

*Hidaka Agriculture Mutual Aid Association, Mitsuishi, Hokkaido, 059–3105, Japan*

## **Effect of Prophylactic Administration of Hyperimmune Plasma to Prevent *Rhodococcus equi* Infection on Foals from Endemically Affected Farms**

T. HIGUCHI<sup>1</sup>, T. ARAKAWA<sup>1</sup>, S. HASHIKURA<sup>1</sup>, T. INUI<sup>1</sup>, H. SENBA<sup>1</sup> and S. TAKAI<sup>2,3</sup>

Addresses of authors: <sup>1</sup>Hidaka Agriculture Mutual Aid Association, Mitsuishi, Hokkaido, 059–3105 Japan; <sup>2</sup>Department of Animal Hygiene, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori, 034–8628 Japan; <sup>3</sup>Corresponding author

*With 2 figures and 2 tables*

*(Received for publication December 17, 1998)*

### **Summary**

The effect on foals of prophylactic administration of hyperimmune plasma to prevent *R. equi* infection was investigated on three farms at which *R. equi* infection was endemic. Sixteen foals between 10 and 39 days of age were intravenously given 1–21 of hyperimmune plasma. ELISA antibody titres against *R. equi* were significantly increased and maintained at high levels for over 30 days in most of the recipient foals. The prevalence of *R. equi* infection was 6.3% (1/16) in the foals that received the immune plasma, and 26.3% (5/19) in the control foals not given the immune plasma on the three farms. For 2 years before and after this field trial on the three farms, 18 of 64 foals (28.1%) showed clinical signs of respiratory tract infection and four of them died of *R. equi* pneumonia. Heavy contamination of horses and their environment with virulent *R. equi* was detected by colony blotting, and plasmid profiles also suggested that foals on the three farms were constantly exposed to virulent *R. equi*. The results of this field trial support previous observations by some researchers that the administration of hyperimmune plasma to foals in the early days of life promotes prevention of *R. equi* infection on endemic farms; however, the mechanism of hyperimmune plasma protection remains unclear.

### **Introduction**

*Rhodococcus equi* infection in foals causes considerable economic losses on horse-breeding farms at which infection is endemic. *R. equi* pneumonia, with or without enteritis, in foals is an insidious disease and early diagnosis of the disease is not easy; moreover, antimicrobial treatment is costly and only moderately effective (GIGUERE and PRESCOTT, 1997). *R. equi* becomes distributed widely in the environment of horse-breeding farms, but only virulent *R. equi*, which expresses virulence-associated antigens and possesses a virulence plasmid, causes the disease in foals (TAKAI, 1997). Foals are infected via the respiratory and/or alimentary routes with virulent *R. equi*, which shows a unique propensity for affecting foals during the first 3 months of life (PRESCOTT, 1991).

Cell-mediated immunity is thought to play an important role in eliminating the facultative intracellular pathogen from foals; however, humoral immunity seems to be critically involved in early protection in young foals (PRESCOTT, 1991; HINES et al., 1997). *In vitro* studies by HIETALA and ARDANS (1987) showed that opsonization of *R. equi* with antiserum significantly enhanced killing of *R. equi* by alveolar macrophages in foals. Recently, passive immunization of foals has proved highly beneficial and cost effective in preventing the disease on endemically affected farms (BECU et al., 1997; MADIGAN et al., 1991; MARTENS et al., 1989). MARTENS et

al. (1989) demonstrated that the survival rate of immunized foals was significantly greater than that of non-immunized foals in experimental *R. equi* infection. MADIGAN et al. (1991) reported that the administration of hyperimmune plasma was significantly protective against *R. equi* in foals on endemic farms. More recently, BECU et al., (1997) evaluated an immunoprophylaxis programme for *R. equi* infection of foals on endemic farms using an *R. equi* vaccine and hyperimmune plasma, and showed that mare immunization with the vaccine significantly prevented *R. equi* pneumonia in foals.

