

CASE REPORT

Cutaneous pyogranuloma in a cat caused by virulent *Rhodococcus equi* containing an 87 kb type I plasmid

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A 2-year-old intact male domestic shorthaired cat presented with a chronic, nodular, ulcerated, cutaneous lesion on the right thoracic limb. Histological and cytological examination revealed a pyogranulomatous inflammation with basophilic organisms in the macrophages. A virulent form of *Rhodococcus equi* containing an 87 kb type I (VapA) virulence plasmid was identified from cultures of biopsy samples. This report describes the clinicopathological features, plasmid profile and virulence of this case of *R equi* infection.

Key words: cat, dermatology, bacterial, *Rhodococcus*, pyogranulomatous

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In many areas of the world, *Rhodococcus equi* is an important cause of pyogranulomatous pneumonia in foals.¹ Since the first isolation of *R equi* from equine lung abscesses by Magnusson in 1923,² subsequent reports have shown that it also causes extrapulmonary lesions.³ *R equi* has been detected in pyogranulomatous lesions of other domestic and companion animals, and its incidence in HIV-infected humans has increased during the last decade.⁴ The clinical manifestations of *R equi* infection in cats are cutaneous lesions with fistulas or ulcerations and purulent drainage, mediastinal and mesenteric lymphadenitis, anorexia, weight loss, pyothorax, pneumonia and disseminated pyogenic lesions of the internal organs.^{5–9} This report describes the clinical, pathological, microbiological and virulence-associated features of a cat with a cutaneous pyogranuloma infected with VapA-positive *R equi*.

Case report

A 2-year-old entire male domestic shorthaired cat was admitted to the Dermatology Service at the Veterinary School of UNESP/Botucatu, State of São Paulo, Brazil. The cat came from a rural

property and had been in contact with numerous other cats and dogs, horses and cattle. On the lateral aspect of the right thoracic limb (just proximal to the elbow), the animal had a painless, firm, well-circumscribed, 6 cm-diameter mass (Figure 1), which had been growing slowly for 2 months. The lesion was not pruritic and had an elevated border, a liquefied, necrotic and ulcerated centre and a copious purulent exudation. The cat had generalised musculo-skeletal weakness and pyrexia (39.6°C). There was no history of previous disease, but the owners had often noticed cutaneous scratches and bite wounds. There had been no response to treatment with local application of povidone–iodine and a 20 day oral course of cephalexin (30 mg/kg twice daily).

Leukocytosis (leukocyte count: $21.0 \times 10^9/L$), neutrophilia (81%) with toxic neutrophils, lymphopenia (7%) and a left shift in the leukogram were found. The erythrogram showed that moderate anaemia was present (erythrocyte count: $4100 \times 10^9/L$). Tests for renal failure (urea and creatinine concentrations) and hepatic failure (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and serum bilirubin concentrations) were negative. A Gram-stained smear showed that basophilic, Gram-positive, coccoid or coccobacillary organisms were present within macrophages and neutrophils of the mass. Biopsies were submitted for histological examination and microbiological culture in defibrinated sheep-blood agar, MacConkey agar, Sabouraud agar or Löwenstein–Jensen medium. Histological samples stained with haematoxylin–eosin, Giemsa and Gomori's methenamine silver showed the presence of a chronic pyogranulomatous inflammatory process involving neutrophilic suppurative foci among plasma cells, a great number of macrophages and few lymphocytes. Macrophages contained vacuolised cytoplasm and coccoid or coccobacillary organisms, and basophilic structures were observed in several cells. Periodic acid–Schiff and Ziehl–Neelsen staining showed no evidence of fungal agents or alcohol–acid-resistant bacilli. Numerous large, irregular, mucoid, non-haemolytic, salmon-pink colonies formed after aerobic incubation of biopsy fragments at 37°C for 48 h in defibrinated sheep-blood agar medium. There was no microbial growth in MacConkey agar or Sabouraud agar or of organisms compatible with *Mycobacterium* genera in the Löwenstein–Jensen medium. Antimicrobial susceptibility tests showed that the organism was susceptible to streptomycin, norfloxacin, gentamicin

