

# 家畜および土壌からのRhodococcus (Corynebacterium) equiの分離

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## The Incidence of *Rhodococcus (Corynebacterium) equi* in Domestic Animals and Soil

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ABSTRACT. Three hundred and eight and 194 *Rhodococcus (Corynebacterium) equi* strains were isolated from 1,129 domestic animal samples and 224 soil samples examined, respectively.—KEY WORDS: *Rhodococcus (Corynebacterium) equi*.

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*Rhodococcus (Corynebacterium) equi* is an important pathogen of foals, being associated with a purulent bronchopneumonia and frequently an enteritis [2, 5]. It has been recovered from pneumonia in sheep [1] and immunosuppressive people [4, 15], and lymphadenitis in cattle [8] and pigs [7]. The selective media [18] introduced during recent years for isolation of *R. equi* have created favorable conditions for wider epidemiological studies [3, 9, 10, 11, 12, 19, 20]. There have been a few reports with regard to the isolation of *R. equi* from horses [11] and pigs [16] in Japan, but precise knowledge of the distribution of *R. equi* in domestic animals and the environment is limited. As part of an ongoing study of the epidemiology of *R. equi* in Japan, we examined the fecal samples of horses and cattle, the submaxillary lymph nodes of pigs, and soil collected from domestic animals' feeding grounds in Aomori prefecture and Hokkaido.

For selective isolation of *R. equi*, NANAT medium [18] was used. This medium consists of YCC agar (Eiken), to which the following is added: nalidixic acid (20 µg/ml), novobiocin (25 µg/ml), cycloheximide (40 µg/ml), and potassium tellurite (0.005%). In Woolcock's NANAT medium,

tryptone soya broth was used as a base medium, but YCC agar was used as a base medium in this experiment because of its good reproductiveness in colony counts. Fecal samples of horses were collected from the pad, those of cattle were removed from the rectum, and placed in a sterile dish for transport to the laboratory. Within a day of collection, each specimen was diluted with sterile saline, inoculated onto the NANAT medium, and incubated for 2 to 3 days at 37°C. Submaxillary lymph nodes were removed from freshly slaughtered pigs in the slaughterhouse and used for isolation. Tissues were placed in sterile dishes and processed within a day of collection. The lymph nodes were immersed in boiling water for three seconds prior to cutting up finely with sterile scissors and then the pieces were placed onto the NANAT medium. Ten grams of soil were collected from domestic animals' feeding grounds. In the laboratory, one gram of the sample was diluted with a ten fold volume of sterile water and inoculated onto the NANAT medium. All suspected colonies of *R. equi* on NANAT medium were subcultured, and the isolates were identified as gram-positive pleomorphic bacilli which were non motile, non spore-forming and non

Table 1. Isolation of *R. equi* from feces of horses and cattle and submaxillary lymph nodes of pigs

Animal	Samples	No. of samples examined	No. of isolates	Isolation rate (%)
Horse	Feces			
Mare		184	84	46.7
Foal		85	21	24.7
Cattle	Feces			
Farm A		283	68	24.0
Farm B		107	33	30.8
Pig	Submaxillary lymph nodes	470	102	21.7

Table 2. Isolation of *R. equi* from soil collected from domestic animals' feeding grounds

Domestic animal's feeding ground	No. of samples examined	No. of isolates	Isolation rate (%)
Horse	122	103	84.4
Cattle	52	52	100
Pig	26	26	100
No feeding ground	24	13	54.2

acid-fast. The colonies were non hemolytic cream mucoid, moist and glistening on blood agar, and after two to three days' growth, the colonies were mucoid and pink. The isolates produced catalase, urease and reduced nitrate, but were oxidase negative, and did not produce gelatinase. Glucose, sucrose, lactose, maltose, mannose, xylose, and rhamnose were not fermented. Organisms conforming with the description of *R. equi* [14] were kept at 4°C.

