NOTE. Bacteriology

The Absence of *Rhodococcus equi* in Mongolian Horses

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**ABSTRACT.** In native Mongolian horses, the incidence and distribution of *Rhodococcus equi* are poorly understood. One hundred and fourteen equine fecal samples and 71 soil samples were collected from the camp sites of 26 nomadic families located in three areas less than 100 km from Ulaanbaatar, Mongolia. Five fecal samples were also collected from foals of Przewalski’s Horses introduced into the Hustai National Park, Mongolia. No *R. equi* was isolated from the Mongolian horses or the soil samples. However, three colonies of *R. equi* were isolated from two fecal samples collected from foals of Przewalski’s Horses. These isolates were avirulent, with neither 15- to 17-kDa antigens (VapA) nor a 20-kDa antigen (VapB) genes being detected. We concluded that native Mongolian horses and their environment appear free from contamination with *R. equi*.

**KEY WORDS:** Mongolian horse, Przewalski’s horse, *Rhodococcus equi*.

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Following its first isolation from equine lung abscesses by Magnusson in 1923 [4], *Rhodococcus equi*, a soil organism, is one of the most important causes of pneumonia in foals [7]. *R. equi* has a worldwide distribution in the feces of domestic animals, especially horses, and their environment [1, 13]. It is ingested by many herbivores [9]. We have reported that *R. equi* has at least two different virulence levels: virulent *R. equi* requires 10^6 bacteria for lethality in mice, while the intermediately virulent form requires 10^7 bacteria [13, 17]. Virulent *R. equi* is characterized by the presence of 15- to 17-kDa antigens (VapA) and a plasmid DNA of 80–90 kb, and has been found in the pulmonary and/or intestinal lesions of foals [21]. *R. equi* strains of intermediate virulence are identified by a 20-kDa antigen (VapB) and a plasmid DNA of 79–100 kb and have been isolated from the submaxillary lymph nodes of pigs [13, 20]. There is also an avirulent form of *R. equi*, widespread in soil, that shows no evidence of either virulence-associated antigens or plasmid DNA [9].

The restriction-enzyme digestion patterns of virulence plasmids in foals have categorized the plasmids of virulent isolates into 12 closely related types, and revealed geographic differences in the distributions of these virulence plasmids in Japan and throughout the world [5, 10, 14, 16, 18, 19]. In a recent study, we demonstrated that the environment of the native Jeju horse farms on Jeju Island, Korea, is contaminated with two types of virulent *R. equi* isolates containing a 90-kb type II plasmid, that has also been found in isolates from the native Kiso horses of Japan [19].

There are eight native horse breeds in Japan (Hokkaido, Kiso, Noma, Misaki, Tokara, Taishu, Miyako, and Yonaguni) and each is thought to have originated principally from the Mongolian horse [6]. On the island of Cheju, the native horse has been present for about 700 years since the Mongolians introduced their horses to the island [6]. We hypothesized that the transmission to Japan of the virulent form of *R. equi*, with its various virulence plasmid types, may have occurred with the migration of native horses from the Mongolian grasslands. The presence of a 90-kb type II plasmid in Korean and Japanese isolates also partially supports the above hypothesis of a common origin and ancestry of the native horses of northeast Asia [19]. However, there has been no information about the distribution of virulent *R. equi* in Mongolian horses in Mongolia. According to a field survey by local veterinarians, the disease was not recognized, as there has been no outbreak of *R. equi* infection in foals. To investigate the virulence of *R. equi* in Mongolian horses, we studied the feces of foals and soil samples collected from horses raised by nomadic families in three areas less than 100 km from Ulaanbaatar, Mongolia.

One hundred and fourteen fecal samples and 71 soil samples were collected from the camp sites of 26 families who raised horses (Table 1). The details were as follows: 54 fecal samples and 46 soil samples were collected from horses raised by 15 nomadic families camped southwest of the Tuul river in Turgen, Bokhog, Sujig, and Khar khugir; 40 fecal samples and 16 soil samples were collected from horses raised by six nomadic families camped northwest of the Tuul river in Baruun turuu, Davaaanii zolr and Jargalant; and 20 fecal samples and nine soil samples were collected from horses raised by five nomadic families camped east of Ulaanbaatar in Khonkhor and Bus nuur. Five fecal samples from foals of the Przewalski’s Horses in the Hustai National Park were also examined.

