Paecilomycosis in two Australian dogs

L Woolford,a K Parker,b K Lee,a T Westermann,a P Hicks,a Q Mackie,a A Derksa and A Kessella

CASE REPORT

Paecilomyces variotti, a common environmental saprophytic mould, is one of several fungal genera associated with hyalohyphomycosis. An emerging disease of immunocompromised human patients, paecilomycosis is rarely reported in veterinary species. Here we describe contrasting presentations of P. variotti infection in two Australian dogs. Case 1 was a 3-year-old pregnant female German Shepherd Dog presenting with anorexia and generalised pain subsequently localised to the cervical vertebrae. Postmortem revealed destructive pyogranulomatous discospondylitis, meningitis and osteomyelitis at C5–C6 and disseminated granulomatous disease with intralesional fungi. Case 2 was a 10-year-old female spayed Australian Silky Terrier that presented with chronic lameness and multicentric osteolytic lesions in the fore- and hindlimb. Amputation of the hindlimb and draining lymph nodes was performed and histopathology revealed multicentric granulomatous osteomyelitis and synovitis and granulomatous lymphadenitis, with intralesional fungi. This dog was treated with itraconazole and survived to 14 years of age. Cultures confirmed P. variotti in both cases.

CONCLUSION

Although rare, paecilomycosis may be under-recognised in Australia.

KEYWORDS
dogs; disseminated granulomatous disease; fungal disease; Paecilomyces variotti; paecilomycosis

ABBREVIATION
GSD, German Shepherd Dog

Paecilomyces variotti is a common environmental filamentous, saprophytic fungus found worldwide and is one of several fungal agents associated with hyalohyphomycosis, a disseminated granulomatous disease caused by opportunistic, non-pigmented, non-Aspergillus spp. hyphal fungal organisms. It is a rare case of mycosis in companion animal species, with canine systemic and localised mycoses more typically associated with Aspergillus spp., the Dermatiaceae and pathogenic dimorphic fungi. Paecilomyces spp. are common environmental moulds and are seldom associated with disease; however, P. variotti and P. marquandii are emerging as causative agents of mycotic keratitis and hyalohyphomycosis in immunocompromised human patients.1 Paecilomyces variotti is a rare yet important cause of morbidity in dogs, cats, horses and rodents, and a range of other species from this genera have been isolated from exotic and wildlife species.2

Here we describe contrasting presentations of paecilomycosis in two Australian dogs. Infections were diagnosed on the basis of clinical, radiographic, cytological, histopathological and microbiological investigations.

Case 1

A 3-year-old female entire German Shepherd Dog (GSD) was presented to the referring clinic for evaluation of anorexia and mild generalised pain. Approximately 3 weeks prior to presentation she had been bred to dog based in New South Wales. Despite no evidence of illness during the breeding or immediately after, on return home to South Australia, she was hesitant to jump into the trailer and her appetite was reduced. A hindlimb lameness and mild swelling of the left ankle and pain on flexion of the digits were found on clinical examination at the first-opinion veterinarian and initial haematology and biochemistry showed a mild increase in urea only. Spinal radiographs revealed calcification on the ventral aspect of the thoracic lumbar vertebrae and a semicircular and distinctly margined lucent focus in the endplates surrounding the L2–L3 disc. This lucency had a thin rim of sclerosis, but there was no evidence of bone lysis or exuberant bone reaction and therefore was considered to be an old discospondylitis lesion or Schmorl’s node. Abdominal ultrasound revealed a questionable area on the left kidney that did not appear to affect the shape of the left kidney; there were no other significant findings. Urine analysis was unremarkable, and bacterial and fungal culture, which revealed no growth after 48 h. The dog was placed on amoxicillin-clavulanic acid and improved slightly, but returned on day 10 inappetent and uncomfortable. On physical examination she was in lean body condition (body condition score 2/5), but was bright, alert and responsive with a normal temperature, pulse, respiration and heart and lung auscultation. There was no abdominal pain evident on palpation, but she was mildly uncomfortable on spinal palpation in the mid-lumbar region. The rest of her spinal palpation was unremarkable. Differential diagnoses at this stage included fungal discospondylitis, multiple disc extrusion, immune-mediated polyarthritis multiple myeloma and meningitis. Serum was submitted for Aspergillus titres. At recheck 3 days later (day 13) examination revealed marked cervical pain localised to the C5–C7 disc spaces. While awaiting results of Aspergillus titres, the dog was given an injection of methadone 0.5 mg/kg IM, a 75-µg fentanyl patch (2.7 mg/kg/hr) was placed on her left hindleg and carprofen at 4 mg/kg/day and gabapentin 3 mg/kg three times daily were prescribed. She was given food and water and drank readily throughout the day. Her appetite was decreased, but she ate when hand fed. On day 14 the dog was not-febrile and reflexes were within normal limits, but she would not support weight on the forelimbs. Aspergillus antibody titre was found to be negative. The owners elected euthanasia and a necropsy was performed the next day.

