Systemic *Rasamsonia piperina* infection in a German shepherd cross dog

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**Abstract**

**Introduction.** Infection with the *Rasamsonia argillacea* species complex represents an emerging problem in human and veterinary medicine with systemic mycoses presenting with significant clinical complications and being a cause of death.

**Case presentation.** In this report, a case of systemic *Rasamsonia piperina* infection discovered in a 3-year-old male neutered, German shepherd cross dog is described together with the clinical presentation, the course of the disease and diagnosis. This report describes the first case of veterinary mycosis due to *R. piperina* in Europe and the first case in humans or animals in the UK.

**Conclusion.** Although seemingly rare, *R. argillacea* species complex infection should be a differential diagnosis for dogs, especially German shepherds with the described presenting signs, and radiographic and ultrasonographic findings.

INTRODUCTION

*Rasamsonia argillacea* species complex infections are an emerging problem in human medicine. In the absence of molecular diagnostics, morphological similarity to other fungi may cause misidentification of *Rasamsonia* species and thus their role as pathogens may be underappreciated. Veterinary *Rasamsonia* species infections have so far been limited to two previous cases and here we report a case of systemic *Rasamsonia piperina* infection in a 3-year-old, male neutered, German shepherd cross dog. In addition to being the first case of veterinary *R. piperina* mycosis in Europe and the first *R. piperina* case in humans or animals in the UK, this is also the first case of systemic mycosis due to *R. piperina* in a dog with macroscopic pulmonary, pleural, splenic, hepatic and lymphoid involvement.

CASE REPORT

A 3-year-old, male neutered, German shepherd cross dog presented with a 4-week history of intermittent left hind limb, non-weight-bearing lameness. On physical examination the patient was resenting extension of hip joints and pain reaction was elicited on palpation of the left humerus. Initial treatment with meloxicam and tramadol did not resolve the clinical signs and the dog presented again 3 months later. On the second assessment the left hind limb lameness persisted and pain was also found on palpation of the left humeral, and both femoral diaphyses. Radiographs revealed a moderate, generalized and patchy increase in radiopacity in the medulla of the left femoral diaphysis. Smooth, continuous periosteal new bone formation extended along the medial and caudal cortex of the proximal third of the diaphysis. A diagnosis of left femoral panosteoitis was made and the patient was discharged with exercise restriction and analgesia. The lameness failed to improve and the dog presented again a few days later, this time with shifting lameness involving left front and hind limbs and pyrexia. Recent lethargy, hyporexia, vomiting and restlessness were also reported.

An investigation of pyrexia of unknown origin was initiated. Blood analysis revealed monocytosis [12.2 × 10⁹ l⁻¹, reference interval (RI) 0–1.5], hypoalbuminaemia (21.5 g l⁻¹, RI 26–35) and hyperglobulinaemia (42 g l⁻¹, RI 18–37). Radiography of the right femur showed an aggressive proximal diaphyseal lesion, which had progressed compared to previous images, with secondary disuse muscle atrophy (Fig. 1). Differential diagnoses included fungal or bacterial osteomyelitis. An adjacent soft tissue neoplasm was considered less likely. A clinical suspicion of mycotic infection was raised.

Blood cultures were negative. Repeat blood analysis showed no clinical changes except for thrombocytosis (700 × 10⁹ l⁻¹, RI 150–450). A sample of peripheral blood mononuclear cells from a peripheral blood smear was inoculated into Sabouraud’s dextrose agar and macroscopic growth of a yeast was observed in 18 hours. The isolate was identified as *Rasamsonia piperina* (GenBank accession number MG700428) using standard molecular techniques. Histopathology of a biopsy sample of the femur revealed ostitis of the bone cortex and extraordinary periosteal new bone formation. An adjacent soft tissue neoplasm was considered less likely.

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**Abbreviations:** ITS, internal transcribed spacer; RI, reference interval.

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likely. Thoracic radiographs revealed partial right middle lung lobe consolidation with lobar pneumonia and small- to moderate-volume right-sided pleural effusion.

On abdominal ultrasound there was marked mass effect in the cranial abdomen associated with marked generalized lymphadenopathy, steatitis and minimal peritoneal effusion. The spleen was enlarged with irregular margins, and moderately heterogeneous. Thoracentesis was performed and fine needle aspirates were taken from the spleen, lymph nodes and mass in the cranial abdomen.

