Mucormycosis in the platypus

Fact sheet

Aetiology

*Mucor amphibiorum* is a dimorphic fungus in the *Mucorales* order of the *Zygomycetes* class of fungi. Its sporangiospores, when found in infected tissues occur as the yeast form (spherule-like structures which may contain daughter spherules) or develop into the more usual hyphal form on culture medium (Frank et al. 1974) or in the environment. Positive and negative mating types occur. *Mucor amphibiorum* will only grow *in vitro* at a maximum temperature of 36°C (Obendorf et al. 1993; Scholer et al. 1983).

Natural hosts

Natural infections have been reported in a range of anuran amphibians (frogs, toads), and the platypus (Frank et al. 1974; Frank 1976; Munday and Peel 1983; Obendorf et al. 1993, Speare et al. 1994; Connolly et al. 1998). Salamanders exposed to infected captive anurans were also infected (Frank 1976).

World distribution

*Mucor amphibiorum* occurs in Australia and was suspected of being introduced to European captive anurans in a German Zoo from Australia. It has not been isolated from cane toads from Hawaii or Costa Rica to date (Speare et al 1994).

Occurrences in Australia

*Mucor amphibiorum* infection occurs in free-ranging platypuses from Tasmania. Infection has also been reported in free-ranging cane toads and green tree frogs from mainland Australia (see anuran mucormycosis fact sheet).
Epidemiology

Morbidity and mortality rate

Thought to be high in platypuses at infected sites. During a twelve-month Tasmanian study (Connolly et al 1998b), one platypus survived and was captured three times within 97 days, another was captured three times and later recovered dead and scavenged, 24 days after initial capture, and a third was recovered dead 72 days after its initial capture. Both dead platypuses were recovered in an emaciated and autolytic state.

Distribution

The distribution of the disease is expanding, with mucormycosis observed in at least eleven catchments since the index cases were seen in the Elizabeth River at Campbell Town in the South Esk River catchment in 1982 (Figure 1).

Locations of platypus with mucormycosis (laboratory confirmed cases, 1 sightings by the author, 2 reports in literature 3 and sighting reports from the public 4) include:

- South Esk River catchment
- Elizabeth River (Campbell Town) 1, Meander River (Deloraine 1,2,3, Westbury 1,3), South Esk River (Perth 1, Hadspen 2, Evandale 2,3), Macquarie River (Cressy 3,4, Campbell Town 4, Epping Forest 4), Brumbys Creek (Cressy 1,3), Liffey River (Carrick) 1,3, Gunns Lake 3,4 and Arthurs Lake 3,4.
- North Esk River catchment – North Esk River, Upper Blessington 1.
- Supply River catchment – Supply River, Glengarry 1.
- Piper River catchment – Hogans Brook, Karoola 1.
- Mersey River catchment – Mersey River 1, near Lake Parangana.
- Upper Derwent River catchment – Dee Lagoon 4, Bradys Lake 4 and near Derwent bridge 4.
- Inglis River catchment – Inglis River, Wynyard 4.
- Emu River catchment – tributary at Ridgley 1.
- Hatfield River catchment – Hatfield River, east of Murchison Highway crossing 4.
- Lower Derwent catchment – Lachlan River at Lachlan 4 and Hobart rivulet, South Hobart 4.
- Wilmot catchment – Lake Lea 4.

More recent reports of platypuses displaying raw chronic skin ulcers typical of mucormycosis in river catchments to the south and west of the original incident sites is of concern. Over time it appears there has been spread from catchment to catchment.

Prevalence

The prevalence of disease is high in affected sites. The prevalence of mycotic granulomatous dermatitis among platypuses captured from Brumby’s Creek, Cressy was 33% (12/36 platypuses captured) and from Liffey River, Carrick was 66% (2/3) during the twelve-month Tasmanian study. An 8% prevalence (2/25) was observed in platypuses necropsied during the same period (Connolly et al 1998).

Incubation period

Unknown, but one platypus developed an ulcer at the site of tick attachment when last captured 161 days previously.
The duration of the skin lesions for most captured affected platypuses was unknown.

**Seasonality**

No apparent seasonality has been identified to date. Index cases occurred in Autumn.