However, little is known about the dynamics of the antibody titres in foals which received hyperimmune plasma during the first 3 months of life (BECU et al., 1997; MADIGAN et al., 1991; MARTENS et al., 1989). The purpose of this study was to determine the effect of parental administration of *R. equi* immune plasma to prevent infection on foals on endemically affected farms, to detect increase and decay of *R. equi*-specific antibody following plasma transfusion by ELISA, and to discuss the optimal age for administrations and the minimum effective dose of the immune plasma.

## Materials and Methods

### *Hyperimmune plasma*

Two clinically healthy thoroughbred mares that were negative for anti-erythrocyte antibodies and the Coggins test were used to produce hyperimmune plasma against virulent *R. equi* strains. The horses were immunized with virulent *R. equi* strains ATCC 33701 and L1 by repeated inoculations as follows: day 1, the donors received 10 ml of bacterial suspension, which contained about  $10^9$  cells, intravenously; day 14, the donors received 10 ml, which contained about  $10^9$  cells, subcutaneously; day 28, the donors received 10 ml, which contained about  $10^9$  cells, subcutaneously (TAKAI et al., 1993). Hyperimmune plasma with ELISA OD value 0.6–1.3 was harvested 35 and 56 days after immunization. The hyperimmune plasma was negative for bacterial culture and stored at  $-80^{\circ}\text{C}$  until use.

### *Farms*

The three farms studied were located in the Hidaka district, where over 80% of Japanese thoroughbreds are bred every year. These farms are endemically affected and have a history of *R. equi* pneumonia.

### *Field trial*

Field trials were conducted on the three farms for 2 years between 1994 and 1995. Hyperimmune plasma was administered to four of seven foals born in 1994 and three of seven foals born in 1995 on farm A, and four of 11 foals born in 1994 on farm B. On farm C, five foals born earlier in 1995 showed clinical signs of respiratory tract disease, and four of them were diagnosed as having *R. equi* pneumonia by ELISA and culture of *R. equi* from tracheal aspirates. Subsequently, five foals born later were given hyperimmune plasma.

Sixteen foals were administered 1–2 l of hyperimmune plasma at 10–39 days of age (average 25 days), and 19 foals were observed as non-treated controls (Table 1). For 2 years before and after the field trials (1993 and 1996), the medical records of all foals kept at the three farms were also examined. A total of 99 foals were surveyed at the three farms for a period of 4 years (Table 2).

### *Clinical observation and sample collection*

Complete blood counts, fibrinogen concentrations, globulin concentrations and *R. equi* ELISA OD values were examined in foals 1 day before and after the plasma transfusion, and tests were continued at 2-week intervals up to 3–4 months of age. All the foals, with or without immune plasma, were observed daily by farm manage, and they were examined physically by veterinarians at the sample collections.

Table 1. Volume of hyperimmune plasma and age at administration for foals used in this study

Farm	Foal No.	Year of birth	Administration of hyperimmune plasma (l)	Age at administration (days)
A	1	1994	2.0	34
	2	1994	1.5	34
	3	1994	2.0	31
	4	1994	2.0	33
	5	1994	—	
	6	1994	—	
	7	1994	—	
	19	1995	1.0	28
	20	1995	1.0	23
	21	1995	1.0	21
	22	1995	—	
	23	1995	—	
	24	1995	—	
	25	1995	—	
	B	8	1994	2.0
9		1994	2.0	39
10		1994	2.0	22
11		1994	2.0	23
12		1994	—	
13		1994	—	
14		1994	—	
15		1994	—	
16		1994	—	
17		1994	—	
18		1994	—	
C	26	1995	1.0	20
	27	1995	1.0	19
	28	1995	1.0	14
	29	1995	1.0	10
	30	1995	1.0	11
	31	1995	—	
	32	1995	—	
	33	1995	—	
	34	1995	—	
	35	1995	—	