Cranial abdominal mass and lymph node fine needle aspirates were consistent with marked pyogranulomatous inflammation with multinucleated giant cells and intralobular fungal hyphae. Hyphae were 2–3 µm in width, and had parallel walls with septate and 45°–90° branching. They stained purple, and had a thin, clear outer cell wall (Fig. 2). The pleural effusion was consistent with eosinophilic to mixed inflammation, consistent with a reaction to the disseminated fungal infection. While waiting for culture results a working diagnosis of aspergillosis was made and treatment with itraconazole (5 mg kg⁻¹ PO q24h), amphotericin B (1 mg kg⁻¹ IV CRI) and terbinafine (20 mg kg⁻¹ PO q12h) was commenced.

The patient was hospitalized and repeat abdominal ultrasound scans revealed new heterogeneous appearance of the liver with numerous hypoechoic lesions. Cytology of the hepatic lesions revealed marked pyogranulomatous inflammation with fungal hyphae and inter-hepatocellular bile casts, indicating cholestasis.

Microbiological examination of hepatic lesion fluid isolated a pure growth of a fungus described as having macro- and micro-morphological characteristics similar to those of Purpureocillium species and Paecilomyces species; no Aspergillus species was isolated. As the identity of the isolate was not clear from colony morphology or microscopic examination, molecular identification was used. An internal transcribed spacer (ITS) region of the ribosomal DNA gene cluster was amplified and sequenced on both strands with primers ITS-F and ITS-R [1]. The resultant 422 bp ITS region sequence was identified using the ISHAM-ITS database (http://its.mycologylab.org) [2]. The highest match was 99.763% nucleotide identity to the ITS region of the type strain of R. piperina (CBS 408.73; NCBI accession no. NR_120176.1, position 71–492). To confirm the identity as R. piperina, fragments of the beta-tubulin and calmodulin genes were amplified and sequenced on both strands using the
German shepherd dogs between 2 and 8 years of age. Systemic aspergillosis has been most commonly reported in this breed. The main similarities in the two cases include age and breed.

R. piperina and R. argillacea have been reported to cause disease in both species. In human medicine, R. argillacea infections are rare, and in veterinary cases, R. piperina has been more commonly reported. This heredity susceptibility may extend to Rasamsonia species complex infection and highlights the need to consider other fungal causes beyond Aspergillus species for systemic mycoses in this breed.

Presenting signs in our case were similar to those of R. argillacea [10] where an initial lameness was the primary complaint. In the previous case report of R. piperina [9], the main presenting signs were acute-onset glaucoma of the right eye together with combined presence of lethargy, spinal hyperpathia, panuveitis, haematuria and pyuria. None of those were seen in our case, where the signs of systemic disease were limited to pyrexia and, later in the disease course, epistaxis and respiratory signs.

Both dogs in previous reports [9, 10] had radiographic signs consistent with discosponydlitis. In the case described here the initial radiographic findings were consistent with panosteitis, and lesions characteristic of osteomyelitis did not develop until later.

Neither of the previous veterinary Rasamsonia species mycosis reported macroscopic pulmonary or pleural involvement although that has been reported in human medicine in patients with cystic fibrosis undergoing lung transplants [4, 5, 8]. There were abnormalities on abdominal ultrasound of both previous dogs. The previously reported case [9] had bilateral renal pelvic dilation with all other organs appearing normal, unlike in our case where a clear splenic, hepatic and lymphoid involvement was present.

In the case described here the diagnosis of disseminated fungal infection was reached following fine needle aspirates from the spleen, lymph nodes and liver. Fungal hyphae were also observed in the pleural effusion. Fungal hyphae were also observed in the pleural effusion following thoracentesis, unlike the previous veterinary reports [9, 10] where the diagnosis was reached following aspirates or biopsies of the vertebral bodies or sternebra. Molecular characterization was pivotal in distinguishing the fungal species in all three reports.

Recent antifungal testing [11] on Rasamsonia species showed that caspofungin had significant activity in vitro, followed by amphotericin B and posaconazole. In another study voriconazole and isavuconazole had poor in vitro activity against Rasamsonia isolates and the echinocandins were found to be the most effective agents [22]. Voriconazole was the least active of the antifungals tested [11, 22] and amphotericin B was added only late in the course of the disease, which may be the reason for the poor outcome in this case.

It is possible that previous cases reported as Aspergillus or Paecilomyces might have actually been Rasamsonia species infections. We agree with other authors [10] and suggest that molecular fungal identification is warranted in future cases, which so far has only rarely been done in veterinary publications. A study has successfully validated a commercially available real-time PCR assay that reliably detected three species from the R. argillacea complex direct from clinical specimens and from culture isolates [23]. We believe...
that the Rasamsonia species complex is potentially an underappreciated and misdiagnosed pathogen and that molecular approaches should be used in suspect cases because of the potentially aggressive disease pattern and refractoriness to medical treatment [6, 9, 11, 24].

References