**Transmission**

Via cutaneous route and/or respiratory route. The route of entry of *M. amphibiorum* is most likely via superficial skin wounds (Munday and Peel 1983; Obendorf et al 1993; Connolly et al 1998), although the respiratory route has also been proposed (Munday, Whittington and Stewart 1998). Skin wounds could be caused by spurring injuries, bites from a water rat, eel or crustacean, the attachment site of ticks or trombiculid mites, skin infestation by nematode larvae, or a result from foraging among sharp stones. Engorging ticks have been reported in association with ulcerative dermatitis in the platypus (Obendorf et al 1993). An ulcer developed at the site of a previous tick attachment and spherule-like structures have been observed in the haemolymph of an engorging tick adjacent to an ulcer (Connolly et al 1998).

**Sources of agent**

Hypothesised source is other infected platypuses and/or other sources such as frogs, soil (ground level, burrow or stream base), water?

Only positive mating type has been isolated to date from infected Tasmanian platypuses.

Is there a carrier state? Unknown.

**Effect of water quality**

Connolly et al (1998) found similar water quality at all 5 Tasmanian field study sites in 1994 (2 affected sites and 3 free of platypus with mucormycosis). Previously a platypus with mucormycosis from South Esk River had very high liver cadmium levels (690 mol/kg compared to 1.2 – 10.2 mol/kg in 9 other platypuses from elsewhere). Stewart and Munday (2004) found that Tasmanian platypus-derived *M. amphibiorum* isolates were more pathogenic to cane toads than mainland frog-derived isolates.
Clinical signs

Munday and Peel (1983) first described four cases of ulcerative dermatitis in dead and debilitated Tasmanian platypuses, but the causative agent was not identified as *M. amphibiorum* until 1993 (Obendorf et al. 1993). *Mucor amphibiorum* causes a severe granulomatous and often ulcerative dermatitis in the platypus, which may progress to involve underlying muscle and occasionally disseminate to internal organs, particularly the lungs (Obendorf et al. 1993) leading to the death of the animal. In the absence of the systemic spread of the organism, death could result from secondary bacterial infections or impaired thermoregulation and mobility.

All platypuses with mucormycosis captured in the 1994 Tasmanian study were alert and displayed normal responses to capture and handling. The gross appearance of the skin lesions in affected platypuses (Connolly et al. 1998) varied from non-ulcerated, hairless nodules and abscesses, to ulcers with underrun or thickened margins, sinuses exuding pus, or exuberant granulation tissue attempting wound repair (Figure 2). Some lesions appeared as discrete entities, whereas others coalesced to form plaques. Lesions were found on haired regions including the hind limbs (38%), fore limbs (6%), tail (19%), trunk (6%) and head (6%), and unhaired regions such as the webbing of the fore limbs (13%) or bill (6%). Some of the affected animals had lesions at more than one site. One platypus had a tail ulcer which reduced in size over a three month period between captures (Connolly unpublished). Munday, Whittington and Stewart (1998) also observed healing mucormycosis lesions in platypuses.
Diagnosis

Platypuses are diagnosed as having mucormycosis based on the presence of lesions, spherule-like structures cytologically or histologically, and culture of *M. amphibiorum* from lesions. An indirect ELISA is also available for detection of *M. amphibiorum*-specific serum immunoglobulin.

Cytologically, spherules typical of *M. amphibiorum* are observed in unstained wet preparations or Diff Quik smears made from material collected aseptically by fine needle aspirate or swabs collected after punch biopsy (Figure 3). Smears from *Mucor*-infected lesions reveal spherule-like structures that produce pseudohyphae (germ tubes) following incubation at 28°C (Frank 1976; Frank *et al*. 1974).

