotracheal lavage fluid from foals with clinical signs of *R. equi* pneumonia (7). Thus, culture of exhaled air samples appears likely to be at least as sensitive for the detection of clinically affected foals as culture of tracheal lavage specimens, which is still seen as the definitive method for the diagnosis of *R. equi* pneumonia. Although the sensitivity of air sampling is comparable to that of tracheal lavage fluid samples, the specificity of this test is poor, as 6/7 (86%) healthy foals also exhaled virulent *R. equi*. These results highlight the difficulties facing clinicians attempting to diagnose *R. equi* pneumonia, as healthy foals may be a source of infection and so a diagnosis based only on bacteriological detection of virulent organisms in the respiratory tract may be erroneous.

Three apparently healthy foals in this study were found to have lesions suggestive of *R. equi* pneumonia by ultrasonographic examination. Ultrasonographic examination of lungs has been used widely on horse farms as a diagnostic aid for investigating suspected cases of *R. equi* pneumonia and as a screening tool on properties with a consistently high prevalence of disease. However, ultrasonographic examination of lungs has limitations in the diagnosis of *R. equi* pneumonia, as other bacterial causes of pneumonia, such as *Streptococcus equi* subsp. *equi*, may induce similar lesions. Furthermore, only superficial lesions are detectable. Deep or mediastinal lesions and small lesions that do not involve the pleural surface of the lung may not be detected (20, 21).

Virulent *R. equi* was detected in samples of exhaled air from 6/7 clinically healthy foals with no ultrasonographically detectable lung abscesses. This suggests that these foals either had lung abscesses that were undetectable by ultrasonographic examination or were subclinically infected and were shedding virulent *R. equi* from their respiratory tracts without any obvious lung pathology. As these foals did not develop clinical respiratory illness after sampling, it is more likely that they had subclinical infections with virulent *R. equi*. Subclinical rhodococcal disease has been seen previously (2). *R. equi* was recovered from transtracheal lavage fluid from 77/216 (36%) clinically healthy foals in one study (1). That study was performed prior to the differentiation of virulent and avirulent *R. equi* and hence could not assess the subclinical carriage of virulent *R. equi*. Subclinical infection may be more common on farms with a high disease prevalence, as these farms are more likely to have elevated environmental burdens of virulent *R. equi*. The reasons that subclinically infected foals do not develop clinical disease and the potential for these foals to act as respiratory sources of virulent *R. equi* need to be explored.

We chose to investigate the likelihood of foal-to-foal transmission under field conditions, using quantitative sampling to distinguish the probable contributions of environmental bacteria and those exhaled by foals, because this method was felt to enable a more accurate assessment of the potential significance of the route of infection than one using experimental infection. The ubiquity of *R. equi* in the environment of horses and suggestions that foals may be infected at a very young age limit the capacity to conduct studies that adequately mimic natural infection and establish contagious transmission under controlled experimental conditions (3, 10). There is no certainty that an intratracheal or intrabronchial bolus of cultured *R. equi*, the method currently used to experimentally infect foals, would accurately mirror natural infection (12, 28). Such a challenge is likely to result in a distribution of lesions in the respiratory tract that is different from that seen after natural exposure to aerosols over an extended period of time, and thus excretion by experimentally infected foals is not likely to be fully representative of the situation in the field. Virulent *R. equi* is commonly isolated from the soil on farms and from bedding material and is ingested and excreted in the feces of both diseased and healthy foals, resulting in some degree of environmental contamination on all farms, regardless of the disease status (18, 22, 27). Therefore, there could be no certainty in an experimental model of transmission that the source of infection was the respiratory tract of the infected foals and not organisms excreted in the feces of the foals and then aerosolized from the environment by their movement.

