

ACUTE NECROTISING ZYGOMYCOTIC GASTRITIS IN AN ECLECTUS PARROT

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INTRODUCTION

Acute necrosis of the ventriculus is a very uncommon lesion in birds. We describe a fatal case of acute necrotising ventriculitis caused by *Rhizopus microsporus* var. *chinensis* in a mature female eclectus parrot (*Eclectus roratus*). The bird presented acutely dull and lethargic, was vomiting and had bright green droppings, suggestive of acute heavy metal poisoning. It was treated with fluids and chelation therapy but died within 12 hours. Necropsy, cytology, histopathology and culture results demonstrated fungal invasion of the ventriculus associated with transmural necrosis, haemorrhage, acute inflammation and abundant *Rhizopus microsporus* var. *chinensis* and lesser numbers of *Candida krusei*.

CASE REPORT

In companion birds there are several well recognised relatively common fungal diseases of the gastrointestinal tract that cause chronic signs of gastric and upper alimentary tract dysfunction. These include candidiasis and macrorhabdosis. Primary candidiasis of the ventriculus is a very common disease of finches and often occurs in association with proventricular cryptosporidiosis and macrorhabdosis. Anecdotal evidence suggests that *Candida albicans* is the most common aetiological agent present in such cases. Clinical signs of these diseases include regurgitation, weight loss and the passing of undigested seeds in the droppings. These findings are relatively non-specific and could be detected with other conditions such as acute toxicoses and alimentary foreign bodies as well as other infectious causes of ventriculitis. In this case report we describe the clinical signs and lesions associated with a fatal case of necrotising ventriculitis caused by *Rhizopus microsporus* var. *chinensis* in a mature female Eclectus parrot (*Eclectus roratus*). The bird was an aggressive, aviary breeding hen purchased 8 days previously. Some 48 hours before veterinary presentation the owner noted that the bird had changed behaviour, turned abnormally friendly and had lost her appetite at which time *per os* doxycycline (Psittavet® Vetafarm) was administered prior to admission.

The bird presented acutely depressed and had yellow to brown vomitus and bright green droppings, suggestive of acute heavy metal poisoning, and was treated with fluids and chelation (parenteral calcium EDTA and *per os* Metamucil) but died within 12 hours. Necropsy demonstrated a dark irregular thickening of the koilin layer within the ventriculus and dark reddish watery fluid within the proventriculus and duodenum. Impression smears of the surface of the ventriculus were stained with Wright's and Gram's revealing a marked infiltration of heterophils and branching septate fungal hyphae including rhizoids.

Histopathological examination of the ventriculus demonstrated diffuse transmural necrosis and haemorrhage with diffuse oedema and a moderate to marked infiltration of heterophils and macrophages. Amongst this were large numbers of branching non-septate fungal hyphae with non-parallel walls. The liver was diffusely congested with foci of rounded hepatocytes which had lost their normal cellular attachments, disrupting the sinusoids. The kidney also appeared moderately congested but otherwise normal and there was an absence of acute renal tubular necrosis as occurs often in cases of heavy metal poisoning. There was widespread degranulation of exocrine cells of the pancreas. The duodenum and proventriculus appeared normal. In the lung there was abundant aspirated foreign body material within major airways, with many budding yeasts consistent with the source being crop or stomach contents.

Swabs taken from the crop, proventriculus and cloaca were cultured aerobically on sheep blood agar and yielded a light to moderate mixed growth of Gram negative and positive bacteria. A heavy pale brownish-grey fungal growth was obtained from the proventriculus. Cytological evaluation of this isolate demonstrated pigmented rhizoids. A heavy growth of *Candida krusei* was cultured from the crop, proventriculus and cloaca. The mould contained pigmented rhizoids and globose sporangia measuring 50 µm in diameter, consistent with *Rhizopus spp.*, subsequently identified as *R. microsporus var chinensis* by the National Reference Centre for Medical Mycology, South Australian Pathology.

