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Quantitative Aspects of Fecal *Rhodococcus* (*Corynebacterium*) *equi* in Foals

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Quantitative aspects of fecal *Rhodococcus* (*Corynebacterium*) *equi* in newborn foals for 12 weeks after birth were investigated on two horse breeding farms. *R. equi* was found in the feces of foals during week 1 of life. The greatest numbers of *R. equi* were present in the feces of foals during the first 8 weeks of their lives, which coincides with the age when foals are most liable to be exposed to *R. equi*.

Rhodococcus (*Corynebacterium*) *equi* is an important pathogen which causes a suppurative pneumonia and enteritis in foals (3, 22). Although infection is thought to occur at a very young age, the foal does not show clinical signs until 1 to 6 months (3, 15, 22). The natural route of *R. equi* transmission is not known but is generally regarded as inhalation and ingestion (1, 10, 17). Attempts to induce experimental infections by both routes were reported successful by Johnson et al. (8, 9). *R. equi* is known to be a soil saprophyte with widespread distribution, and domestic animals probably come in contact with the organism by ingestion and inhalation (1, 2, 20, 24). Recently, we reported that in a naturally infected foal the number of *R. equi* in the feces increased markedly after the initial development of clinical signs of illness at 5 weeks of age, and specific antibodies against *R. equi* were detected at 6 weeks of age as shown by enzyme-linked immunosorbent assay (19). The quantitative fecal culture of *R. equi* was indicated as a prospective study for the diagnosis of *R. equi* infection in foals (19). The organism is commonly found in the feces of horses (2, 11, 13, 20, 24). Previous studies investigated only the prevalence of *R. equi* in the feces of adult horses and older foals, which are thought to be resistant to the infection (2, 11, 13, 20, 24). There is no information concerning the first colonization of *R. equi* in newborn foals and its quantitative aspects.

The purpose of this study was to investigate the quantitative aspects of *R. equi* in newborn foals for 3 months after birth, the time during which foals are most susceptible to the infection (1, 10, 15, 17, 22).

Fourteen foals of thoroughbred breed on farm A with a sporadic incidence of *R. equi* infection and 11 foals of thoroughbred breed on farm B with a persistent incidence of *R. equi* infection were examined by fecal culture of *R. equi* on a selective medium (23). The foals were born from late February to early June 1985 in Aomori prefecture, Japan. Twenty-five adult horses (males and females over 1 year old) on farm A and 27 adult horses (males and females over 1 year old) on farm B were also examined one and three times, respectively.

For the selective isolation of *R. equi*, nalidixic acid-novobiocin-actidione (cycloheximide)-potassium tellurite (NANAT) medium, which was described by Woolcock et al. (23), was used. Yeast extract-caseine-cystine agar (Eiken, Tokyo, Japan) was used as the base medium. To the base medium the following were added: nalidixic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 20 μg/ml; novobiocin (Sigma Chemical Co., St. Louis, Mo.), 25 μg/ml; cycloheximide (Sigma), 40 μg/ml; and 0.005% potassium tellurite (E. Merck AG, Darmstadt, Federal Republic of Germany).

Fecal specimens were collected from the 14 foals on farm A and the 11 foals on farm B at weekly intervals for 7 months from February to August 1985. About 10 g of feces was removed from the rectum of each foal and taken to a laboratory in a sterile dish. All other fecal specimens were taken from freshly passed materials. Specimens were examined within a day of collection as described previously (20). One gram of feces was diluted serially with a 10-fold volume of sterile saline. Dilutions were then each inoculated onto two plates of NANAT medium. The plates were incubated at 37°C for 2 or 3 days. All suspect colonies of *R. equi* were counted, and the number of viable organisms per gram of feces was calculated. One of the suspect colonies on each plate was subcultured, and the isolates were identified by accepted criteria (14).

Quantitative culture of *R. equi* from the feces of the 14 foals on farm A and the 11 foals on farm B was done individually at weekly intervals for 12 weeks after birth. The results are indicated as the mean ± standard error according to the age of the foals examined (Fig. 1). On farm A, *R. equi* was first isolated from the feces of four foals at 2 weeks of age. The mean numbers of *R. equi* in the feces of foals on farm A increased to 10^4/g of feces at 5 weeks of age, were maintained for 3 weeks, and gradually decreased to 10^3/g at 11 weeks of age. The isolation rate of *R. equi* from the feces of foals on farm A reached 100% at 7 weeks of age and then gradually decreased. On farm B, *R. equi* was isolated from the feces of three foals at 3 and 7 days of age, much earlier than on farm A. The mean number of *R. equi* and the isolation rate of *R. equi* from the feces of foals on farm B were similar to those for farm A.

