



Epidemiology of *Rhodococcus equi* infections: A review

Shinji Takai *

Department of Animal Hygiene, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034, Japan

Abstract

An overview of epidemiology of *R. equi* infection in foals is presented, emphasizing the importance of the virulence-associated antigens and plasmids as epidemiological markers. The monoclonal antibody-based colony blot test has been developed to identify rapidly and accurately virulent *R. equi*. Epidemiological studies conducted during the recent 5 years have revealed that: (1) avirulent *R. equi* are widespread in the feces of horses and their environment on every farm; (2) the feces of horses and the environment of the horse farms having endemic *R. equi* infections demonstrated heavy contamination with virulent *R. equi*, but the farms without the problem did not, thus suggesting that foals bred on a farm with endemic disease are exposed more frequently to virulent *R. equi* in their environment than those of a farm without the problem; (3) only virulent *R. equi* are isolated from lesions of naturally infected foals, showing that natural infections in foals are principally by virulent *R. equi*, but not avirulent organisms; (4) infected foals which constantly shed large quantities of virulent *R. equi* in their feces are the major source of virulent *R. equi*, which this may be the mechanism of progressive development of infection on farms with a history of the disease. At present, farms with a potential for endemic infection can be distinguished on the basis of the contamination with virulent *R. equi*, so regular examination of foals and their environment by virulence markers might be the most practical approach to control *R. equi* infection on endemic farms. © 1997 Elsevier Science B.V.

Keywords: Epidemiology; *Rhodococcus equi*; Foal; Review

* Corresponding author. Tel.: +81-176-234371 ext. 459; fax: +81-176-238703; e-mail: takai@vmas.kitasato-u.ac.jp.

1. Introduction and historical aspects

Rhodococcus equi, formerly known as *Corynebacterium equi*, was initially described by Magnusson (1923) as a causative agent of purulent bronchopneumonia in young foals in Sweden. The disease has worldwide distribution, and in general causes sporadic disease, though it has become endemic on some farms. Three clinical forms of the disease have been recognized: an acute pneumonic form, a more characteristic chronic pneumonic form showing pyogranulomatous abscesses, and the less common intestinal form associated with mesenteric lymphadenitis (Zink et al., 1986; Prescott, 1991). *R. equi* is a soil organism that is widespread in the feces of herbivores, especially horses, and their environment (Barton and Hughes, 1980; Woolcock et al., 1980; Prescott et al., 1984; Takai and Tsubaki, 1985b). Inhalation and ingestion are considered to be of infection routes (Martens et al., 1982; Johnson et al., 1983a; Johnson et al., 1983b). *R. equi* infection has been considered an opportunistic infection in foals, since the increased susceptibility of young foals to *R. equi* infection is thought to be the failure of transfer or waning of maternal immunity, and immaturity or impairment of cell-mediated immunity (Yager, 1987). Furthermore, the type strain of *R. equi*, ATCC 6939, which was isolated from an infected foal by Magnusson (1923), could not induce pneumonia experimentally in foals (Takai et al., 1987b). In such circumstances, researchers and clinical veterinarians have generalized that a combination of the increased susceptibility of young foals to infection and other predisposing factors are the most important factors in the pathogenesis of the disease (Yager, 1987).

1.1. Quantitative aspects of *R. equi* on horses and their environment

R. equi has been thought to be a soil organism; but until the development of a selective NANAT medium by Woolcock et al. (1979) there was no effective way to isolate it from the contaminated samples, such as soil and feces. This medium has led to advanced epidemiological and ecological studies, in which *R. equi* has been shown to multiply in soil and the feces of foals and to be widespread in horses and their environment (Woolcock et al., 1980; Woolcock and Mutimer, 1981; Barton and Hughes, 1984; Prescott et al., 1984; Takai et al., 1986b, 1987a; Debey and Bailie, 1987; Hughes and Sulaiman, 1987).