Each fecal sample was collected from the ground immediately after defecation and a small spoon was used to scrape each soil sample from the surface of the ground. Each sample was placed in a sterile tube. In the laboratory at State
Central Veterinary Laboratory, 1 g of the feces or soil was diluted serially with a tenfold volume of sterile saline. Each dilution was inoculated onto two plates of nalidixic acid-novobiocin-actidione (cycloheximide)-potassium tellurite (NANAT) medium, as previously described by Woolcock et al. [22]. The plates were incubated at 30 °C for two or three days. The \textit{R. equi} colonies were counted, and the numbers of viable organisms per gram of feces or soil were calculated. Suspected colonies of \textit{R. equi} were subcultured and examined for \textit{vapA} and \textit{vapB} genes by polymerase chain reaction (PCR). The target DNAs for PCR amplification were the published sequences of the 15- to 17-kDa antigen (VapA) gene and a 20-kDa antigen (VapB) gene (Genebank database accession numbers D21236l and D44469) from \textit{R. equi} strains ATCC 33701 and 5, respectively [8, 13]. Primer 1 (5'-GACTCTTCACAAGACG GT-3') corresponded to the sense strand at position 6 to 23, and primer 2 (5'-TAG-GCGTTGTGCCAGCTA-3') corresponded to the antisense strand at position 569 to 552 in the sequence of the cloned fragment containing the 20-kDa antigen gene [13]. PCR amplification was performed using previously described methods [12, 20].

No \textit{R. equi} was isolated from any of the fecal and soil samples from Mongolian horses (Table 1). However, three colonies of \textit{R. equi} were isolated from two fecal samples collected from foals of Przewalski’s Horses, though neither 15- to 17-kDa antigens (VapA) or a 20-kDa antigen (VapB) genes were detected by PCR. These Przewalski’s horses were isolated from native horses owned by the nomadic families and their native horses could not enter the national park.

We have isolated virulent \textit{R. equi} from the feces of foals and their environment on horse-breeding farms in the Americas, Europe, Africa, Oceania, and Asia [5, 10, 12, 14, 16, 18, 19, 21]. In the present study, no \textit{R. equi} was isolated from fecal samples of Mongolian foals raised by the nomadic families and their native horses could not enter the national park. In a previous study, we isolated \textit{R. equi} from the feces of foals at one to two weeks of age, and from all of the foals by four weeks of age [15]. The mean number of \textit{R. equi} in the fecal samples increased progressively with age to \(10^4\) to \(10^5\) g of feces, was maintained up to the age of eight to 10 weeks, which was significantly different from the number of \textit{R. equi} in the fecal samples from native horses.
and then gradually decreased to the level found in the dams’ feces [15]. Also in the present study, no R. equi was found in soil samples around the camp sites. These results were of special interest, since avirulent R. equi is widespread in parks, yards, and cultivated land located in temperate zones in Japan and European countries [1, 5, 11]. Possible reasons for the absence of R. equi in soil in Ulaanbaatar include the severe climate and the large areas of grazing land over which R. equi is dispersed. The evidence that there have been no reports of R. equi infection in foals at the State Central Veterinary Laboratory, Ulaanbaatar also supports these unexpected findings. The prediction is therefore that adult horses in Mongolia will be fully susceptible to R. equi infection. This would address the issue of whether R. equi is a problem because of some age-related impairment of cellular immunity in foals [3].

The Przewalski’s Horse (Equus przewalski poliakov) is the only known surviving wild species of Equidae remaining on earth since the extinction of the last wild Tarpan (E. p. gmelini) in 1879 [2, 6]. In June of 1992, through the herculean efforts of the Przewalski Foundation in Holland and the breeding reserves in Askania Nova, Ukraine, two combined breeding groups of Przewalski’s Horses were released at the Hustai National Park in Mongolia [2]. Three colonies of avirulent R. equi were grown from the fecal samples of foals of these horses. It is not known whether these isolates were introduced into the national park with the Przewalski’s Horses.

In conclusion, this study showed no evidence of R. equi in either fecal samples of Mongolian foals or soil samples collected from their environment, however, avirulent R. equi were isolated from the feces of descendants of the introduced Przewalski’s Horses. It thus appears unlikely that the transmission to Japan of the virulent form of R. equi occurred with the migration of native horses from the Mongolian grasslands. However, further studies are needed to investigate the ecology of R. equi and the immunity against R. equi in Mongolian horses in other regions of Mongolia.

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