The mean numbers of *R. equi* in the feces of foals and adult horses were compared (Table 1). The results of the quantitative study of the feces of foals up to 3 months of age on farms A and B were used. The mean numbers of *R. equi* in the feces of foals and adult horses were $2.1 \times 10^4 \pm 0.4 \times 10^4$ and $5.6 \times 10^2 \pm 2.6 \times 10^2$ on farm A, respectively, and $2.8 \times 10^4 \pm 0.7 \times 10^4$ and $5.3 \times 10^2 \pm 1.3 \times 10^2$ on farm B, respectively. There was a significant difference in the mean number of *R. equi* in the feces between adult horses and foals on farms A and B. There was no significant difference

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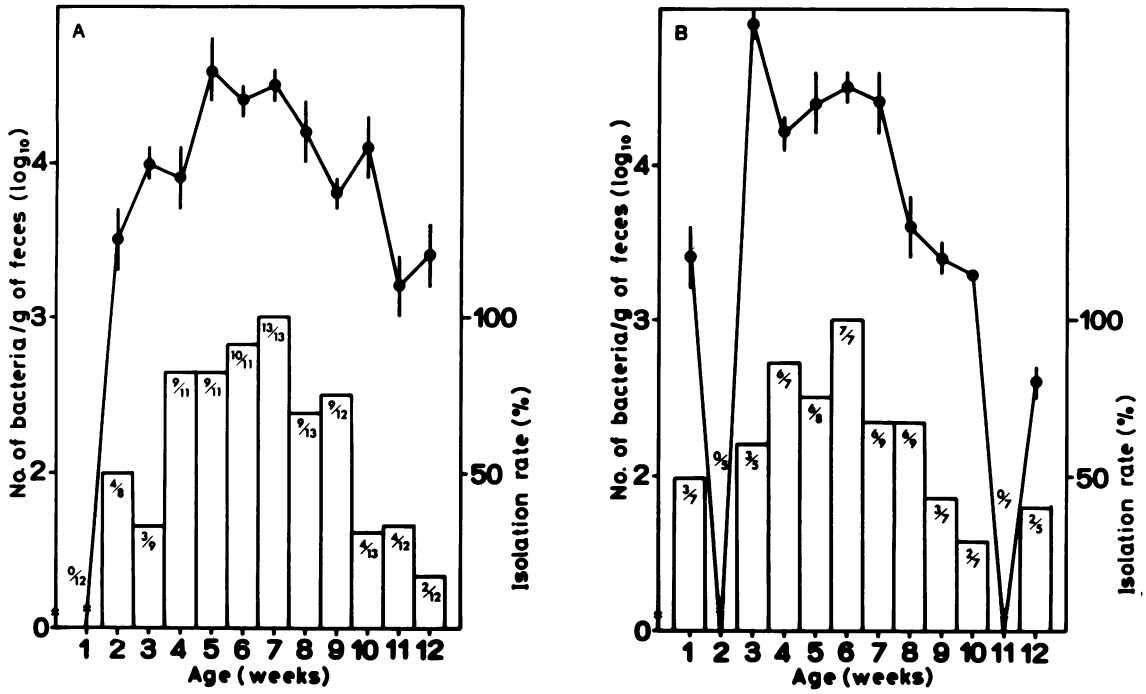

FIG. 1. Mean counts of *R. equi* in the feces of foals from 1 to 12 weeks of age on farms A and B. Symbols represent the mean (± standard error of the mean) $\log_{10}$ of the viable number of *R. equi* per gram of feces in foals with positive cultures at each interval. Bars represent the isolation rate. The ratio of the number of foals with positive cultures to the number of foals examined in each age group is indicated in the bar.

in the isolation rate between foals and adult horses and between farms A and B.

