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Virulence of *Rhodococcus equi* Isolated from Cats and Dogs

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Nine cat isolates and nine dog isolates of *Rhodococcus equi* from clinical material were investigated for the presence of the virulence-associated antigens (VapA and VapB) and virulence plasmids. Five of the cat isolates and one dog isolate were VapA positive and contained an 85-kb type I or an 87-kb type I plasmid. The remaining 12 isolates were avirulent *R. equi* strains and contained no virulence plasmids.

Rhodococcus equi is a facultative, intracellular, gram-positive coccobacillus that causes chronic suppurative bronchopneumonia and enteritis and is associated with a high mortality rate in 1- to 3-month-old foals (1, 15, 16). It has also been isolated from the submaxillary lymph nodes of pigs (18) and the lymph nodes of cattle (8). *R. equi* infection is increasing in prominence in immunocompromised humans, particularly those infected with human immunodeficiency virus (9, 12, 27). Although *R. equi* infections in species other than horses (foals) are rare and are regarded as opportunistic, they have recently been increasingly reported in cats and dogs (5, 6, 7, 10, 11, 13, 14). In cats, pyogranulomatous lesions are the characteristic symptom, with primary involvement of the extremities in most affected cats (14).

The discovery of virulence-associated antigens and virulence plasmids has allowed the virulence of *R. equi* strains to be classified (24, 26). At least three levels of virulence have been identified for *R. equi*: virulent, intermediately virulent, and avirulent (16). Virulent *R. equi* is characterized by the presence of 15- to 17-kDa virulence-associated antigens (VapA) and virulence plasmid DNA of 80 to 90 kb (21, 24, 25), and these strains are found in the pulmonary or intestinal lesions of foals and in the pulmonary lesions of AIDS patients (murine 50% lethal dose [LD_{50}] = 10^6 bacteria) (23). *R. equi* strains of intermediate virulence are identified by a 20-kDa virulence-associated antigen (VapB) and virulence plasmid DNA of 79 to 100 kb and are found in the submaxillary lymph nodes of pigs (murine LD_{50} = 10^7 bacteria) and the pulmonary lesions of AIDS patients (22). In contrast, avirulent *R. equi* shows evidence of neither virulence-associated antigens nor plasmid DNA (murine LD_{50} = $>10^8$ bacteria) and is widespread in soil (16). It is noteworthy that the majority of *R. equi* isolates from AIDS patients are virulent or intermediately virulent, whereas those from patients with immunosuppression derived from other causes are avirulent (22, 23).

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Exposure to manure and soils contaminated with the manure of domestic animals such as horses, cattle, and pigs may be one of the possible routes of infection in humans (15, 16). Cats and dogs infected with *R. equi* have not been considered to be a source of infection for humans, who usually acquire the infection from environmental exposure. Nevertheless, infected cats and dogs with discharges may pose some theoretical risk to immunocompromised owners (9, 12, 27). There have been few studies that include the plasmid profiles or report the presence of VapA in isolates from companion animals (19, 26). Therefore, the pathogenicity of *R. equi* isolates from cats and dogs remains unclear. The purposes of this study were to investigate the presence of *vap* genes in 18 *R. equi* isolates from cats and dogs and to examine the plasmid profiles of these isolates.

The clinical isolates of *R. equi* used in the present study are listed in Table 1. Five cat isolates and seven dog isolates were obtained from the College of Veterinary Medicine, Texas A&M University; two cat isolates and two dog isolates were from Ontario Veterinary College, University of Guelph; and one cat isolate each was from New Zealand and Brazil. Although the respiratory tract is not a principal site of infection in these animals, nasal swabs were collected from three dogs. Extrapulmonary infection was more common in both cats and dogs, including wound infections, subcutaneous abscesses, vaginitis, hepatitis, osteomyelitis, myositis, and joint infections. Swabs were also collected from the eyes and ears of these animals. Unfortunately, details of the clinical manifestations were not available in most cases.