*ELISA for detection of anti-R. equi antibody*

ELISA antigen was prepared from *R. equi* ATCC 6939 as described previously (TAKAI et al., 1985). Briefly, strain ATCC 6939 was grown on brain heart infusion agar. Bacteria were harvested after 5 days of incubation at 38°C. *R. equi* (2 g wet weight) was suspended in 10 ml of 0.0125 M sodium phosphate buffer (pH 7.4) containing 0.1% (wt/vol) Tween 20, incubated at 37°C for 90 min in a water bath with agitation and centrifuged at 20 000 *g* for 30 min at 4°C. The supernatant was used as the antigen, which was adjusted to 1.0 g of protein/ml in carbonate-bicarbonate buffer (pH 9.6). ELISA was performed as described previously: the positive limit was set at OD 0.3, which is the average OD of healthy foals plus 3 standard deviations (TAKAI et al., 1985; SANADA et al., 1992).

*Isolation and identification of virulent R. equi from faeces of foals and soil samples*

Fifty-three faecal samples were collected from foals on each farm during the study. Seventy-three soil samples were collected from five to 10 sites in small paddocks that were used for housing a mare and her

Table 2. Number of foals with *R. equi* infection with and without administration of hyperimmune plasma on farms A, B and C in years 1993–96

Farm	Administration of hyperimmune plasma	Year				Total
		1993	1994	1995	1996	
A	+	—	0/4 <sup>a</sup>	0/3	—	0/7
	—	3/14	0/3	0/4	ND	3/21
B	+	—	0/4	—	—	0/4
	—	4/12	1/7	2/9	2/11	9/39
C	+	—	—	1/5	—	1/5
	—	ND	1/7	4/5	6/11	11/33
All three farms	+	—	0/7 (0) <sup>b</sup>	1/9 (11.1)	—	1/16 (6.3)
	—	7/26 (26.9)	2/17 (11.8)	6/18 (33.3)	8/22 (36.4)	23/83 (27.7)

<sup>a</sup>Number of foals with *R. equi* infection/number of foals in each group.

<sup>b</sup>%.

foal. Soil was scraped from the surface with a small spoon and placed in sterile tubes. One gram of faeces or soil was diluted serially with a 10-fold volume of sterile saline (0.9% NaCl) solution. Each dilution was inoculated onto two plates containing *R. equi*-selective media, and the plates were incubated at 30°C for 2 or 3 days. All suspected colonies of *R. equi* were counted, and the number of viable organisms per gram of faeces or soil was calculated. Two to 10 colonies per specimen were subcultured, identified in our laboratory and examined for virulence-associated antigens and virulence plasmids (TAKAI et al., 1994).

## Results

### *Dynamics of antibody levels in foals with and without hyperimmune plasma*

ELISA was used to evaluate the dynamics of antibody levels against *R. equi* in foals with and without hyperimmune plasma. Before the administration of hyperimmune plasma, almost all foals showed ELISA OD values below 0.2. Hyperimmune plasma was administered to foals at 10–39 days of age (typical results on farm A are shown in Fig. 1), and they showed a significant increase in ELISA OD values ranging from 0.3 to 1.4 (mean  $0.85 \pm 0.32$ ). The half-life of the *R. equi* antibodies was 30–35 days, and the majority of foals having 2 l of hyperimmune plasma were maintaining an ELISA OD value above 0.3 by 90 days of age. On the other hand, foals that received only 1 l of hyperimmune plasma could not maintain the antibody titres above 0.3 by 60 days of age. Some foals showed transitory trembling during the infusion of hyperimmune plasma; however, no foals showed side-effects except during and after the intravenous administration of hyperimmune plasma.

### *Clinical findings*

On farm A, no foals showed clinical signs of *R. equi* pneumonia in 1994 and 1995, and seven control foals had low *R. equi* ELISA OD values during the observation period (Table 2, Fig. 1). However, in 1993, 1 year before this field trial, three of 14 foals showed clinical signs of respiratory tract infection and then died of *R. equi* pneumonia.