Barton and Hughes (1984) pointed out that dung was important as a source of infection, since *R. equi* multiplies to 1000 times or more in the affected horses' dung which is left on the paddock or the pasturing ground. Prescott et al. (1984) investigated *R. equi* contamination of the loafing paddocks and pasture areas on 6 farms in Ontario. They pointed out the danger of progressive development of infection in affected soil on horse farms with prolonged use, because conditions favorable to the proliferation of *R. equi* were provided in the environment of a horse-breeding farm which had been established for 30 years. Takai et al. (1986b) studied the ecology of *R. equi* in soil on a horse-breeding farm and indicated that *R. equi* could multiply in the soil and flourish in the cycle existing between horses and their soil environment. The highest numbers of *R. equi* were found in surface soil, whereas almost no bacteria were found in soil observed 30 cm or more underground. In addition, *R. equi* grows as well in soil exudate broth as

in nutrient broth, so that it is able to proliferate in soil when growth conditions such as temperature and humidity are suitable for growth (Takai et al., 1986b). Moreover, *R. equi* was isolated from air in the stalls at the end of March, and the number of *R. equi* in the air increased particularly on dry and windy days (Takai et al., 1987a). Falcon et al. (1985) pointed out that prevalence of the disease appears to increase in dusty environments and dry weather.

R. equi was also isolated from the feces of dams at various isolation rates (Debey and Baillie, 1987; Takai et al., 1986c; Woolcock et al., 1980). The mean numbers of *R. equi* obtained from the feces of these adults ranged from 10^2 to 10^3 per gram of feces. On the other hand, *R. equi* was isolated first from the feces of foals at 1 to 2 weeks of age, and then it was isolated from all of the foals by 4 weeks of age (Takai et al., 1986c). The mean number of *R. equi* increased to 10^4 to 10^5 /g of feces, was maintained up to the age of 8 to 10 weeks, and then gradually decreased to the level of the dams. The greatest numbers of *R. equi*, which were 100- to 1000-fold greater than those of the dams, were present in the intestinal tract during the first 8 weeks of life, when foals are most liable to the infection (Takai et al., 1986c). In addition, foals with *R. equi* pneumonia shed a large number of *R. equi* (10^6 – 10^8 /g of feces) in the feces, and the quantitative culture of *R. equi* in the feces was useful for making a supplementary diagnosis of *R. equi* infection (Takai et al., 1986a). The excretion of this huge number of *R. equi* almost certainly plays an important role in contamination and expansion of the source of infection. However, these studies conducted at the environment of horse-breeding farms in 1980's were based on the quantitative aspect of *R. equi*, but not qualitative aspect, since virulence markers of *R. equi* were unclear at that time (Prescott, 1991).

2. Molecular epidemiology of *R. equi* infection in foals

The recent interest in virulence mechanism of *R. equi* has undoubtedly been stimulated by the discovery of the virulence-associated antigens and plasmids (Takai et al., 1991a,c; Tkachuk-Saad and Prescott, 1991). Virulent *R. equi* contains a large plasmid of 85 or 90 kb, which contains the gene responsible for the expression of 15 to 17 kDa antigens (Table 1). These findings led to a breakthrough in studies on the epidemiology, pathogenesis, and diagnosis of *R. equi* infection in foals (Takai et al., 1995c; Tan et al., 1995).

Table 1
Virulence markers and pathogenicities of *R. equi* in mice and foals

Strain	Virulence-associated antigens	Virulence plasmid (kb)	Pathogenicity for mice (LD ₅₀)	Pathogenicity for foals (MID ^a)
Virulent	15–17 kDa	85, 90	10^6	10^4
Avirulent	—	—	$> 10^8$	$> 10^9$
ATCC 6939	—	—	$> 10^8$	$> 10^9$

^a Minimum Infective Dose.

2.1. 15 to 17 kDa antigens and plasmids are useful markers in epidemiological studies of *R. equi* infection