Representative histological samples are collected aseptically from the edge of the skin lesions by punch biopsy performed under lignocaine local anaesthesia and/or isoflurane gaseous general anaesthesia (5%
induction, 2% maintenance). Samples from skin lesions are then fixed in 10% buffered formal saline, processed and embedded in paraffin, sections are cut at 6 μm and stained with haematoxylin and eosin (H&E) and Periodic acid Schiff (PAS). Sections for immunohistological staining are cut at 4 μm, mounted on slides coated with 3-aminopropyltriethoxysilane and dried at 37°C. Primary antibodies used for the immunohistological staining of lymphocytes are polyclonal (rabbit anti-human CD3) and monoclonal (mouse anti-human CD5, CD79a and CD79b) species cross-reactive antibodies raised against intracytoplasmic peptide sequences of human T or B lymphocyte associated molecules. A platypus specific monoclonal antibody produced by Dr Susan McClure (mouse anti-platypus serum, clone number A6C2, isotype as IgG1) is also used (Connolly et al 1999a).

Representative mycological samples are collected from lesions aseptically, and may include fine needle aspirates, swabs and punch biopsies. Specimens are plated on Sabouraud’s dextrose agar (SDA) with and without added gentamicin (50 IU/ml) and incubated at 28°C for initial fungal isolation. Pure cultures are then subcultured onto plates containing Sabouraud’s dextrose agar with no added antibiotic. Potato dextrose agar is utilised for more detailed morphological studies and mating experiments. Mucor amphibiorum isolates are identified on the basis of their colonial and microscopic morphology on potato dextrose agar at 28°C, according to the descriptions of Schipper (1978). Two mating strains, CBS 763.74 (positive type strain) and CBS 185.77 (negative type strain) are used to assess zygospore production in the aerial hyphae (Schipper 1978). By definition, positive strains produce zygospores only in test matings with negative strains.

Figure 4. Mucor amphibiorum culture on SDA and a culture smear stained with lactophenol cotton blue.

Detection of M. amphibiorum-specific serum immunoglobulin by indirect ELISA may also be performed (Professor Richard Whittington, University of Sydney). Blood samples are collected from the bill sinus of conscious animals as described by Whittington and Grant (1983) or from the heart of platypuses submitted for necropsy. The serum is separated and stored at -70°C until used for serology. A serological survey may be a useful method for detecting the prevalence of exposure to Mucor amphibiorum and humoral immunity in platypus populations both in Tasmania and the mainland of Australia.

Clinical pathology and serology

Platypuses with mucormycosis showed haematological and serum biochemical changes when compared to clinically normal animals from the same Tasmanian sites (Connolly et al 1999). Affected animals had significantly smaller packed red cell volumes, haemoglobin concentrations, lymphocyte counts, serum cholesterol and calcium concentrations, and higher serum globulin and potassium concentrations than clinically normal animals. The lymphopenia and hyperkalaemia were thought to be clinically significant. Numbers of Trypanosoma binneyi protozoa in blood smears were similar between the two groups.
An enzyme-linked immunosorbent assay (ELISA) demonstrated the ability of the platypus to mount a humoral immune response to *M. amphibiorum* (Whittington et al 2002). Platypus with cutaneous ulcers containing *M. amphibiorum* had higher concentrations of serum antibody against the fungus, as determined by ELISA, compared to clinically normal platypuses (one-way ANOVA *P*<0.001). The effect of internal lesions on antibody levels could not be evaluated in the capture-release study of living platypuses. Seroconversion was observed in a platypus coincident with the development of skin ulcers. The results also suggested that platypuses from affected sites were exposed to *M. amphibiorum* more commonly than the clinical disease occurred. This ELISA could be used to confirm the cause of cutaneous ulcers in the platypus and potentially to monitor the progress of treatment, particularly where internal lesions were suspected.

**Pathology**

Histologically, skin lesions examined from 7 cases of platypus mucormycosis were in the form of discrete, poorly encapsulated granulomas or more commonly of diffuse granulomatous or pyogranulomatous inflammation (Connolly et al 2000). Polymorphonuclear cells varied in being either neutrophil or eosinophil. Plasma cells and lymphocytes were limited in numbers. Fibrovascular tissue was consistently present and diffusely and irregularly scattered in the granulomatous regions. Multinucleated giant cells were present in four biopsy lesions. Spherules characteristic of *M. amphibiorum* infection were observed in all biopsied skin lesions. Daughter spherules were roughly spherical and measured 11.3 ± 2.5 μm (mean ± standard deviation, *n*=77) with an actual range of 7.7 to 17.9 μm. Mother spherules measured 18.0 ± 5.8 μm (range of 12.8 to 33.2 μm, *n*=24). The number of daughter spherules within a mother spherule was 4.7 ± 3.2 (*n*=24).

