NOTE  Bacteriology

Isolation of Rhodococcus equi from the feces of indigenous animals and soil from the Lower Zambezi National Park and Lochinvar National Park, Zambia

Shinji TAKAI1, Michelo SYAKALIMA2, Jun YASUDA3, Yukako SASAKI1, Hisako TSUTSUMI1, Emiko MIYAGAWA1, Kaya WADA1, Tsutomu KAKUDA1, Shiro TSUBAKI1 and Chihiro SUGIMOTO4

1) Department of Animal Hygiene, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034-8628, Japan, 2) Department of Disease Control, School of Veterinary Medicine, University of Zambia, P. O. Box 32379, Lusaka, Zambia, 3) Veterinary Teaching Hospital, School of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka 020-8550 and 4) National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro 080-8555, Hokkaido, Japan

(Received 12 December 2003/Accepted 19 January 2004)

ABSTRACT. Rhodococcus equi is an important pathogen in foals; however, its incidence in African indigenous animals is poorly understood. Fecal samples (92 from nine indigenous species) and 43 soil samples were collected from two Zambian National Parks. The presence of R. equi was investigated and 533 isolates were tested for the presence of 15- to 17-kDa antigens (VapA) and a 20-kDa antigen (VapB) by immunoblotting and PCR. R. equi was isolated (102–104 colony forming units/g) from 75% of fecal and 74% of soil samples. Neither antigen was detected; however, about 20% of the isolates contained cryptic plasmids of various sizes. There was no evidence of virulent R. equi, but the avirulent form was widespread in the animals and the soil.

KEY WORDS: Africa, Rhodococcus equi, wildlife.


Rhodococcus equi is one of the most important bacterial pathogens of foals less than three months of age [1, 10]. Infections caused by this organism are characterized by a chronic, suppurative bronchopneumonia and/or enteritis [10, 12]. In horses, the disease is distributed worldwide, but it is quite rare in other species, except for pigs and immunosuppressed humans, especially humans infected with human immunodeficiency virus (HIV) [1, 10, 12, 13, 20, 23]. R. equi has been reported occasionally in cattle, goats, llama, dogs, cats and in indigenous animals, but only as an opportunistic infection [1, 4, 5, 7, 10, 17, 22].

The discovery of virulence-associated antigens and virulence plasmids has permitted classification of the virulence of R. equi strains [18, 21]. At least three virulence levels of R. equi have been identified: virulent, intermediate virulence, and avirulent [16]. Virulent R. equi is characterized by the presence of virulence-associated 15- to 17-kDa antigens (VapA), and virulence plasmid DNA of 80–90 kb [18, 19]. The virulent form has been found in pulmonary and/or intestinal lesions of foals and in the pulmonary lesions of AIDS patients (murine LD50=108) [12, 16]. R. equi strains of intermediate virulence are identified by a virulence associated 20-kDa antigen (VapB) and virulence plasmid DNA of 79–100 kb [16, 20]. The intermediate virulence form has been found in the submaxillary lymph nodes of pigs (murine LD50=108) and in the pulmonary lesions of AIDS patients [13, 20]. In comparison, avirulent R. equi shows no evidence of either virulence-associated antigens or plasmid DNA (murine LD50>108), and is widespread in soil [12].

In a recent study, we observed that the soil of a horse-breeding farm in South Africa was contaminated with a virulent R. equi containing an 85-kb type I or an 87-kb type I plasmid [14]. There are no indigenous horses in South Africa, and they were first imported by Europeans from Java in 1653 [3]. The introduction of European horses into South Africa may be correlated with the arrival of a virulent form of R. equi [14]. In Africa, little is known about the spread of virulent R. equi to the indigenous animals, especially the Equidae. Zebras (Equus zebra) are abundant in certain areas and it would be interesting to know whether the virulent form of R. equi occurs in this species or other indigenous animals. The purpose of this study was to isolate R. equi from the feces of a range of indigenous animals and from soil samples collected from the local environment in the Lower Zambezi and Lochinvar National Parks in Zambia. We also investigated the plasmid profiles of the isolates of R. equi.

In the Lower Zambezi National Park, 27 fecal samples were collected from the following species: zebras (Equus zebra; 11 samples), African elephants (Loxodonta africana; four samples), impalas (Aepyceros melampus; four samples), baboons (Papio hamadryas; three samples), African buffalos (Syncerus caffer; two samples), and single samples from a greater kudu (Tragelaphus strepsiceros), a warthog (Phacochoerus aethiopicus), and an African porcupine (Hystrix cristata). In addition, soil samples were collected from 16 sites in the Park. In the Lochinvar National Park, 65 fecal samples were collected from zebras (Equus zebra; 45 samples) and lechwes (Kobus leche; 20 samples), and soil samples were collected from 27 sites. The fecal samples were collected from the ground immediately after defecation. A small spoon was used to scrape each soil sample from the surface of the ground and each sample was poured into a sterile tube. In the laboratory, 1 g of the feces or soil was diluted serially with a 10-fold 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. [24]. The plates were incubated at 30°C for two or three days. The *R. equi* colonies were counted, and the numbers of viable organisms per gram feces and soil were calculated. Between one and 10 colonies of *R. equi* per sample were subcultured and examined for VapA and VapB by a colony blot enzyme-linked immunosorbent assay with monoclonal antibodies [16]. For colony blot analysis, bacterial strains were injected onto immunosorbent assay with monoclonal antibodies [16].