In 1991, we analyzed antigens from clinical and soil isolates of *R. equi* with naturally infected sera (Takai et al., 1991a). Whole-cell antigens of the clinical isolates revealed major protein bands of molecular masses of 15 to 17 kDa, and all isolates containing these antigens were virulent for mice. In contrast, 77 of 102 soil isolates lacked the 15 to 17 kDa antigens and were avirulent for mice. Next, we further evaluated 10 isolates of *R. equi* comparing the immunoblot assays for the 15 to 17 kDa antigens, plasmid profiles, and mouse pathogenicity (Takai et al., 1991c). All isolates containing the 15 to 17 kDa antigens contained a large plasmid of approximately 85 kb and were virulent for mice. Curing of this plasmid coincided with a loss of detectable 15 to 17 kDa antigens and a striking decrease in lethality in mice. In addition, the 15 to 17 kDa antigens were not present in type strain ATCC 6939, which was avirulent for mice (Takai et al., 1991c). At the same time, Tkachuk-Saad and Prescott (1991) isolated plasmids from 54 isolates from different clinical sources. A plasmid of approximately 80 kb was isolated from 21 of 22 isolates from horses and 20 of 28 isolates from pigs. A larger plasmid, of approximately 105 kb, was isolated from 7 of 28 pig isolates. There was a significant but not exact association between the presence of the 80 kb plasmid and the production of a diffuse 17.5 kb protein. In 1993, we surveyed for plasmid DNA among isolates obtained from foals at postmortem examination (Takai et al., 1993c). Of the 23 clinical isolates, 19 contained an 85 kb plasmid and the remaining 4 contained a 90 kb plasmid. All of the isolates contained the 15 to 17 kDa antigens and were virulent for mice. When these 85 and 90 kb plasmids were examined by restriction enzyme and Southern blot analyses, there were large regions of DNA homology, which suggested that they have a common origin.

2.2. Development of rapid identification methods of virulent *R. equi*

Monoclonal antibodies against virulence-associated 15 to 17 kDa antigens were produced (Takai et al., 1993b) and a monoclonal antibody-based colony blot test was developed for rapid and specific identification of virulent *R. equi* (Takai et al., 1994b), since these antigens are located on the externally exposed surface of cells (Takai et al., 1992). More recently, the plasmid gene encoding the 15 to 17 kDa antigens has been cloned, and sequenced (Kanno et al., 1993; Sekizaki et al., 1995) and described as VapA (Tan et al., 1995). This terminology will be used in the rest of this review. A PCR assay was developed for the rapid identification of virulence plasmids of *R. equi* based on the DNA sequence obtained (Takai et al., 1995a). During a survey of the prevalence of virulent *R. equi* at horse-breeding farms by plasmid and protein profiles, cryptic plasmids of various sizes were found in isolates from feces of horses and from soil at the rate of 3 to 5% (Takai et al., 1993c, 1994a). Some cryptic plasmids were very similar in size to the virulent plasmids of *R. equi*. Then, we tested 20 different plasmids of various sizes in isolates from horses and their environment by PCR, but none of these plasmids, except 85 and 90 kb, were found to possess the gene (Takai et al., 1995a). The PCR is a rapid, sensitive, and specific test for identification of virulent *R. equi* from environmen-

tal isolates compared with plasmid and protein profiles and the mouse pathogenicity test, and is considered to be a useful tool for epidemiological studies. These tests might help accelerate progress in molecular epidemiology of *R. equi* infection.

2.3. *Natural infections in foals are caused principally by virulent R. equi but not by avirulent organisms*

Empirically, clinical isolates have been noted to be virulent for mice (Nakazawa et al., 1983; Takai et al., 1985a; Bowles et al., 1987), and freshly isolated *R. equi* has been shown to induce pneumonia experimentally in foals (Martens et al., 1982; Johnson et al., 1983a; Takai et al., 1987b). However, we have not known whether all of clinical isolates from infected foals are virulent or not. In 1993, we surveyed for virulent *R. equi* among the isolates obtained from 23 foals having the infection at the postmortem examination (Takai et al., 1993c). All of the isolates contained virulence-associated antigens and plasmids, and were virulent for mice. Thirteen of stock strains of *R. equi* from 12 independent clinical cases collected from 1984 to 1985 by Equine Research Institute, Japan Racing Association were also analyzed for the presence of virulence plasmid, and all clinical isolates except one were virulent *R. equi* (Takai et al., 1993a).