Representative tissues from all major affected and unaffected organs were fixed in 10% neutral buffered formalin for routine histological processing and microscopic examination and duplicate fresh tissues archived at −20°C. The C4–C7 vertebral bodies were subjected to a short period of decalcification in formic acid decalcifying fluid (Australian Biostain) prior to histological processing. Aseptically collected swabs from the C5–C6 vertebral disc space and kidneys were submitted for aerobic and anaerobic bacterial cultures and fungal culture.

Necropsy revealed pyogranulomatous discospondylitis and osteomyelitis at intravertebral space C5–C6, with extensive bony lysis evident in the adjacent C5 and C6 vertebral bodies (Figure 1).
Cytological examination of exudate from the C5–C6 disc space (Wright Giemsa stain) revealed neutrophilic and histiocytic inflammation admixed with numerous fungal hyphae. There were no significant gross findings at the L2–3 disc space. Granulomatous foci, which followed vascular tracts, were also identified in the epicardium/myocardium (Figure 2) and throughout the cortices of the left and right kidneys. There was multicentric subcutaneous, mediastinal, tracheobronchial and mesenteric lymphadenomegaly and follicular lymphoid hyperplasia in the spleen. The uterus was gravid and contained four fetuses estimated at 5 weeks’ gestational age.

Fungal hyphae were detected in the wet mount examinations of the C5–C6 vertebral disc swab but not from the swab of the kidney. No bacteria were isolated aerobically or anaerobically from either swab. Cultures from the vertebral disc swab (only) began to show pure growth of small, white to yellow colonies at 48 h, at both 37°C and 25°C, which at 96 h were 7–8 mm diameter on Sabouraud’s agar and had matured to a dark suede-like olive-brown. Microscopic examination of cultures showed septate hyphae with conidiophores with tapering phialides terminating with oval conidia in long vertical chains. Fungal subcultures were submitted to a mycology reference laboratory for definitive identification and found to be P. variotii. Urinalysis (cystocentesis at necropsy) revealed minimally concentrated urine concentration (urine specific gravity 1.015, pH 6), but was otherwise unremarkable. There were no significant findings on sediment examination.

Microscopically, there was extensive, severe degeneration of the C5–C6 intervertebral disc body, with expansion and effacement of the disc, adjacent vertebral bodies and subdural space by highly cellular infiltrates of degenerate and non-degenerate neutrophils, macrophages, multinucleated giant cells, lesser lymphocytes and plasma cells, as well as haemorrhage and fibrin (Figure 3). Extensive bone necrosis and increased osteoclastic activity along the scalloped margins of thinned vertebral trabeculae were noted in regions adjacent to inflammation. Refractile yellow-brown pigmented fungal hyphae were admixed with inflammatory infiltrates and periodic acid-Schiff staining highlighted extracellular and phagocytosed fragments of fungal hyphae (Figure 4). Hyphae were septate with

**FIGURE 1.** Case 1. Discospondylitis and osteomyelitis at C5–C6 intravertebral space with bony destruction evident in the adjacent C5 and C6 vertebral bodies.

**FIGURE 2.** Case 1. Disseminated granulomatous inflammatory foci following the vascular tracts of the myocardium.

**FIGURE 3.** Case 1. Photomicrograph of a section through the C5–C6 intervertebral disc space. There is pyogranulomatous and necrotising discospondylitis and osteomyelitis (H&E, ×2). IVD, intervertebral disc; M, meninges; SC, spinal cord.