The role of foal-to-foal transmission of virulent *R. equi* in the epidemiology of *R. equi* pneumonia requires further investigation. The inhalation of dust contaminated with virulent *R. equi* is still likely to be an important source of infection, as a high environmental burden of virulent *R. equi* is correlated with a higher disease prevalence (15, 22). However, foals are often confined in crowded areas such as holding pens for many hours while awaiting veterinary attention and farriery, and even within large paddocks, the likelihood of aerosol transmission from foal to foal may be relatively high. In one study, foals kept in small groups (fewer than five mare-foal pairs per group) were found to have a reduced risk of developing *R. equi* pneumonia. However, once the year of birth and farm effects were accounted for, the association was not significant ($P = 0.071$) (4). Even though this finding was not statistically significant, the effect of group size on prevalence warrants further investigation. Management strategies to limit the likelihood of foal-to-foal aerosol transmission, such as decreasing the length of time foals are confined in crowded areas and reducing group sizes, may need to be considered as methods to reduce the prevalence of *R. equi* pneumonia on farms.

ACKNOWLEDGMENTS

This work was supported by the Rural Industries Research and Development Corporation of Australia.

We thank Garry Anderson for assistance with statistical analysis.

REFERENCES

1. **Ardens, A. A., S. K. Hietala, M. S. Spensley, and A. Sansome.** 1986. Studies of naturally occurring and experimental *Rhodococcus equi* (*Corynebacterium equi*) pneumonia in foals. Proc. Am. Assoc. Equine Pract. **32:**129–144.
2. **Barton, M. D., and D. H. Embury.** 1987. Studies of the pathogenesis of *Rhodococcus equi* infections in foals. Aust. Vet. J. **64:**332–339.
3. **Barton, M. D., and K. L. Hughes.** 1984. Ecology of *Rhodococcus equi*. Vet. Microbiol. **9:**65–76.
4. **Chaffin, K. M., N. D. Cohen, R. J. Martens, R. F. Edwards, and M. Nevill.** 2003. Foal-related risk factors associated with development of *Rhodococcus equi* pneumonia on farms with endemic infection. J. Am. Vet. Med. Assoc. **223:**1791–1799.
5. **Giguere, S., and J. F. Prescott.** 1997. Clinical manifestation, diagnosis, treatment and prevention of *Rhodococcus equi* infections in foals. Vet. Microbiol. **56:**313–334.
6. **Giguère, S., M. K. Hondalus, J. A. Yager, P. Darrah, D. M. Mosser, and J. F. Prescott.** 1999. Role of the 85-kilobase plasmid and plasmid-encoded virulence-associated protein A in intracellular survival and virulence of *Rhodococcus equi*. Infect. Immun. **67:**3548–3557.
7. **Hashikura, S., T. Higuchi, S. Taharaguchi, Y. Orita, Y. Nanao, and S. Takai.** 2000. Evaluation of nasotracheal aspiration as a diagnostic tool for *Rhodococcus equi* pneumonia in foals. Equine Vet. J. **32:**560–564.
8. **Hillidge, C. J.** 1987. Use of erythromycin-rifampin combination in treatment of *Rhodococcus equi* pneumonia. Vet. Microbiol. **14:**337–342.
9. **Hondalus, M. K.** 1997. Pathogenesis and virulence of *Rhodococcus equi*. Vet. Microbiol. **56:**257–268.
10. **Horowitz, M. L., N. D. Cohen, S. Takai, T. Becu, K. M. Chaffin, K. K. Chu, K. G. Magdesian, and R. J. Martens.** 2001. Application of Sartwell's model (lognormal distribution of incubation periods) to age at onset and age at death of foals with *Rhodococcus equi* pneumonia as evidence of perinatal infection. J. Vet. Intern. Med. **15:**171–175.
11. **Hughes, K. L., and I. Sulaiman.** 1987. The ecology of *Rhodococcus equi* and physicochemical influences on growth. Vet. Microbiol. **14:**241–250.
12. **Jacks, S., S. Giguere, P. C. Crawford, and W. L. Castleman.** 2007. Experimental infection of neonatal foals with *Rhodococcus equi* triggers adult-like gamma interferon induction. Clin. Vaccine Immunol. **14:**669–677.