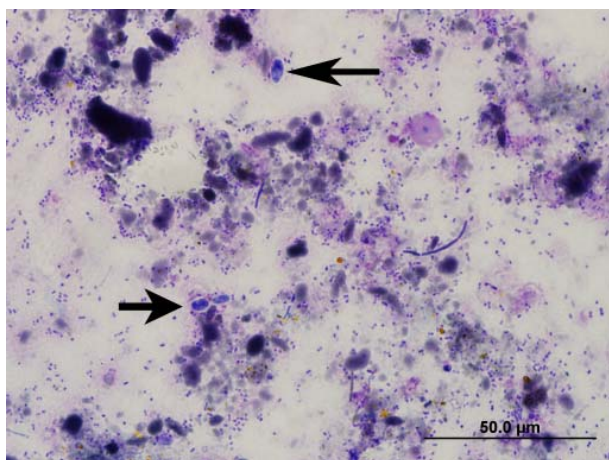


Figure 1. Budding yeasts in crop smear consistent with *Candida* sp. amongst a background of abundant mixed bacterial morphologies.

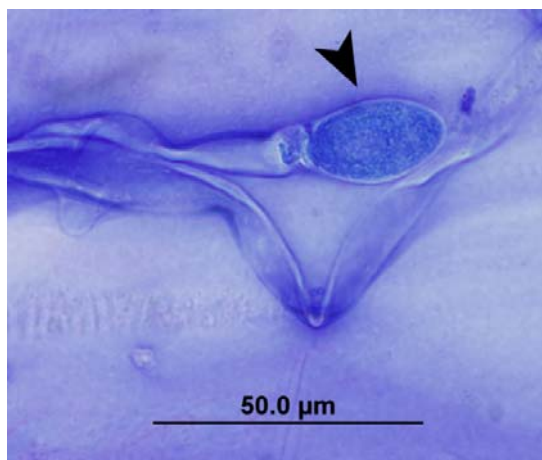


Figure 2. Wright's stained smear of ventriculus demonstrating fungal hyphae and non-parallel walls and sporangia (arrow head) within the koilin.

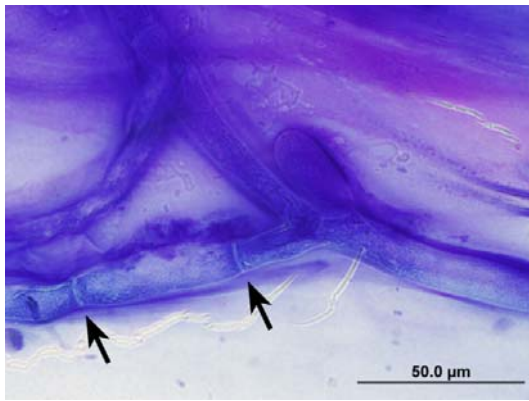


Figure 3. Wright's stained smear of ventriculus demonstrating fungal hyphae and occasional septae (arrows).



Figure 4. Luxol fast blue sticky tape preparation of cultured mould demonstrating a developing sporangium

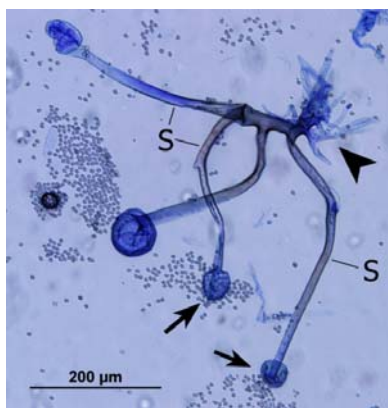


Figure 5. Luxol fast blue sticky tape preparation of *Rhizopus* isolated from the ventriculus demonstrating ruptured sporangia releasing sporangiospores (arrows) and elongate sporangiophores (S) emanating from branching rhizoids (arrow head).