**FIGURE 4.** Case 1. Photomicrograph of intralesional fungal organisms admixed with inflammatory cells in the C5–C6 discospondylitis and osteomyelitis lesion (periodic acid-Schiff, ×40). Note the terminal septate, non-parallel-walled hyphae exhibiting acute angle dichotomous branching and terminal chlamydospores (arrows).
non-parallel walls and occasionally showed dichotomous branching and terminal budding structures (chlamydospores), consistent with *Paecilomyces* spp. Granulomatous or pyogranulomatous inflammation with intrallesional fungal organisms was also present in the myocardium, kidneys, spleen, the mediastinal, mesenteric and subcutaneous lymph nodes, myocardium, adrenal glands, pancreas and liver.

**Case 2**

A 10-year-old female spayed Australian Silky Terrier was presented to the consulting veterinarian with 3-week history of left hindlimb lameness. On clinical examination the dog had enlarged left popliteal and inguinal lymph nodes. Cytological examination of the lymph nodes revealed lymphoid hyperplasia and granulomatous inflammation admixed with fungal hyphae (Figure 5). Radiographic examination of the left hindlimb revealed an enlarged patella with many irregularly-shaped lytic spaces (Figure 6). A single lateral radiograph of the dog including chest, thorax and neck but excluding distal parts of the limbs was unremarkable. A mild non-regenerative anaemia, mild monocytosis and a moderate hyperglobulinaemia were the only significant findings noted in the presurgical haematology and biochemistry results. The left hindlimb was subsequently amputated at the coxofemoral joint together with the left and right inguinal lymph nodes, and submitted for histopathology. Microscopically, the bone marrow of the patella was extensively replaced by multifocal granulomas with intrallesional fungal hyphae (Figure 7). Fungal hyphae were characterised by septate non-parallel walls with terminal chlamydospores, consistent with *Paecilomyces* spp. There was extension of inflammation into surrounding ligamentous structures and synovium through breaks in the articular surface of the patella. Microscopic examination of the popliteal lymph node revealed multifocal granulomatous lymphadenitis and lymphoid hyperplasia with intrallesional fungal hyphae. The right and left inguinal lymph nodes were unremarkable.

Tissue taken aseptically at surgery from the patella and left popliteal lymph node was plated onto sabouraud agar. For both tissues, a pure profuse growth of a fungi subsequently identified as *P. variotii* was obtained.

At 2 days post-surgery the dog was observed to be lame on the left forelimb and radiographic studies revealed multiple lytic and sclerotic lesions in the left distal radius, highly suggestive of osteomyelitis. The owners elected to treat conservatively with pain relief (meloxicam; Boehringer Ingelheim) and antifungal medication (itraconazole 20 mg/kg/day; Janssen-Cilag) and monitor the dog periodically with radiographs and urinalysis. At examination 1 month later the dog was noted to be much improved in demeanour and no impediment to mobility was noted.

The dog survived with good quality of life. He was maintained on a reduced dose of itraconazole for a further 4 years; he would become intermittently lame when the owners forgot to dose him for more than a week, only to return to three-legged soundness on re-institution of the drug. He was euthanased at 14 years of age for an unrelated cause and a follow-up postmortem examination was not performed.

**DISCUSSION**

Typically associated with infections in immunocompromised human patients, *P. variotii* is a rare infection in companion animal species. Reports of disease in dogs range from localised infections, such as keratitis, cutaneous lesions, endocarditis, sinusitis, nephritis, and osteomyelitis, through to severe disseminated infections.3,4 As in case 1, the most common presentation of paecilomycosis in dogs in the literature is discospondylitis, with or without disseminated disease,25 with

**FIGURE 5.** Case 2. Photomicrograph of cytological preparation from popliteal lymph node (Diff Quik stain). Note the central, large, multinucleate macrophage that contains numerous negative-staining hyphal fungal organisms.

**FIGURE 6.** Case 2. Lateral radiograph of the left and right stifles. Note the enlarged left patella bone with many irregularly-shaped lytic spaces (arrow).

**FIGURE 7.** Case 2. The bone marrow of the patella is extensively replaced by multifocal granulomas with intrallesional fungal hyphae (periodic acid-Schiff).
Paecilomyces spp. are anamorphic ascomycete moulds that are close relatives of the *Penicillium* spp. They are common environmental saprophytic fungi found airborne and in soil, vegetative material, dust and food products worldwide, as well as being part of the normal microflora of canine hair. The primary lesion or site of infection in paecilomycosis is usually not determined, although in the previous reports of paecilomycosis and in disseminated fungal infections generally, cutaneous or mucosal wounds are often suspected as the site of entry. No history of penetrating wounds was reported in either of the described cases, but in the GSD in case 1, development of disease was precipitated by a unilateral hindlimb lameness and lower limb swelling, which subsequently resolved; therefore a penetrating wound and fungal entry at this site cannot be excluded.