As shown in Table 1, *R. equi* was isolated from 84 (46.7%) of 184 fecal samples of the mares and from 21 (24.7%) of 85 fecal samples of the foals. There were no foals showing a pneumonia at the time of collection of the samples. *R. equi* was isolated from 68 (24.0%) of 283 fecal samples of cattle at Farm A where horses had been bred for an age. At Farm B where horses had never been bred, *R. equi* was isolated from 33 (30.8%) of 107 fecal samples of cattle. *R. equi* was isolated from 102 (21.7%) of 470 submaxillary lymph nodes of apparently healthy pigs. As shown in Table 2, *R. equi* could be

isolated from samples examined in the feeding grounds of cattle and pigs. In areas examined where animals did not feed, such as streets, river sides and cultivated fields, the incidence of *R. equi* was much lower than other samples examined.

The studies presented here have demonstrated that *R. equi* was recovered frequently from healthy horses, cattle, and pigs, and soil in Japan using the selective medium. This is the first report regarding the incidence of *R. equi* in domestic animals and soil in Japan. The selective medium reported by Woolcock and Mutimer [18] made possible the isolation of the organisms from fecal samples and soil. Our isolation rates from the mares and foals were lower than Woolcock's [20] and Nakazawa's [11]. The bacterial numbers per gram of fecal samples investigated here ranged from  $10^2$  to  $10^4$  colony forming units (data not shown), the same as that of Nakazawa's [11]. The finding of *R. equi* in normal equine feces also confirms the observation that *R. equi* is part of the normal equine fecal flora [11, 20]. *R. equi* was also recovered fre-

quently from bovine feces as Mutimer and Woolcock described [10, 19]. Though the bovine fecal samples were collected from 2 different farms, the distribution of *R. equi* on each farm was shown to be almost the same. The recovery of *R. equi* from porcine lymph nodes resembling tuberculosis [6, 7, 16, 17] and normal lymph nodes [6, 13] have been reported by many researchers, and the isolation rates of *R. equi* from normal lymph nodes were varied from 2.0% to 8.0% [7, 13]. We examined the 470 submaxillary lymph nodes, which were derived from apparently healthy pigs, and the isolation rate was 21.7%, which was as high as we expected. On the microscopical examination of the lymph nodes there were no tuberculosis like lesions. It would seem that the porcine submaxillary lymph nodes are a suitable milieu for *R. equi*. Takeuchi *et al.* [16] reported the isolation of *R. equi* from swine lymph nodes which showed tuberculosis like lesion and the detection of antibodies against *R. equi* in the sera of normal piglets and pigs by indirect fluorescent antibody technique. They concluded that *R. equi* was normal flora in pigs. The oft-recurring question of whether *R. equi* is spread by animals or whether it is primarily a germ of the soil prompted us to examine both domestic animals and soil samples for *R. equi*. Woolcock and Mutimer [19, 20] investigated *R. equi* in the gastrointestinal tracts of ruminants, feces of horses, and soil samples, and concluded that *R. equi* is primarily gut associated. Nakazawa *et al.* [11] also counted the number of *R. equi* in feces of mares and foals and described *R. equi* as a member of the normal intestinal flora in the horse. On the other hand, Burton and Hughes [3] debated the concept and described *R. equi* as a soil organism because of the physical characteristics of *R. equi* and the nature of its distribution in the environment. *R. equi* is present in the soil of every feeding ground, and the higher incidence of *R. equi* in soil samples than in domestic animals leads us to accept

the latter concept. But, there is a need for further research to elucidate this concept. In conclusion, these results suggest that *R. equi* is a normal organism of the intestine and that *R. equi* flourishes in the cycle existing between domestic animals and their soil environment.

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## 要 約

家畜および土壌からの *Rhodococcus (Corynebacterium) equi* の分離 (短報): 高井伸二・椿 志郎 (北里大学獣医畜産学部家畜衛生学教室)——馬の糞便 269 検体, 牛の糞便 390 検体, 豚の正常下顎リンパ節 470 検体および土壌 224 検体から, *Rhodococcus (Corynebacterium) equi* の分離を行なったところ, それぞれから, 105 株, 101 株, 102 株および, 194 株が分離され, 特に家畜飼育土壌から高率に *R. equi* が分離され, *R. equi* が家畜の正常細菌叢として広く分布していることが示唆された。