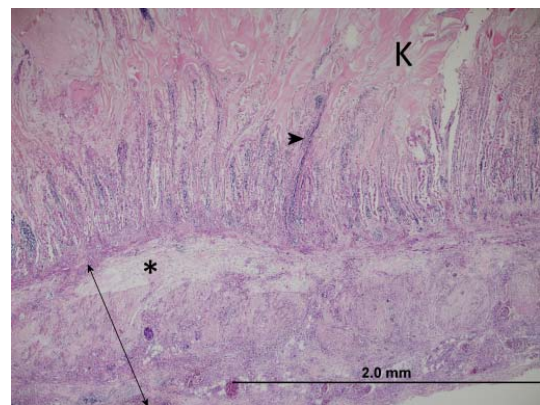


Figure 6. Haematoxylin and eosin-stained section demonstrating transmurial necrosis, haemorrhage, oedema (*) and disruption of the koilin (K).

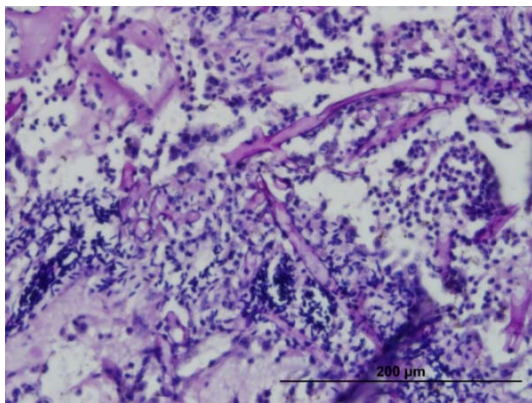


Figure 7. PAS-stained section of ventriculus demonstrated disrupted koilin containing areas of haemorrhage (arrows) and branching septate fungal hyphae with non-parallel walls.

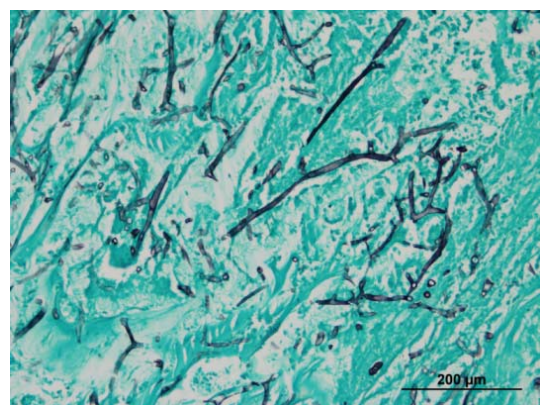


Figure 8. Fungal hyphae stained with silver in the koilin

DISCUSSION

A rich diversity of fungal agents have been found in the avian alimentary system (Cafarchia et al., 2008, Cafarchia et al., 2006) and many are known causes of disease. Proventricular colonisation by the ascomycetous yeast *Macrorhabdus ornithogaster* (Tomaszewski et al., 2003) is a very common cause of gastric dysfunction in a wide range of bird species including companion birds (Baker, 1992, Marlier et al., 2006), wildlife (Filippich and Parker, 1994) and commercially important production species (Hanka et al., 2010, Jansson et al., 2008, Phalen and Moore, 2003). Whilst acute disease can occur in macrorhabdosis, when full-thickness ulceration of the proventricular isthmus occurs most cases of infection are typically associated with clinical signs of chronic gastric dysfunction, proventricular and ventricular dysplasia, haemorrhage and/or chronic inflammation. Acute gastritis is a relatively uncommon lesion in companion birds and, as exemplified in this present case, the clinical signs that can be associated with acute gastric dysfunction are relatively non-specific and can occur with a range of non-infectious causes including heavy metal toxicosis and penetrating gastric foreign bodies. Whilst diagnostic testing such as haematology, serum biochemistry and radiography might be useful for differentiating such causes, the likelihood of obtaining a definitive *ante mortem* diagnosis in our present case was unlikely given the acuteness and localised nature of the lesion. Cytology of crop contents alone revealed occasional budding yeasts with insufficient evidence of fungal hyphae to have relied on this for diagnosis.