Eighteen isolates were examined for VapA and VapB by colony blot enzyme-linked immunosorbent assay with monoclonal antibodies (20, 22). The target DNAs for PCR amplification were the published sequences of the 15- to 17-kDa antigen (VapA) gene and the 20-kDa antigen (VapB) gene (GenBank database accession numbers D21236l and D44469, respectively) from *R. equi* strain ATCC 33701 and isolate 5, respectively (4, 21). PCR amplification was performed as described previously (4, 21). Plasmid DNA was isolated from *R. equi* by the alkaline lysis method (3), with some modifications as described previously (24). Plasmid DNAs were analyzed by

TABLE 1. Source, presence of *vapA* and *vapB* genes, and plasmid profiles of *R. equi* isolates from cats and dogs

Isolate	Yr of isolation	Animal	Sex	Age	Lesion or symptom	Presence of *vapA*	Presence of *vapB*	Plasmid[c]	Reference and/or site of isolation
C98037174	1998	Cat	Male	10 yr	Unknown	−[a]	−		United States
C98326079	1998	Cat	Male	Unknown	Abdominal wound	−	−		United States
D9210985	1999	Cat	Female	Unknown	Abscess with an associated adenocarcinoma	+	−	85	United States
C000910124	2000	Cat	Male	1 yr	Ear swab	−	−		United States
C012570132	2001	Cat	Male	Unknown	Wound	+	−	85	United States
Ruakura 1	2000	Cat	Male	Unknown	Necrotic tissue on leg	+	−	85	New Zealand
Isolate F1 FD-118	1977	Cat	Unknown	Unknown	Unknown	+	−	85	Canada
Isolate F2 1979–6048	1979	Cat	Unknown	Unknown	Abscess	−	−		Canada
Isolate 8	1994	Cat	Male	2 yr	Ulcerated tissue on leg	+	−	87	Brazil
Isolate 102	Unknown	Cat	Unknown	Unknown	Clinical material	+	−	85	26; Canada[b]
Isolate 23	Unknown	Cat	Unknown	Unknown	Clinical material	+	−	85	26; Canada[b]
C98219287	1998	Pekinese	Male	11 yr	Nasal swab	−	−		United States
C98293165	1998	Golden retriever	Female	8 yr	Nasal lesion	−	−		United States
TAMU135318	2000	Dog	Unknown	Unknown	Joint	−	−		United States
18711	2001	Dog	Unknown	Unknown	Mass	−	−		United States
C001150191	2000	Dog	Female	12 yr	Eye	+	−	87	United States
C012340021	2001	Dog	Female	5 yr	Nasal lesion	−	−		United States
C012630142	2001	Dog	Male	7 mo	Eye	−	−		United States
Isolate CI 1977–3725	1977	Dog	Unknown	Unknown	Skin swab	−	−		Canada
Isolate C2 1998-28764	1998	Dog	Unknown	Puppy	Vaginitis	−	−		Canada
Isolate	Unknown	Basenji dog	Female	3 mo	Necrotizing pyogranulomatous hepatitis, osteomyelitis, myositis	−	−		5; United States[b]
4527	1997	Dog	Unknown	Unknown	Chronic tracheitis	−	−		19; South Africa[b]
ATCC 33702	Unknown	Dog	Unknown	Unknown	Clinical material (skin)	−	−		26; Canada[b]
Isolate 5	Unknown	Dog	Unknown	Unknown	Clinical material (skin)	−	−		26; Canada[b]

[a] Symbols: +, present; −, not present.
[b] Data are from Cantor et al. (5), Takai et al. (19), and Tkachuk-Saad et al. (26).
[c] 85, 85-kb type I; 87, 87-kb type I.

digestion with restriction endonucleases *Eco*RI, *Eco*T22I, and *Hin*dIII for detailed comparisons and to estimate plasmid sizes. The bacterial strains used as reference strains in this study were *R. equi* ATCC 33701 (85-kb type I plasmid), 96E35 (85-kb type II plasmid), T47-2 (85-kb type III plasmid), T43 (85-kb type IV plasmid), 222 (87-kb type I plasmid), 96B6 (87-kb type II plasmid), and L1 (90-kb type I plasmid) (17, 25).