Farm B was also endemically affected, and four of 12 foals born in 1993 showed clinical signs of respiratory tract infection (Table 2). At this farm, hyperimmune plasma was administered to four of 11 foals born in 1994, and none of these four foals showed any clinical signs of respiratory tract disease. On the other hand, the seven remaining control foals showed slight tachypnea and moderate fever in mid-August, and they were diagnosed with heat stroke by clinical symptoms. One of the seven foals had an ELISA OD value of 0.6 and was diagnosed

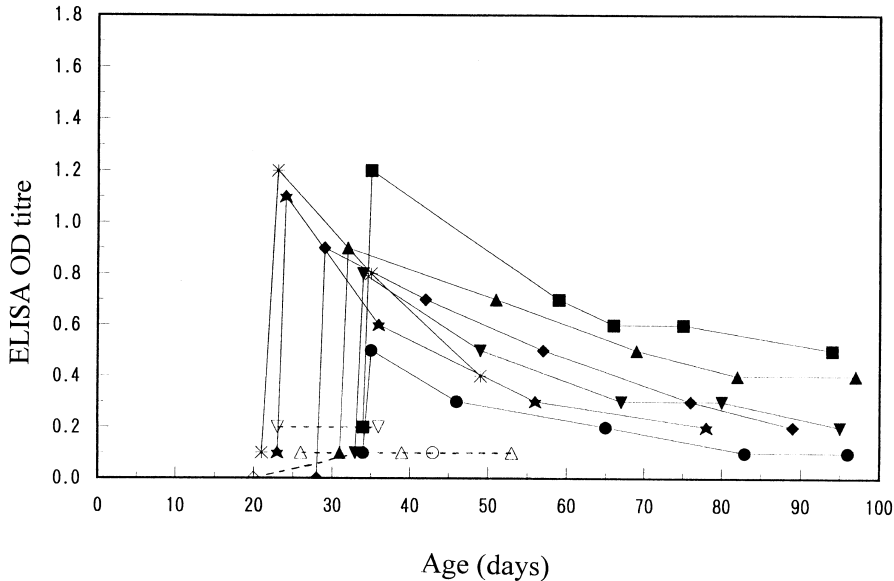


Fig. 1. ELISA titres against *R. equi* ATCC 6939 antigens in the seven foal sera obtained after the administration of hyperimmune plasma and in the four control foal sera on farm A. Symbols: ■ foal 1, ● foal 2, ▲ foal 3, ▼ foal 4, ◆ foal 19, ★ foal 20, \* foal 21, ○ foal 22, △ foal 23, ▽ foal 24, ◇ foal 25.

with *R. equi* pneumonia. During the following 2 years, two of nine foals born in 1995 and two of 11 foals born in 1996 showed clinical signs of respiratory tract disease and were diagnosed as having *R. equi* pneumonia by ELISA and bacteriological culture of tracheal aspirates. These foals had inflammatory characteristics, such as increases in WBC counts and  $\alpha$ -globulin and fibrinogen concentrations.

On farm C, five foals born in February and March of 1995 showed febrile and respiratory symptoms at 29–46 days of age, and four of them were definitively diagnosed with *R. equi* pneumonia by ELISA and bacteriological culture of tracheal aspirates. These four foals showed high ELISA OD values above 0.3 (Fig. 2). Next, hyperimmune plasma was administered to five newborn foals born in April and May of 1995. However, one of the foals, to which hyperimmune plasma was administered at 19 days of age, showed febrile and respiratory symptoms at 31 days old and was diagnosed with *R. equi* pneumonia by bacteriological culture of tracheal aspirates. The ELISA OD value of the foal showing clinical signs increased just after administration of the immune plasma at 19 days old, and then it increased at 30 days of age (Fig. 2). No hyperimmune plasma was administered to foals born in 1996; in this year, six of 11 foals showed clinical signs of respiratory tract disease and were diagnosed as having *R. equi* pneumonia by ELISA and bacteriological culture of tracheal aspirates. These foals had inflammatory characteristics, such as increases in WBC counts and  $\alpha$ -globulin and fibrinogen concentrations.