The *Paecilomyces* genus comprises multiple species, including *P. variotii*, *P. viridis*, *P. tenuipes*, *P. pericinuis*, *P. marquandii*, *P. javanicus*, *P. fumosoroseus*, *P. flavinosus*, *P. carneus*, *P. canecens* and *P. aerugineus*. Given the widespread environmental presence of these organisms, interpretation of its isolation must consider the possibility of sample or laboratory contamination, particularly if fungal cultures are obtained without concurrent cytological or histopathological investigations; conversely, dismissal of this organism as a contaminant may contribute to *Paecilomyces* being overlooked as a pathogen. Culture may be variably sensitive, depending on tissue concentrations of fungi; however, it is always beneficial to perform because the fungal morphology may be confused with that of *Aspergillus* spp., *Penicillium* spp. or *Candida* spp.in tissue or cytological samples. Speciation of *Paecilomyces* is also important because it carries therapeutic implications, with *P. variotii* being susceptible to most common antifungals. In case 2 the dog was successfully managed with itraconazole; antifungal treatment was not attempted in case 1. Prognosis and response to antifungal therapy is reportedly poor and pneumonia, discospondylitis or disseminated infection carries a grave prognosis, with reported mortality rates ranging from 57% to 100%. Despite this, some treatment successes are reported, as in case 2, and are optimised with knowledge of antifungal susceptibility data and minimum inhibitory concentrations. Such data for *P. variotii* are available for human isolates, with limited information available for veterinary species. Clinical pathological findings were generally unremarkable or mild in both dogs. In case 1 the dog presented with a mild and transient increase in urea only early in the course of the disease, whereas in case 2 the dog had an inflammatory leucogram and hyperglobulinaemia in blood samples collected prior to surgery, suggestive of a response to antigen/s or infection. Despite granulomatous nephritis in case 1, urine sediment examination did not identify *P. variotii* in the urine and renal fungal culture was negative. This is in contrast to two reports of paecilomycosis with renal involvement in GSDs, in which fungal hyphae were able to be both visualised and cultured from urine obtained via cystocentesis. Other studies report low sensitivity of urine sediment examination and renal culture in disseminated infection with renal involvement, which may reflect the method of collection as well as the tissue fungal load and chronicity of infection.

As observed for other disseminated fungal infections, the GSD breed is overrepresented for paecilomycosis. The mechanisms behind the breed predisposition are unknown, but depressed local cellular responses, and IgA dysregulation resulting in decreased mucosal immunity, have been proposed. A clear sex predisposition for paecilomycosis is unclear, although females appear to be overrepresented in the studies examined here. The role, if any, of immunomodulation by pregnancy in case 1 is uncertain; in humans, pregnancy is a reported risk factor for development of disseminated coccidioidomycosis, particularly when infection is acquired late in pregnancy. There are only two reported cases of paecilomycosis during and after pregnancy in women, comprising a nail infection and a postoperative infection of an incision.

**CONCLUSION**

*Paecilomyces variotii* is a rare but important cause of hyalectrophomycoses in dogs and may present as disseminated granulomatous disease or miscellaneous localised infections. Disseminated disease is more common and dogs presenting with localised *Paecilomyces* infection should also be evaluated for systemic involvement. Definitive diagnosis may be hindered by culture sensitivity and the similar appearance of this organism to other fungal agents in tissue or cytological specimens, and dismissal of this organism as a contaminant may contribute to *Paecilomyces* being overlooked and underrecognised as a pathogen.

**ACKNOWLEDGMENTS**

We thank Dr Helen Alexiou of the National Mycology Reference Laboratory for technical assistance with identifying the fungal isolate from case 1. Thanks also to Adrian Hines for technical assistance during the necropsy of case 1 and to Jim Manavis of SA Pathology for skilful production of histology slides. Thanks to veterinarians Richard Lucy and Richard Malik for advice on treatment in case 2 and to staff at the North Shore Hospital, Sydney, for definitive fungal identification in case 2.

**REFERENCES**