Epidemiological surveillance of infected foals with *R. equi* has been conducted in Hidaka, a breeding area for race horses in Japan, from 1992 by our laboratory. Of 54 cases of *R. equi* infection in foals submitted for necropsy by 1994, almost all cases were diagnosed as suppurative pneumonia, and of these, 24 had associated ulcerative enterocolitis and mesenteric and/or colonic lymphadenitis with and without abscesses (Takai et al., 1994e). The average age at death of the 54 foals was 70 ± 21 days old. All of the isolates from the lung lesions of these foals were found to be virulent *R. equi*. Of the 54 foals, 44 foals were infected with virulent *R. equi* containing an 85 kb plasmid and 10 foals were infected with *R. equi* containing a 90 kb plasmid. The ratio of 85 and 90 kb plasmids in clinical isolates was similar to that in the environmental isolates from the studfarms (Takai et al., 1994a). Half of the foals showed intestinal lesions at necropsy. Large amounts of virulent *R. equi* were also isolated from intestinal contents collected from the stomach to the rectum in these infected foals (Takai et al., 1994e). It has been thought that the intestinal changes seen with *R. equi* infection in foals often follow pneumonic infection when infected sputum is swallowed by a pneumonic foal; we have confirmed bacteriologically this phenomenon in naturally infected foals. These bacteriological studies suggest that natural infections in foals are caused principally by virulent *R. equi*, but not by avirulent organisms. In our recent experimental infections in foals, strain ATCC 33701 caused severe lesions in foals following aerosolization, but a plasmid-cured derivative of strain ATCC 33701 failed to induce lesions (Wada et al., 1997). These results also confirm the above conclusion.

2.4. *Mechanism of the attenuation of ATCC 6939: A rational speculation*

Strain ATCC 6939, which was isolated from a foal with fatal pneumonia by Magnusson (1923), could not experimentally reproduce *R. equi* infection in foals (Takai et al., 1987b). The type strain consistently failed to yield a plasmid and the virulence-as-

sociated antigens (Takai et al., 1991c; Tkachuk-Saad and Prescott, 1991). The type strain has been passaged repeatedly since it was isolated. It is very likely that the attenuation of strain ATCC 6939 occurred as a result of curing the plasmid in the strain by repeated passage but the phenomenon of attenuation is not fully understood (Takai et al., 1991c). We demonstrated that attenuation of virulent strains of *R. equi* by repeated passage at 38°C is attributable to the selection of spontaneous plasmid-cured derivatives which exhibit a higher growth rate than do their virulent parents (Takai et al., 1994d). At 30°C, the growth rate of neither strain was affected by the presence or absence of the plasmid. Workers who intend to examine the virulence of isolates in their epidemiological studies therefore need to be aware that growth temperature is an important factor in maintaining the virulence of *R. equi*.

2.5. Horse-breeding farms with endemic infection are contaminated heavily with virulent R. equi

One characteristic of *R. equi* infection is that it occurs endemically on some farms and sporadically on others, but is not found on the majority of farms (Prescott, 1987). Before the finding of virulence markers of *R. equi*, it had been thought that endemic farms have a greater number of *R. equi* in stable soil than non-endemic ones, and that the number of cases of *R. equi* pneumonia tended to correlate with the number of *R. equi* in soil (Prescott et al., 1984; Takai et al., 1986c). We have proposed that the incidence of infection depends on the magnitude of contamination of horses and their environment with virulent *R. equi* (Takai et al., 1991b). To demonstrate this hypothesis, we investigated the prevalence of virulent *R. equi* contamination in horses and their environment (Takai et al., 1994a; Takai et al., 1994e). Virulent *R. equi* has been isolated from soil in paddocks and feces of foals at a high frequency on farms with the problem, whereas the environment of farms without a history of *R. equi* infection was only slightly contaminated with virulent *R. equi* (Takai et al., 1991b, 1994e). Susceptible foals on farms with endemic disease may therefore be at a higher risk of infection for infection than the same foals located on a farm without a history of endemic disease. Wada et al. (1997) described that the minimum infective dose of virulent *R. equi* for establishment of experimental infection in foals was about 10^4 /foal, or less. This number of virulent *R. equi* in the minimum dose is not high as that per gram of soil or feces of horses on farms with the problem! These studies strongly suggest an important fact to explain the characteristic of *R. equi* infection in foals: the difference in the prevalence of the disease on the farms is related not just to the number of *R. equi* in the environment, but also to the prevalence of virulent *R. equi* in the environment (Table 2).