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Figure 1. *Rhodococcus equi* pyogranuloma in a cat. Chronic cutaneous lesion on the right thoracic limb.

and cephalexin, had intermediate susceptibility to rifampicin, amikacin, erythromycin, ceftriaxone and amoxicillin–clavulanate, and was resistant to penicillin, amoxycillin and trimethoprim–sulfamethoxazole. Tests for feline immunodeficiency virus and feline leukaemia virus (SNAP FIV Antibody/FeLV Antigen Combo Test kit, IDEXX Laboratories, Westbrook, Maine, USA) were negative.

Virulence-associated antigens (VapA and VapB) and the plasmid profile of the *R equi* strain were analysed according to methods described previously.¹³ Virulence antigens were examined using a colony-blot enzyme-linked immunosorbent assay and monoclonal antibodies. The target DNAs for PCR amplification were based on the 15 to 17 kDa antigen (VapA) gene and the 20 kDa antigen (VapB) sequences from *R equi* strain ATCC 33701 and isolate 5, respectively. Plasmid DNAs were analysed using digestion with restriction endonucleases (*EcoRI*, *EcoT22I* and *HindIII*) for the estimated plasmid sizes. The biochemical characteristics, virulence-associated antigens and the plasmid profile all indicated that the micro-organism was a virulent *Rhodococcus equi* containing an 87 kb type I virulence plasmid.

Surgical excision was recommended and the cat was started on gentamicin (5 mg/kg subcutaneously twice daily) while awaiting surgery. After 3 days the clinical signs suddenly worsened, with the cat becoming hypotensive, dyspnoeic and hypothermic. There was no response to aggressive fluid therapy and the cat was euthanased. At necropsy, there was no macroscopic evidence of involvement of other organs, and microbiological cultures of tissue from the lymph nodes, spleen, liver, kidney and lung were negative.

Discussion

In the present study, we describe the clinicopathological characteristics of a cat with cutaneous pyogranuloma caused by virulent VapA-positive *R equi*. This was one of the cases in previously published larger study, in which the virulence-associated antigens and the plasmid profile of this *R equi* strain, together with eight other *R equi* strains isolated from cats and nine *R equi* strains isolated from dogs in various countries, were described.¹³ The molecular characteristics of the virulence of these strains were compared with those of common immunosuppressive diseases in cats and humans.¹³

In previous reports of sporadic cases of *Rhodococcus* infections in cats, this disease was associated with pneumonia,⁹ mediastinal and mesenteric lymphadenitis,⁶ cellulitis and abscesses of the extremities and neck.^{7,8} The clinical features of our case were similar to those of previously described cases in which the skin and subcutaneous tissue of the thoracic and pelvic limbs were affected with oedema, erythema, abscesses and purulent exudation.⁸ In the present case, the firm tumour-like lesion and its elevated borders, liquefied necrotic centre, purulent exudation and granulomatous histological characteristics resembled the cutaneous lesions caused by *Actinomyces* and *Mycobacterium*.⁹ We used culture media for isolation of cutaneous opportunistic bacteria, mycobacterial or fungal agents, however, only *R equi* grew on the blood agar. The associated involvement of regional lymph nodes draining the cutaneous lesion has been described;^{8,10} in one cat, the infection spread to the spleen and peritoneal cavity.⁶ In the present study, there was no evidence of involvement of regional lymph nodes. Microbiological cultures of lymph nodes, spleen, liver, kidney and lung were also negative.