There remains some controversy in human medicine whether or not *Candida* species are causally related to alimentary tract disease or are opportunistic pathogens secondary to altered host immunity or luminal microbial conditions such as occurs with antibiotic administration (Dahlhausen, 2006, Schulze and Sonnenborn, 2009). The vast majority of human infections are due to *Candida albicans* (Dorko et al., 2000, Kusne et al., 1994) and presumably the same applies to avian patients. In one study of human liver transplantation, 100% of patients had *Candida* species isolated from at least one site of the gastrointestinal tract, with the majority (64%) of isolates being *Candida albicans*, whilst less common species included *C tropicalis* (19%), *C krusei* (10%), and *C glabrata* 11 (7%) (Kusne et al., 1994). At least 16 other *Candida* species are known to occur in humans (Dorko et al., 2000), mostly associated with other severe medical conditions. *Candida spp.* are common inhabitants of the avian alimentary tract and it is not unusual to find budding yeasts in crop contents. The role of *C krusei* in the pathogenesis of the lesions depicted in this present case report is unclear and as far as we are aware this is the only documentation of this species as a potential invader of the koilin layer in birds. A previous study of 60 cockatiels indicated that *C krusei* might be far less important than *C. albicans*, *C. tropicalis*, or *C. parapsilosis* (Sidrim et al., 2010). In finches and other birds *Candida* colonisation of the ventricular koilin layer is also a common lesion and perhaps this may have been present in the eclectus parrot as a primary predisposing element leading to *Rhizopus* invasion. There have been few studies of the causal factors of ventricular candidiasis in birds, with perhaps many cases assumed to be due to *C albicans*. Our findings indicate that there is value in speciation of *Candida* isolates obtained from clinically affected birds, in order to better understand virulence factors that might be associated with their ability to survive the chemical conditions of the avian stomach as well as colonisation and invasion of the koilin.

As far as we are aware this is the first report of acute transmural necrotising ventriculitis associated with *Rhizopus spp.* infection in a bird and one of only a few cases of zygomycosis confirmed by cytological, histological examination, fungal culture and identification. Gastrointestinal zygomycosis due to *Rhizopus microsporus* var. *rhizopodiformis* has been described in humans associated with gastric carcinoma (Kimura et al., 1995) and chronic granulomatous disease (Dekkers et al., 2008) and in a pig associated with porcine circovirus infection (Szeredi and Szentirma, 2008). Zygomycetes are

ubiquitous typically fast-growing, relatively thermotolerant fungi which can be cultured at temperatures between 45-50°C. The potential sources of infection with zygomycetes are broad and it is noteworthy that wooden spatulae colonised with *Rhizopus* spp. were speculated as a cause of an outbreak of infection in a London human paediatric referral hospital (Holzel et al., 1998).