In conclusion, one (6.7%) of 16 foals to which hyperimmune plasma was administered was diagnosed with *R. equi* pneumonia; however, five (26.3%) of 19 foals without hyperimmune plasma administration and 18 (28.1%) of 64 foals for 2 years before and after this field trial on the three farms were diagnosed with *R. equi* pneumonia by ELISA and bacteriological culture of tracheal aspirates during the 4-year observation period. The differences observed were not statistically significant between the two groups of foals; those given hyperimmune plasma (1/16) and those not given hyperimmune plasma (5/19).

#### *Prevalence of virulent R. equi in foal faeces and soil*

Prevalence of virulent *R. equi* in isolates from faeces of foals and soil collected from the farms was investigated during field trials in 1994 and 1995. *R. equi* was isolated from almost all

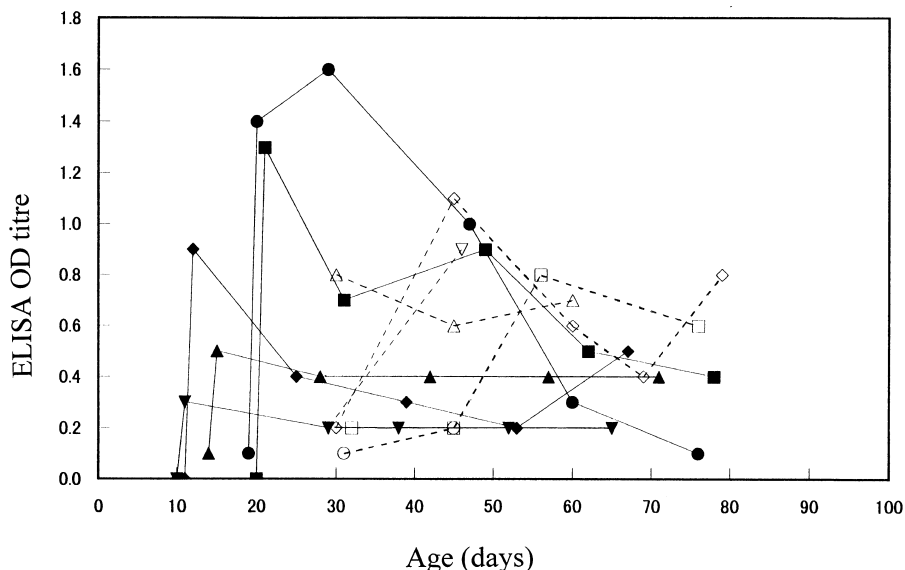


Fig. 2. ELISA titres against *R. equi* ATCC 6939 antigens in the five foal sera obtained after the administration of hyperimmune plasma and in the five control foal sera on farm C. Symbols: ■ foal 26, ● foal 27, ▲ foal 28, ▼ foal 29, ◆ foal 30, □ foal 31, ○ foal 32, △ foal 33, ▽ foal 34, ◇ foal 35.

of the foal faeces tested and from soil samples obtained from  $10^2$  to  $10^5$  per gram of sample. Virulent *R. equi* appeared in 26.7 (16 of 60 isolates), 5.4 (three of 56 isolates) and 5.1% (three of 59 isolates) of soil isolates from farms A, B and C, respectively. On the other hand, virulent *R. equi* appeared in 27.5% (41 of 149 isolates) and 19.7% (31 of 157 isolates) of isolates from the faeces of foals maintained at farms A and C, respectively. Isolates were analysed for virulence plasmid DNA by agarose-gel electrophoresis to determine the plasmid size, and analysis of these results confirmed the results of immunoblot tests for the 15- to 17-kDa antigens.