A pneumonic foal on a farm with the disease can constantly shed virulent *R. equi* in its feces at over 10^6 /g because its intestinal contents contained a very high percentage of virulent *R. equi* (Takai et al., 1994e, Anzai et al., 1997). This observation indicates that infected foals are the major source of virulent *R. equi* and that they contaminate the environment of farms by shedding large quantities of *R. equi* in their feces. This is probably be the mechanism of progressive development of infection on stud farms with a history of the disease (Takai et al., 1994e). These findings demonstrate the usefulness of virulence markers such as VapA antigens and virulence plasmids in epidemiological

Table 2

The difference in the incidence of the disease on the farms is related to the prevalence of virulent *R. equi* in the environment

Farm type	Contamination with <i>R. equi</i>	Prevalence of virulent <i>R. equi</i> in soil/feces
Endemic	Heavy?	> 20%?
Sporadic	Slight?	5–10%?
None	Low?	< 5%?

studies of *R. equi* contamination on horse-breeding farms. Therefore, it is important to know whether or not the environment of horse-breeding farms is contaminated by virulent *R. equi*, and to anticipate the danger of infection.

3. Intermediately virulent *R. equi* is isolated from submaxillary lymph nodes of pigs

R. equi has emerged as an important pulmonary pathogen among immunosuppressed patients, especially those with human immunodeficiency virus infection (Prescott, 1991). We have recently reported that some isolates from AIDS patients were found by immunoblotting to contain a 20 kDa antigen and were intermediately virulent (10^7 bacteria needed for lethality) in mice (Takai et al., 1995b). Moreover, these isolates contained one of four distinct large plasmids of 79 to 100 kb. The majority of the isolates from patients with AIDS were virulent and of intermediate virulent (Takai et al., 1994c, 1995b). Contact with farm animals and manure was reported in about one-third of the human cases (Takai et al., 1994c). However, the route of infection in majority of human cases remains obscure. To examine the distribution of intermediately virulent *R. equi* in the human environment, we attempted to isolate *R. equi* from soil and sand at parks and gardens (Takai et al., 1996b). *R. equi* was isolated from these samples with a high frequency, but no virulent or intermediately virulent *R. equi* was found in these isolates. *R. equi* has been isolated from the submaxillary lymph nodes of pigs with and without lesions resembling those of tuberculosis lesions, and the causative role of *R. equi* in granulomatous lymphadenitis in swine remains unclear. In our recent study, we isolated *R. equi* with the 20 kDa antigen from the submaxillary lymph nodes of pigs and demonstrated that these isolates were intermediately virulent for mice and contained one of five large plasmids of 79 to 95 kb (Takai et al., 1996a). It is very important to reveal the route of infection in AIDS patients with *R. equi* from the standpoint of zoonotic potential.

4. Conclusion

Epidemiological studies of *R. equi* infection in foals have been advanced since discovery of virulence-associated antigens and plasmids. We can measure the contami-

nation of virulent *R. equi* in horses and their environment on farms with the problem by means of virulence markers, and judge the risk of infection on these farms. Since infected foals are the major source of contamination, isolation of infected foals from the rest and removal of manure, especially to that from infected foals, are very important for farmers to prevent the progressive contamination of virulent *R. equi* on farms, and might be not so difficult to carry out (Prescott, 1987). It is very difficult to clean up the contaminated farms with virulent *R. equi*, but soil dressing of the contaminated paddocks, where susceptible foals are reared, might be one solution to the problem. We need studies of the efficacy, cost, and choice of disinfectants for pasture decontamination. At present, regular examination by virulence markers might be the most practical approach to control *R. equi* infection on endemic farms on the basis of current epidemiological understanding.

Acknowledgements

This study was supported by a grant-in-aid from the Equine Research Institute of Japan Racing Association, and a grant-in-aid for general scientific research (06454119, 06807028) from the Ministry of Education, Science and Culture, Japan. I thank T. Sekizaki, M. Kamada, H. Madarame, T. Anzai, R. Wada, T. Kanemaru, and T. Higuchi for helpful discussions over the years. We also thank Y. Sasaki, S. Tsubaki and our undergraduate students at the Department of Animal Hygiene, School of Veterinary Medicine and Animal Sciences, Kitasato University, for their assistance in this work.