R equi are intracellular, facultative, opportunistic organisms. In animals and humans, they cause severe pyogranulomatous lesions that are refractory to conventional therapy. The combination of rifampicin and erythromycin is considered to be the therapy of choice for rhodococcosis, especially in foals. These drugs are lipid soluble and penetrate caseous material and phagocytic cells.^{1,3} Drugs such as gentamicin, lincomycin and amoxicillin have also been recommended for treatment of rhodococcosis in cats.^{5,6} A combination of amoxicillin and clavulanate was successful in curing cutaneous lesions in five of six cats within 14 to 16 days.⁹ In the present case, the *R equi* strain displayed intermediate susceptibility to rifampicin, erythromycin and amoxicillin–clavulanate. The referring veterinarian treated the cat with oral cephalexin (30 mg/kg, twice a day for 20 days), but the clinical response was poor. Based on in vitro susceptibility testing, we used gentamicin (5 mg/kg, SC, twice a day) while awaiting the scheduled surgical excision, however, the condition of the cat deteriorated, probably as a result of toxæmia, and it was euthanased.

Previous examination of cats with cutaneous and disseminated *R equi* infections have demonstrated the presence of pyogranulomatous inflammation, neutrophils and macrophages. The macrophages were associated with numerous Gram-positive, pleomorphic organisms and giant cells in the dermis, subcutaneous tissue

and spleen.^{6,8-10} In one cat with an extra-cutaneous lesion, the mesenteric and mediastinal lymph nodes exhibited chronic granulomatous lymphadenitis characterised by extensive histiocytic proliferation and the presence of neutrophils and epithelioid cells.¹⁰ Our case also showed a pyogranulomatous inflammatory process with large numbers of neutrophils and macrophages, and basophilic coccoid or coccobacillary organisms.

The primary mechanism of transmission of *R equi* in companion animals remains unclear. It has been suggested that infection may be established by exposure to *R equi* present in soil or manure^{1,11} or by direct contact with infected animals, particularly horses.^{1,4} In our case, the cat had access to horses, cattle and other cats and could have become infected with *R equi* by direct exposure of a cutaneous injury to *R equi* in the environment (faeces or aerosols) or by direct cutaneous inoculation with *R equi* via a scratch or bite from another cat.

Three virulence levels of *R equi* have been identified: virulent, intermediate and avirulent.¹¹ Virulent *R equi*, characterised by the presence of VapA and virulence plasmid DNA 80 to 90 kb long, has been found in lesions of foals (murine LD₅₀ = 10⁶). *R equi* strains of intermediate virulence are characterised by the presence of VapB and virulence plasmid DNA 79 to 100 kb long, and have been isolated from the submaxillary lymph nodes of pigs (murine LD₅₀ = 10⁷). Avirulent *R equi* shows no evidence of either virulence-associated antigens or plasmid DNA (murine LD₅₀ > 10⁸) and is widespread in soil.

In Brazil, a molecular epidemiological study of *R equi* strains isolated from foals with pyogranulomatous pneumonia revealed that 87 kb type I (VapA) *R equi* is the most important strain in foals in this country.¹² The cat described in the present study also displayed the 87 kb type I, VapA-positive virulence plasmid profile. Recently, Takai et al¹³ documented pathogenicities and plasmid profiles of cat and dog isolates obtained from various countries. Five of nine cat isolates consisted of VapA-positive *R equi* that contained 85 or 87 kb type I plasmids, and one of nine dog isolates consisted of VapA-positive *R equi* that contained an 87 kb type I plasmid. The remaining 12 isolates were avirulent *R equi* and contained no virulence plasmids. The prevalences of

virulent *R equi* among cat and dog isolates were similar to those of isolates from humans with and without AIDS, respectively.⁴ These results may reflect differences between cats and dogs in the source and route of infection.

Although molecular studies of virulence-associated antigens and plasmids have contributed to the epidemiology, pathogenesis, diagnosis and control of rhodococcosis in horses, pigs and humans,¹¹ few such investigations have been conducted in companion animals.¹³ Factors predisposing cats to *R equi* infections remain obscure, however, virulence characterisation of *R equi* strains isolated from infections in cats and from their environment may elucidate some of the risk factors for rhodococcosis in cats.

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