### Discussion

The present field study conducted in Japan demonstrated the effectiveness of hyperimmune plasma administration to protect foals from *R. equi* infection at three farms with endemic infection, and supported previous observations in the United States and Argentina that the administration of hyperimmune plasma to foals in the early days of life offers prophylactic protection from infection on endemic farms (BECU et al., 1997; MADIGAN et al., 1991; MARTENS et al., 1989).

MADIGAN et al. (1991) reported that 68 foals in 1988 and 98 foals in 1989 given hyperimmune plasma did not develop *R. equi* pneumonia on an endemic farm, whereas six of 14 (43%) non-treated foals in 1989, 90 non-treated foals in 1986 and 92 non-treated foals in 1987 developed the disease. Hyperimmune plasma was administered to foals by 30 days old in 1988, and at 30–60 days old in 1989, since these foals possessed maternal antibodies against *R. equi* from vaccinated mares during the first month of life. The optimal days after birth for administration of hyperimmune plasma to foals has not been examined in detail, but the majority of foals usually become infected by *R. equi* very early in life because of heavy contamination with virulent *R. equi* on endemic farms (TAKAI, 1997), so the administration of hyperimmune plasma to foals soon after birth might be more useful on endemic farms. Recently, similar results of administration of hyperimmune plasma to foals were obtained in the field studies conducted in Argentina by BECU et al. (1997).

Foals received 1000 ml of hyperimmune plasma in the study by MADIGAN et al. (1991)

and 600–1200 ml in the study by BECU et al. (1997). In the present study, we administered 1000 or 2000 ml of hyperimmune plasma to foals, which then maintained their ELISA antibody titres above 0.3 (OD values) for 30 days or more. The minimum effective dose of hyperimmune plasma to prevent *R. equi* pneumonia in foals is difficult to determine, since many factors such as infection dose, host susceptibility, age, sex, weight, etc. may differ in natural infections. The hyperimmune plasma produced by MADIGAN et al. (1991), BECU et al. (1997) and us contains specific antibodies against virulence-associated 15- to 17-kDa antigens. Although the role of these antibodies in the prevention of *R. equi* pneumonia remains unclear, opsonizing antibodies might be considered (HINES et al., 1997). The increased opsonization prior to exposure to *R. equi* by administration of hyperimmune plasma may play an important role in the first stage of host defence in foals. The dose used in this study, which increased the ELISA titre to the established infection level, might be enough to cause the opsonizing effect. On the other hand, other factors such as fibronectin, complement and cytokines in the hyperimmune plasma might also play a role (HINES et al., 1997).

Vaccination of mares did not provide protection against *R. equi* pneumonia despite a significant increase in colostral specific antibody, and vaccination of mares and their foals with virulence-associated proteins also did not protect foals (BECU et al., 1997; MARTENS et al., 1991). These results suggested the presence of important immune factors which do not transfer to foals through colostrum, and which might be contained in hyperimmune plasma. Further studies are needed to elucidate which factors in plasma are able to confer immunity.

More recently, HIGUCHI et al. (1998) proposed that physical and serologic examinations of foals at 30 and 45 days of age are useful for early diagnosis of *R. equi* infection, especially in foals on endemic farms. However, these protocols are more useful for early diagnosis than for prevention. As a prophylactic measure, however, hyperimmune plasma can help decrease both the size of infectious challenges and the risk of exposure of susceptible foals to *R. equi* infection. The results of the present study demonstrated an appreciable improvement in morbidity and mortality from the disease on the three endemically affected farms studied over a 2-year period. In conclusion, on farms endemically affected by *R. equi* infection, the following prophylactic and hygienic measures are recommended: (1) putting foals, especially within the first month of life, in well-ventilated stalls and clean paddocks known to have no contamination with virulent *R. equi*; (2) conducting physical and serologic examination of foals at 30 and 45 days old, and (3) if possible, administering hyperimmune plasma to newborn foals soon after birth to compensate for their immature immunity.