13. **Jain, S., B. R. Bloom, and M. K. Hondalus.** 2003. Deletion of *vapA* encoding virulence associated protein A attenuates the intracellular actinomycete *Rhodococcus equi*. Mol. Microbiol. **50:**115–128.
14. **Martens, R. J., R. A. Fiske, and H. W. Renshaw.** 1982. Experimental subacute foal pneumonia induced by aerosol administration of *Corynebacterium equi*. Equine Vet. J. **14:**111–116.
15. **Muscatello, G., G. A. Anderson, J. R. Gilkerson, and G. F. Browning.** 2006. Associations between the ecology of virulent *Rhodococcus equi* and the epidemiology of *R. equi* pneumonia on Australian thoroughbred farms. Appl. Environ. Microbiol. **72:**6152–6160.
16. **Muscatello, G., and G. F. Browning.** 2004. Identification and differentiation of avirulent and virulent *Rhodococcus equi* using selective media and colony blotting DNA hybridization to determine their concentrations in the environment. Vet. Microbiol. **100:**121–127.
17. **Muscatello, G., J. R. Gilkerson, and G. F. Browning.** 2007. Comparison of two selective media for the recovery, isolation, enumeration and differentiation of *Rhodococcus equi*. Vet. Microbiol. **119:**324–329.
18. **Muscatello, G., S. Gerbaud, C. Kennedy, J. R. Gilkerson, T. Buckley, M. Klay, D. P. Leadon, and G. F. Browning.** 2006. Comparison of concentrations of *Rhodococcus equi* and virulent *R. equi* in air of stables and paddocks on horse breeding farms in a temperate climate. Equine Vet. J. **38:**263–265.
19. **Prescott, J. F.** 1991. *Rhodococcus equi*: an animal and human pathogen. Clin. Microbiol. Rev. **4:**20–34.
20. **Ramirez, S., G. Lester, and G. Roberts.** 2004. Diagnostic contribution of thoracic ultrasonography in 17 foals with *Rhodococcus equi* pneumonia. Vet. Radiol. Ultrasound **45:**172–176.
21. **Reef, V. B.** 1998. Thoracic ultrasonography, p. 187–214. *In* Equine diagnostic ultrasound. WB Saunders Company, Philadelphia, PA.
22. **Takai, S.** 1997. Epidemiology of *Rhodococcus equi* infections: a review. Vet. Microbiol. **56:**167–176.
23. **Takai, S., J. Takahagi, Y. Sato, K. Yamaguchi, S. Kakizaki, F. Takehara, S. Matsukura, Y. Tamada, A. Tani, Y. Sasaki, S. Tsubaki, and M. Kamada.** 1994. Molecular epidemiology of virulent *Rhodococcus equi* in horses and their environment, p. 183–187. *In* H. Nakajima and W. Plowright (ed.), Proceedings of the 7th International Conference on Equine Infectious Diseases, R&W Publications, Newmarket, United Kingdom.
24. **Takai, S., M. Shoda, Y. Sasaki, S. Tsubaki, G. Fortier, S. Pronost, K. Rahal, T. Becu, A. P. Begg, G. F. Browning, V. N. Nicholson, and J. F. Prescott.** 1999. Restriction fragment length polymorphisms of virulent plasmids in *Rhodococcus equi*. J. Clin. Microbiol. **37:**3417–3420.
25. **Takai, S., S. A. Hines, T. Sekizaki, V. N. Nicholson, D. C. Alperin, M. Osaki, D. Takamatus, M. Nakamura, K. Suzuki, N. Ogino, T. Kakuda, H. Dan, and J. F. Prescott.** 2000. DNA sequence and comparison of virulence plasmids from *Rhodococcus equi* ATCC 33701 and 103. Infect. Immun. **68:**6840–6847.
26. **Takai, S., S. Iimori, and S. Tsubaki.** 1986. Quantitative fecal culture for early diagnosis of *Corynebacterium (Rhodococcus) equi* enteritis in foals. Can. J. Vet. Res. **50:**479–484.
27. **Takai, S., S. Ohbushi, K. Koike, S. Tsubaki, H. Oishi, and M. Kamada.** 1991. Prevalence of virulent *Rhodococcus equi* in isolates from soil and feces of horse-breeding farms with and without endemic infections. J. Clin. Microbiol. **29:**2887–2889.
28. **Wada, R., M. Kamada, T. Anzai, and S. Takai.** 1997. Pathogenicity and virulence of *Rhodococcus equi* in foals. Vet. Microbiol. **56:**301–312.
29. **Zink, M. C., J. A. Yager, and N. L. Smart.** 1986. *Corynebacterium equi* infections in horses, 1958–1984: a review of 131 cases. Can. Vet. J. **27:**213–217.