References

- Anzai, T., Wada, R., Nakanishi, S. and Takai, S., 1997. Comparison of transtracheal washing with other tests for diagnosis of *Rhodococcus equi* pneumonia of horses. *Vet. Microbiol.*, this issue.
- Barton, M.D. and Hughes, K.L., 1980. *Corynebacterium equi*: a review. *Vet. Bull.*, 50: 65–80.
- Barton, M.D. and Hughes, K.L., 1984. Ecology of *Rhodococcus equi*. *Vet. Microbiol.*, 9: 65–76.
- Bowles, P.M., Woolcock, J.B. and Mutimer, M.D., 1987. Experimental infection of mice with *Rhodococcus equi*: differences in virulence between strains. *Vet. Microbiol.*, 14: 259–268.
- Debey, M.C. and Bailie, W.E., 1987. *Rhodococcus equi* in fecal and environmental samples from Kansas horse farms. *Vet. Microbiol.*, 14: 251–257.
- Falcon, J., Smith, B.P., O'Brien, T.R., Carlson, G.P. and Biberstein, E., 1985. Clinical and radiographic findings in *Corynebacterium equi* pneumonia of foals. *J. Am. Vet. Med. Assoc.*, 186: 593–599.
- Hughes, K.L. and Sulaiman, I., 1987. The ecology of *Rhodococcus equi* and physicochemical influences on growth. *Vet. Microbiol.*, 14: 241–250.
- Johnson, J.A., Prescott, J.F. and Markham, R.J., 1983a. The pathology of experimental *Corynebacterium equi* infection in foals following intrabronchial challenge. *Vet. Pathol.*, 20: 440–449.
- Johnson, J.A., Prescott, J.F. and Markham, R.J., 1983b. The pathology of experimental *Corynebacterium equi* infection in foals following intragastric challenge. *Vet. Pathol.*, 20: 450–459.
- Kanno, T., Asawa, T., Ito, H., Takai, S., Tsubaki, S. and Sekizaki, T., 1993. Restriction map of a virulence-associated plasmid of *Rhodococcus equi*. *Plasmid*, 30: 309–311.
- Magnusson, H. 1923., Spezifische infektiöse Pneumonie beim Fohlen. Ein neuer Eiterreger beim Pferd. *Arch. Wiss. Prakt. Tierhelkd.*, 50: 22–38.
- Martens, R.J., Fiske, R.A. and Renshaw, H.W., 1982. Experimental subacute foal pneumonia induced by aerosol administration of *Corynebacterium equi*. *Equine. Vet. J.*, 14: 111–116.