#### Acknowledgements

This work was supported by a grant-in-aid (1995–97) from the School of Veterinary Medicine and Animal Sciences (S. Takai), Kitasato University, and by a grant-in-aid from the Equine Research Institute, Japan Racing Association (S. Takai).

#### References

- BECU, T., G. POLLEDO, and GASKIN, 1997: Immunoprophylaxis of *Rhodococcus equi* pneumonia in foals. *Vet. Microbiol.* **56**, 193–204.
- GIGUERE, S., and A. A. J. F. PRESCOTT, 1997: Clinical manifestations, diagnosis, treatment, and prevention of *Rhodococcus equi* infections in foals. *Vet. Microbiol.* **56**, 313–334.
- HIETALA, S. K., and S. ARDANS, 1987: Interaction of *Rhodococcus equi* with phagocytic cells from *R. equi*-exposed and non-exposed foals. *Vet. Microbiol.* **14**, 307–320.
- HIGUCHI, T., S. T. TAHARAGUCHI, S. HASHIKURA, S. HAGIWARA, C. GOJO, S. SATOH, M. YOSHIDA, and S. TAKAI, 1998: Physical and serological examinations of foals at 30 and 45 days of age for early diagnosis of *Rhodococcus equi* infection on endemically infected farms. *J. Am. Vet. Med. Assoc.* **212**, 976–981.
- HINES, S. A., S. KANALY, B. A. BYRNE, and G. H. PALMER, 1997: Immunity to *Rhodococcus equi*. *Vet. Microbiol.* **56**, 177–185.
- MADIGAN, J. E., R. J. HIETALA, and N. MULLER, 1991: Protection against naturally acquired *Rhodococcus*

- equi* pneumonia in foals by administration of hyperimmune plasma. J. Reprod. Fertil. **Suppl. 44**, 571–578.
- MARTENS, J. G., J. G. MARTENS, R. A. FISKE, and S. K. HIETALA, 1989: *Rhodococcus equi* foal pneumonia: protective effects of immune plasma in experimentally infected foals. Equine. Vet. J. **21**, 249–255.
- MARTENS, R. J., H. MARTENS, and R. A. FISKE, 1991: Failure of passive immunization by colostrum from immunized mares to protect foals against *Rhodococcus equi*. Equine Vet. J. **Suppl. 12**, 19–22.
- PRESCOTT, J. F., 1991: *Rhodococcus equi*: an animal and human pathogen. Clin. Microbiol. Rev. **4**, 20–34.
- SANADA, Y., T. NODA, and H. NAGAHATA, 1992: Serological survey of *Rhodococcus equi* infection in horses in Hokkaido. J. Vet. Med. Sci. **54**, 649–652.
- TAKAI, S., 1997: Epidemiology of *Rhodococcus equi* infections. Vet. Microbiol. **56**, 167–176.
- TAKAI, S., M. ANZAI, K. YAMAGUCHI, S. KAKIZAKI, J. TAKAHAGI, Y. SATO, F. TAKEHARA, Y. TAMADA, S. MATSUKURA, A. TANI, M. KATO, N. SENO, Y. SASAKI, S. TSUBAKI, S., and S. KAMADA, 1994: Prevalence of virulence plasmids in environmental isolates of *Rhodococcus equi* from horse-breeding farms in Hokkaido. J. Equine Sci. **5**, 21–25.
- TAKAI, S., Y. KAWAZU, and S. TSUBAKI, 1985: Enzyme-linked immunosorbent assay for diagnosis of *Corynebacterium (Rhodococcus) equi* infection in foals. Am. J. Vet. Res. **46**, 2166–2170.
- TAKAI, S., J. M. WATANABE, T. IKEDA, T. OZAWA, S. MATSUKURA, Y. TAMADA, S. TSUBAKI, and T. SEKIZAKI, 1993: Virulence-associated plasmids in *Rhodococcus equi*. J. Clin. Microbiol. **31**, 1726–1729.