The target DNAs for polymerase chain reaction (PCR) amplification were the published sequences of the 15- to 17-kDa antigen (VapA) gene and a 20-kDa antigen (VapB) [11]. Primer 3 (5'-AACGAGACTTGAGCGACTA-3') corresponded to the sense strand at position 6 to 23, and primer 4 (5'-ACCGAGACTTGAGCGACTA-3') corresponded to the antisense strand at position 1066 to 1048 in the sequence of the cloned fragment containing the 20-kDa antigen gene [16, 20].

PCR amplification was performed with 10 µl of the DNA preparation in a 50-µl reaction containing 10 mM Tris-HCl (pH 8.3 at 25°C), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM (each) deoxynucleotide triphosphates, 1 mM of each primer, and 2.5 U of Taq DNA polymerase (Takara, Tokyo, Japan), as described previously [15]. The samples were subjected to 30 cycles of amplification in a GeneAmp PCR system 2400 (Perkin-Elmer, Norwalk, CT). The cycling conditions were as follows: denaturation for 90 s at 94°C, primer annealing for 1 min at 55°C, and extension for 2 min at 72°C [15].

In the present study, strains ATCC 33701 (equine origin, virulent strain) and strain 5 (human origin, strain of intermediate virulence) were used as reference strains because some of the protein profiles, plasmid characteristics, and virulence levels of these strains have been described [19, 20].

In samples with 10² to 10⁶ colony forming units per gram (Table 1), *R. equi* was isolated from 69 of 92 (75%) of the fecal samples obtained from nine species (zebra, lechwe, African elephant, impala, African buffalo, greater kudu, warthog, baboon, and African porcupine), and from 32 of 43 (74%) of the soil samples. None of the 533 isolates showed the presence of 15- to 17-kDa antigens (VapA), or a 20-kDa antigen (VapB) (Table 2). However, about 20% of the isolates (108 of 533) contained cryptic plasmids of various sizes.

The Lower Zambezi National Park is in a remote region of Zambia and is rarely entered by humans or domestic animals. The Park contains many indigenous animals including zebras, African elephants, hippopotami, Asiatic buffaloes (*Bubalus bubalis*), lions, warthogs, and various antelopes. On the other hand, the Lochinvar National Park has a flat topography and is located along the Kafue River.

### Table 1. Incidence of *R. equi* in the feces of indigenous animals and soil collected from the Lower Zambezi and Lochinvar National Parks in Zambia

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>Lower Zambezi National Park</th>
<th>Lochinvar National Park</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples</td>
<td>No. of samples</td>
</tr>
<tr>
<td>Feces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zebra</td>
<td>11 (10)³</td>
<td>45 (33)</td>
</tr>
<tr>
<td>Lechwe</td>
<td>–</td>
<td>20</td>
</tr>
<tr>
<td>African elephant</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>Impala</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>Baboon</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>African buffalo</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Greater kudu</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Warthog</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>African porcupine</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Subtotal</td>
<td>27</td>
<td>65</td>
</tr>
<tr>
<td>Soil</td>
<td>16 (11)</td>
<td>27 (21)</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>92</td>
</tr>
</tbody>
</table>

- a) The numbers of *R. equi* positive samples are given in parentheses.
In the rainy season, the river flats are flooded. Local people are often seen entering the park, together with domestic animals, including zebras, lechwes and water buffalos. There are no reports of a R. equi infection in indigenous animals in Africa. However, there are several cases of R. equi infection in HIV patients in Uganda and Zimbabwe, and outbreaks of R. equi infection in Thoroughbred foals in Zimbabwe have been described [6, 8, 9]. Unfortunately, the virulence of those human and foal isolates was not determined; however, in a recent study, we found virulent R. equi in isolates obtained in South Africa from clinically affected foals and their environment [14].

Some of the indigenous animals investigated in this study (lechwe, impala, African buffalo and greater kudu) belong to the family Bovidae. Avirulent R. equi has been isolated from domestic members of Bovidae (such as cattle and goats) [4, 5, 22], and it was also isolated from the indigenous members of this family in this study. Although zebras belong to the genus Equus, we did not isolate virulent R. equi from either the feces or the animals’ environment. However, the overall prevalence of cryptic plasmids in the isolates from all indigenous animals and soil was high, and ranged from 13.3 to 30%. In our previous investigation of horse-breeding farms in Hokkaido, Japan, the incidence of cryptic plasmids was lower (3.8% of 1,725 fecal isolates and 0% from either the feces or the animals’ environment).

In conclusion, this study found no evidence of virulent R. equi in either fecal samples of indigenous animals or soil samples collected from their environment in the two national parks in Zambia; however, avirulent R. equi was widespread in both the animals and soil. Further studies may be needed to explain the ecology of virulent R. equi in indigenous animals in other regions of Africa.

ACKNOWLEDGMENTS. This study was supported by a Grant-in-Aid for General Scientific Research (Yasuda:15255021, Takai:14656124) from the Ministry of Education, Science, Sports and Culture of Japan.

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