Of the nine clinical isolates from cats, five isolates from Brazil, Canada, New Zealand, and the United States were positive for *vapA* and virulence plasmid DNA. Of the nine clinical isolates from dogs, one isolate from the United States was positive for *vapA* and virulence plasmid DNA. The remaining four cat and eight dog isolates were negative for both virulence-associated antigens and plasmids. The six isolates expressing VapA and a positive control, ATCC 33701, produced positive results in the PCR, showing a 547-bp amplification product. Plasmid DNA preparations from the six isolates were analyzed by restriction enzyme digestion with the four endonucleases, and four of the six isolates contained an 85-kb type I plasmid and the remaining two isolates contained an 87-kb type I plasmid.

There have been sporadic reports in the literature of *Rhodococcus* infections in cats associated with mediastinal and mesenteric lymphadenitis (11) and cellulitis and abscesses, mainly of the extremities (6, 7, 10, 13) and of the neck (14). More recently, reports of *R. equi* infections in dogs have appeared (5). Although the number of cases is increasing, the virulence of the feline and canine isolates has not previously been characterized. In this study, we examined 18 cat and dog isolates from the Americas and New Zealand and found that five of the nine cat isolates and one of the nine dog isolates were *R. equi* VapA positive and that the remaining isolates were avirulent. Fisher's exact test indicated that there was no statistically significant difference between the nine isolates from cats and the nine isolates from dogs. However, when the data (shown in Table 1) from previous studies (5, 19, 26) were added into the statistical analysis, there was a significant difference between the 11 cat isolates and the 13 dog isolates. These results may reflect differences between the features of cat and dog infections, such as the source or route of infection or the predisposing factors of the hosts.

Virulent, but not avirulent, *R. equi* can produce pneumonic disease in foals experimentally (16), and clinical isolates from naturally infected foals have all been virulent *R. equi* strains expressing VapA (16, 24). On the other hand, the majority of pig isolates are intermediately virulent *R. equi* strains expressing VapB (18). VapA-positive *R. equi* is widespread in the environments of horse-breeding farms (16), and VapB-positive *R. equi* is largely restricted to the environments of pig farms (18). Previous studies thus suggest that VapA-positive *R. equi* isolates from cats and dog should be derived from horses or their environments (16). The plasmid profiles of VapA-positive isolates from cats and dogs also indicate that these isolates may be closely associated with those from horses, because either 85-kb type I or 87-kb type I plasmids have been found in clinical isolates from foals in North and South America (17, 25). However, little is known about the source and mode of *R. equi* infections in cats and dogs. Exposure to soil contaminated

with livestock manure is likely to be the major route of infection for these companion animals. The most probable mode of infection could involve the establishment of *R. equi* in subcutaneous tissues after a penetrating wound is contaminated from environmental sources, with subsequent hematogenous dissemination to the spleen and local spread to the peritoneal cavity (14). In the present study, a Brazilian cat from which isolate 8 was recovered had had contact with horses and cows, but we have no information about the exposure of the other animals from which isolates were taken.

In the nine dog isolates, one isolate was from a puppy and three were from old dogs, suggesting that immaturity and impairment of the immune system may be predisposing factors. Cantor et al. (5) also discussed the possibility of intrinsic defects of the immune system in their 3-month-old animal.

In humans, *R. equi* infections have primarily been reported in association with human immunodeficiency virus infections and other immunosuppressive diseases or therapy (27). The majority of cat isolates in this and previous studies were virulent *R. equi* strains (26). It is very interesting that the prevalence of virulent *R. equi* in cats and dogs is very similar to that in patients with and without AIDS, respectively (22, 23). Feline immunodeficiency virus (FIV)-infected cats are found worldwide (2). In the United States, approximately 1.5 to 3% of healthy cats are infected with FIV (2). Infection rates rise significantly in cats that are sick; up to 15% of cats with clinical signs of other diseases are also infected with FIV (2). In the present study, there was no information on the FIV status of the nine cats analyzed, but the high seroprevalence of FIV in clinically ill cats suggests that some of the feline *R. equi* infections studied were probably associated with FIV infections (2).

R. equi infections in companion animals have been thought to be very rare, but they may be increasing in cats and dogs. It is possible, however, that in the past, laboratories have misidentified colonies of *R. equi* as contaminants in routine bacteriological examination of specimens from cats and dogs or that such isolates were not reported in the literature, even when correctly identified. Further epidemiological surveillance may clarify the incidence of *R. equi* infection and the factors predisposing cats and dogs to this infection.

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