- Nakazawa, M., Haritani, M., Sugimoto, C. and Isayama, Y., 1983. Virulence of *Rhodococcus equi* for mice. *J. Vet. Med. Sci.*, 45: 679–682.
- Prescott, J.F., 1987. Epidemiology of *Rhodococcus equi* infection in horses. *Vet. Microbiol.*, 14: 211–214.
- Prescott, J.F., 1991. *Rhodococcus equi*: an animal and human pathogen. *Clin. Microbiol. Rev.*, 4: 20–34
- Prescott, J.F., Travers, M., Yager-Johnson, J.A., 1984. Epidemiological survey of *Corynebacterium equi* infections on five Ontario horse farms. *Can. J. Comp. Med.*, 48: 10–13.
- Sekizaki, T., Takai, S., Egawa, Y., Ikeda, T., Ito, H. and Tsubaki, S., 1995. Cloning and nucleotide sequence of *Rhodococcus equi* virulence-associated 15- to 17-kilodalton antigens gene. *Gene*, 155: 135–136.
- Takai, S., Anzai, T., Sasaki, Y., Tsubaki, S. and Kamada, M., 1993a. Virulence of *Rhodococcus equi* isolates from lesions of infected foals. *Bull. Equine Res. Inst.*, 30: 9–14.
- Takai, S., Anzai, T., Yamaguchi, K., Kakizaki, S., Takahagi, J., Sato, Y., Takehara, F., Tamada, Y., Matsukura, S., Tani, A., Kato, M., Seno, N., Sasaki, Y., Tsubaki, S. and Kamada, M., 1994a. Prevalence of virulence plasmids in environmental isolates of *Rhodococcus equi* from horse-breeding farms in Hokkaido. *J. Equine Sci.*, 5: 21–25.
- Takai, S., Fujimori, T., Katsuzaki, K. and Tsubaki, S., 1987a. Ecology of *Rhodococcus equi* in horses and their environment on horse-breeding farms. *Vet. Microbiol.*, 14: 233–239.
- Takai, S., Fukunaga, N., Ochiai, S., Imai, Y., Sasaki, Y., Tsubaki, S. and Sekizaki, T., 1996a. Identification of intermediately virulent *Rhodococcus equi* isolates from pigs. *J. Clin. Microbiol.*, 34: 1034–1037.
- Takai, S., Fukunaga, N., Ochiai, S., Sakai, T., Sasaki, Y. and Tsubaki, S., 1996b. Isolation of virulent and intermediately virulent *Rhodococcus equi* from soil and sand on parks and yards in Japan. *J. Vet. Med. Sci.*, 58: 669–672.
- Takai, S., Iie, M., Kobayashi, C., Morishita, T., Nishio, Y., Ishida, T., Fujimura, T., Sasaki, Y. and Tsubaki, S., 1993b. Monoclonal antibody specific to virulence-associated 15- to 17-kilodalton antigens of *Rhodococcus equi*. *J. Clin. Microbiol.*, 31: 2780–2782.
- Takai, S., Iie, M., Watanabe, Y., Tsubaki, S. and Sekizaki, T., 1992. Virulence-associated 15- to 17-kilodalton antigens in *Rhodococcus equi*: temperature-dependent expression and location of the antigens. *Infect. Immun.*, 60: 2995–2997.
- Takai, S., Iimori, S. and Tsubaki, S., 1986a. Quantitative fecal culture for early diagnosis of *Corynebacterium (Rhodococcus) equi* enteritis in foals. *Can. J. Vet. Res.*, 50: 479–484.
- Takai, S., Ikeda, T., Sasaki, Y., Watanabe, Y., Ozawa, T., Tsubaki, S. and Sekizaki, T., 1995a. Identification of virulent *Rhodococcus equi* by amplification of gene coding for 15- to 17-kilodalton antigens. *J. Clin. Microbiol.*, 33: 1624–1627.
- Takai, S., Imai, Y., Fukunaga, N., Uchida, Y., Kamisawa, K., Sasaki, Y., Tsubaki, S. and Sekizaki, T., 1995b. Identification of virulence-associated antigens and plasmids in *Rhodococcus equi* from patients with AIDS. *J. Infect. Dis.*, 172: 1306–1311.
- Takai, S., Kawazu, S. and Tsubaki, S., 1987b. Humoral immune response of foals to experimental infection with *Rhodococcus equi*. *Vet. Microbiol.*, 14: 321–327.
- Takai, S., Koike, K., Ohbushi, S., Izumi, C. and Tsubaki, S., 1991a. Identification of 15- to 17-kilodalton antigens associated with virulent *Rhodococcus equi*. *J. Clin. Microbiol.*, 29: 439–443.
- Takai, S., Michizoe, T., Matsumura, K., Nagai, M., Sato, H. and Tsubaki, S., 1985a. Correlation of in vitro properties of *Rhodococcus (Corynebacterium) equi* with virulence for mice. *Microbiol. Immunol.*, 29: 1175–1184.
- Takai, S., Morishita, T., Nishio, Y., Sasaki, Y., Tsubaki, S., Higuchi, T., Hagiwara, S., Senba, H., Kato, M., Seno, N., Anzai, T. and Kamada, M., 1994b. Evaluation of a monoclonal antibody-based colony blot test for rapid identification of virulent *Rhodococcus equi*. *J. Vet. Med. Sci.*, 56: 681–684.
- Takai, S., Narita, K., Ando, K. and Tsubaki, S., 1986b. Ecology of *Rhodococcus (Corynebacterium) equi* in soil on a horse-breeding farm. *Vet. Microbiol.*, 12: 169–177.
- Takai, S., Ohbushi, S., Koike, K., Tsubaki, S., Oishi, H. and Kamada, M., 1991b. Prevalence of virulent *Rhodococcus equi* in isolates from soil and feces of horses from horse-breeding farms with and without endemic infections. *J. Clin. Microbiol.*, 29: 2887–2889.
- Takai, S., Ohkura, H., Watanabe, Y. and Tsubaki, S., 1986c. Quantitative aspects of fecal *Rhodococcus (Corynebacterium) equi* in foals. *J. Clin. Microbiol.*, 23: 794–796.
- Takai, S., Sasaki, Y., Ikeda, T., Uchida, T., Tsubaki, S. and Sekizaki, T., 1994c. Virulence of *Rhodococcus equi* isolates from patients with and without AIDS. *J. Clin. Microbiol.*, 32: 457–460.