REFERENCES

- Baker, JR. 1992. Megabacteriosis in exhibition budgerigars. *Veterinary Record* 131, 12-14.
- Cafarchia, C, Camarda, A, Romito, D, Campolo, M, Quaglia, NC, Tullio, D. and Otranto, D. 2006. Occurrence of yeasts in cloacae of migratory birds. *Mycopathologia* 161, 229-234.
- Cafarchia, C., Romito, D., Coccioli, C., Camarda, A. and Otranto, D. 2008. Phospholipase activity of yeasts from wild birds and possible implications for human disease. *Medical Mycology* 46, 429-434.
- Dahlhausen, RD. 2006. Implications of Mycoses in Clinical Disorders. IN HARRISON, G. J. & LIGHTFOOT, T. L. (Eds.) *Clinical Avian Medicine* Palm Beach Florida, Spix Publishing Inc.
- Dekkers, R, Verweij, PE, Weemaes, CMR, Severijnen, R, Van Krieken, J. and Warris, A. (2008) Gastrointestinal zygomycosis due to *Rhizopus microsporus* var. *rhizopodiformis* as a manifestation of chronic granulomatous disease. *Medical Mycology* 46, 491-494.
- Dorko, E., Kmetova, M., Pilipcinec, E., Bracokova, I., Dorko, F., Danko, J., Svicky, E. and Tkacikova, L. (2000) Rare non-albicans *Candida* species detected in different clinical diagnoses. *Folia Microbiologica* 45, 364-368.
- Filippich, LJ and Parker, MG. 1994. Megabacteria in wild birds in Australia. *Australian Veterinary Practitioner* 24, 84-84.
- Hanka, K., Kohler, K., Kaleta, EF., Sommer, D. and Burkhardt, E. 2010. *Macrorhabdus ornithogaster*: detection in companion birds, poultry and pigeons, morphological characterisation and examination of in vitro cultivation. *Praktische Tierarzt*, 91, 390-400.
- Holzel, H., Macqueen, S., Macdonald, A., Alexander, S., Campbell, CK., Johnson, EM. and Warnock, DW. 1998. *Rhizopus microsporus* in wooden tongue depressors: a major threat or minor inconvenience? *Journal of Hospital Infection* 38, 113-118.
- Jansson, D. S., Brojer, C., Mattsson, R., Feinstein, R., Morner, T. and Hard Af Segerstad, C. 2008. Mycotic proventriculitis in gray partridges (*Perdix perdix*) on two game bird Farms. *Journal of Zoo and Wildlife Medicine* 39, 428-437.
- Kimura, M., Udagawa, S., Toyazaki, N., Imori, M. and Hashimoto, S. 1995. Isolation of *Rhizopus microsporus* var. *rhizopodiformis* in the ulcer of human gastric carcinoma. *Journal of Medical and Veterinary Mycology* 33, 137-139.
- Kusne, S., Tobin, D., Pasculle, AW., Vanthiel, DH., Ho, M. and Starzl, TE. 1994. *Candida* carriage in the alimentary-tract of liver-transplant candidates. *Transplantation* 57, 398-402.
- Marlier, D., Leroy, C., Sturbois, M., Delleur, V., Poulipoulis, A. and Vindevogel, H. 2006. Increasing incidence of megabacteriosis in canaries (*Serinus canarius domesticus*). *Veterinary Journal* 172, 549-552.
- Phalen, DN. and Moore, RP. 2003. Experimental infection of white-leghorn cockerels with *Macrorhabdus ornithogaster* (Megabacterium). *Avian Diseases* 47, 254-260.

- Schulze, J. and Sonnenborn, U. 2009. Yeasts in the gut: from commensals to infectious agents. *Deutsches Aerzteblatt international* 106, 837-842.
- Sidrim, JJC., Maia, D., Brilhante, RSN., Soares, GDP., Cordeiro, RA., Monteiro, AJ. and Rocha, MFG. 2010. *Candida* species isolated from the gastrointestinal tract of cockatiels (*Nymphicus hollandicus*): In vitro antifungal susceptibility profile and phospholipase activity. *Veterinary Microbiology* 145, 324-328.
- Szeredi, L. and Szentirma, C. 2008. Gastric zygomycosis in a pig affected with postweaning multisystemic wasting syndrome - Case report. *Acta Veterinaria Hungarica* 56, 207-213.
- Tomaszewski, EK., Logan, KS., Snowden, KF., Kurtzman, CP. and Phalen, DN. 2003. Phylogenetic analysis identifies the 'megabacterium' of birds as a novel anamorphic ascomycetous yeast, *Macrorhabdus ornithogaster* gen. nov., sp. nov. *International Journal of Systematic and Evolutionary Microbiology* 53, 1201-1205.