The present study indicates that *R. equi* had already colonized in the intestinal tract of foals by week 1 of life. It is known that intestinal flora are derived solely from the environment. During normal birth, the newborn picks up microbes from the vagina and external genitalia of the mother and other environmental sources to which it is exposed (16). The intestinal colonization of the neonate is rapid, and the bowel lumen soon becomes the site of extensive bacterial multiplication (16, 18). In the establishment of the normal flora of newborn foals, environmental conditions can have a marked effect on the extent and timing of initial colonization (16, 21). This may explain the difference in the timing of initial colonization between foals of farm A, with a sporadic incidence, and farm B, with a persistent incidence of the infection. There seems little doubt that the feces of dams and the soil environment on the farms play a very important role in the initial colonization of *R. equi* in foals. Coprophagia is a behavior pattern recognized as occurring in the foal. Francis-Smith and Wood-Gush (6) reported that four thoroughbred foals quickly ate part of the feces deposited by their own dams between week 2 and 5 after birth. *R. equi* multiplies in soil, and the greatest numbers of the organism are present in the soil in April and May, which coincides with the foaling period (S. Takai, K. Narita, K. Ando, and S. Tsubaki, Vet. Microbiol., in press). In this study, the number of *R. equi* in the feces of foals increased to 10^4/g of feces at 3 weeks of age, was maintained for 4 to 5 weeks, and then gradually decreased to 10^2 or 10^3/g on farms A and B. The greatest numbers of *R. equi* were present in the intestinal tract during the first 8 weeks of life, which coincides with the age at which foals are most liable to *R. equi* infection (1, 10, 15, 17, 22). Susceptible foals may therefore be exposed to many dangers of infection.

In naturally occurring cases, the disease has been found in foals between 1 and 6 months of age, with most cases occurring between 2 and 3 months (1, 3, 10, 15, 17, 22). Recently, Falcon et al. (5) showed that foals between 2 and 4 months old were more often affected; they accounted for 84.6% of the clinical cases studied. The infection is thought to occur at a very young age (10, 17, 22), and the onset of infection may be insidious, with many foals not being diagnosed until extensive lesions are established (1, 15, 17). Johnson et al. (9) reported that in experimental infections the interval between infection and development of lesions was approximately 3 weeks. Therefore, our proposed maximum time of exposure, the first 8 weeks after birth, may be reconciled with the age for maximum prevalence of the disease, 2 to 4 months of age, as observed in the field (1, 3, 10, 15, 17, 22).

TABLE 1. Comparison of *R. equi* isolation from feces of foals and adult horses on farms A and B

Farm	Horse	No. of specimens	No. of isolates	Isolation rate (%)	No. of bacteria per g of feces (mean ± SE)[a]
A	Foal	159	79	49.7[b]	$2.1 \times 10^4 \pm 0.4 \times 10^4$[c]
	Adult	25	13	52.0	$5.6 \times 10^2 \pm 2.6 \times 10^2$
B	Foal	83	43	51.8[b]	$2.8 \times 10^4 \pm 0.7 \times 10^4$[c]
	Adult	81	32	39.5	$5.3 \times 10^2 \pm 1.3 \times 10^2$

[a] Mean (± standard error of the mean) of the viable number of *R. equi* per gram of feces in horses with positive cultures.
[b] $P > 0.05$, compared with rates for adult horses by the chi-square test.
[c] $P < 0.01$, compared with results for adult horses by Student's *t* test.

The mean number of bacteria in the feces of foals on farms A and B began to decrease after 7 weeks of age. The reason for the decrease in the number of *R. equi* in the feces of the foals is not known. Foals develop significant lymphocyte stimulation indices to *R. equi* antigen at approximately 2 to 3 months of age (12). Antibody against *R. equi* was demonstrated in normal foals by enzyme-linked immunosorbent assay (4, 7, 19). These findings indicate that the gut immunity of foals might be evoked by the initial colonization of *R. equi* (4, 7, 12). If the immunological system of the animal at all influences the composition of the microbiota in the gastrointestinal tract, this might partly explain the decrease in the number of *R. equi* in the feces of the foals after 7 weeks of age. It has been noted that marked changes occur in the gut flora of mice coincident with the transition from a milk to a solid diet (16, 21). Once the animal begins to sample solid food, strict anaerobes can be detected, usually in the large intestine (16). As the level of the populations of strict anaerobes rises, the level of facultatives, such as *Escherichia coli* and *Streptococcus faecalis*, may decline concomitantly (16). This might occur in the case of foals.

Nakazawa et al. (11) reported that the mean viable count of *R. equi* in 1 g of feces determined by quantitative culture was 8.42×10^2 in mares and 7.52×10^2 in foals from 1 to 7 months of age. Their results for mares were similar to ours, but their results for foals were approximately 40 times lower than ours. These difference might partly be owing to the different sampling ages of the foals in the two studies.

In conclusion, this study suggests that the intestinal *R. equi* in foals during the first 8 weeks after birth must be considered when investigating the mode and route of transmission of the disease in foals.

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