- Takai, S., Sasaki, Y. and Tsubaki, S., 1995c. *Rhodococcus equi* infection in foals: a review. *J. Equine Sci.*, 6: 105–119.
- Takai, S., Sekizaki, T., Ozawa, T., Sugawara, T., Watanabe, Y., Tsubaki, S., 1991c. Association between a large plasmid and 15- to 17-kilodalton antigens in virulent *Rhodococcus equi*. *Infect. Immun.*, 59: 4056–4060.
- Takai, S., Sugawara, T., Watanabe, Y., Sasaki, Y., Tsubaki, S. and Sekizaki, T., 1994d. Effect of growth temperature on maintenance of virulent *Rhodococcus equi*. *Vet. Microbiol.*, 39: 187–192.
- Takai, S., Takahagi, J., Sato, Y., Yamaguchi, K., Kakizaki, S., Takehara, S., Matsukura, S., Tamada, Y., Tani, A., Sasaki, Y., Tsubaki, S. and Kamada, M., 1994e. Molecular epidemiology of virulent *Rhodococcus equi* in horses and their environments. In: ed. H. Nakajima and P.D. Rossdale, *Equine Infectious Diseases VII*. R and W Publications (Newmarket) Limited, pp. 183–187.
- Takai, S. and Tsubaki, S., 1985b. The incidence of *Rhodococcus (Corynebacterium) equi* in domestic animals and soil. *J. Vet. Med. Sci.*, 47: 493–496.
- Takai, S., Watanabe, Y., Ikeda, T., Ozawa, T., Matsukura, S., Tamada, Y., Tsubaki, S., Sekizaki, T., 1993c. Virulence-associated plasmids in *Rhodococcus equi*. *J. Clin. Microbiol.*, 31: 1726–1729.
- Tan, C., Prescott, J.F., Patterson, M.C. and Nicholson, V.M., 1995. Molecular characterization of a lipid-modified virulence-associated protein of *Rhodococcus equi* and its potential in protective immunity. *Can. J. Vet. Res.*, 59: 51–59.
- Tkachuk-Saad, O. and Prescott, J., 1991. *Rhodococcus equi* plasmids: isolation and partial characterization. *J. Clin. Microbiol.*, 29: 2696–2700.
- Wada, R., Kamada, M., Anzai, T. and Takai, S., 1997. Pathogenicity and virulence of *Rhodococcus equi* in foals. *Vet. Microbiol.*, this issue.
- Woolcock, J.B., Farmer, A.M. and Mutimer, M.D., 1979. Selective medium for *Corynebacterium equi* isolation. *J. Clin. Microbiol.*, 9: 640–642.
- Woolcock, J.B. and Mutimer, M.D., 1981. *Corynebacterium equi* in the gastrointestinal tract of ruminants. *Vet. Res. Commun.*, 4: 291–294.
- Woolcock, J.B., Mutimer, M.D. and Farmer, A.M., 1980. Epidemiology of *Corynebacterium equi* in horses. *Res. Vet. Sci.*, 28: 87–90.
- Yager, J.A., 1987. The pathogenesis of *Rhodococcus equi* pneumonia in foals. *Vet. Microbiol.*, 14: 225–232.
- Zink, M.C., Yager, J.A. and Smart, N.L., 1986. *Corynebacterium equi* infections in horses 1958–1984: a review of 131 cases. *Can. Vet. J.*, 27: 213–217.