Immunohistologically, T cells were the predominant infiltrating lymphoid cell in lesions, indicating the importance of the cell-mediated response. T cells labeled in larger numbers and more intensely for CD3 than CD5. Sparse B cells (positive for CD79a) were observed in a number of lesions together with small numbers of plasma cells, (variable intensity of staining with CD79b) as single cells or clusters.

**Figure 5.** Histopathological features of mucormycosis in the platypus.

**a)** Pseudoepitheliomatous epidermal hyperplasia in a thigh lesion from a platypus. H&E. x 140.

**b)** Multinucleate giant cell from a platypus with mucormycosis affecting the thigh. H&E. x 550.

**c)** Central neutrophils and a ruptured spherule (arrowhead) surrounded by macrophages, lymphocytes and plasma cells in a discrete granuloma from a platypus with mucormycosis. H&E. x 280.

**Differential diagnosis**

Other causes of cutaneous ulcers would be included in the differential diagnosis of mucormycosis in the platypus. Fungal culture, histopathology and/or the ELISA described earlier could be used to confirm the cause of cutaneous ulcers in the platypus.
Laboratory diagnostic specimens

Samples needed
- For culture: Aseptically collected swab, fine needle aspirate or biopsy material from cutaneous ulcers from living platypuses or skin and/or internal lesions from dead platypuses.
- For cytology: As for culture.
- For histopathology: Skin lesions or internal tissues, fixed in 10% buffered neutral formalin.
- For ELISA: Blood sample collected from the bill sinus, the serum is separated and stored at -70°C until used for serology.

Laboratory procedures
- Culture: Aseptically collected specimens are plated on Sabouraud’s dextrose agar (SDA) with and without added gentamicin (50 IU/ml) and incubated at 28°C for initial fungal isolation. Pure cultures are then subcultured onto plates containing Sabouraud’s dextrose agar with no added antibiotic. Potato dextrose agar is utilised for more detailed morphological studies and mating experiments.
- Cytology: Spherules typical of M. amphibiorum are observed in unstained wet preparations or Diff Quik smears made from material collected aseptically by fine needle aspirate or swabs collected after punch biopsy.
- Histopathology: Samples from skin lesions or internal tissues are fixed in 10% buffered formal saline, processed and embedded in paraffin, sections cut at 6 μm and stained with haematoxylin and eosin (H&E) and Periodic acid Schiff (PAS).
- ELISA: Performed by Professor Richard Whittington, University of Sydney (Whittington et al 2002).
- For more detail see diagnosis section.

Treatment

Treatment of mucormycosis in the free-living platypus has to date not been attempted and no captive platypuses have been diagnosed with this condition or treated.

Limited antifungal sensitivity testing found Mucor amphibiorum to be sensitive to Amphotericin B at <0.002 mg/L, but resistant to itraconazole and fluconazole (Connolly et al 1998). If treatment of mucormycosis in a captive platypus was desired, a small cutaneous lesion could be removed surgically and/or Amphotericin B could be injected into the site twice weekly. Blood urea, creatinine and electrolyte levels should be determined to monitor for nephrotoxicity. Treatment would be discontinued if azotaemia was observed. The ELISA described earlier could be used to monitor the progress of treatment if it was attempted, particularly where internal lesions were suspected.

Prevention and control

Temperature: Mucor amphibiorum will only grow in vitro at a maximum temperature of 36°C (Obendorf et al. 1993; Scholer et al. 1983).

Disinfection: Mucor amphibiorum appears to be susceptible in vitro to many standard disinfectants including phenol (Biogram®), gluteraldehyde (Parvocide®) and sodium hypochlorite (bleach).
General hygiene and disinfection of equipment, footwear etc is recommended when moving from diseased to disease-free zones.

Further work to identify the niche of *Mucor amphibiorum* in the environment, the means of transmission and risk factors for platypus infection are required before prevention and control measures can be enacted.

**Surveillance and management**

Wildlife disease surveillance in Australia is coordinated by Wildlife Health Australia. The National Wildlife Health Information System (eWHIS) captures information from a variety of sources including Australian government agencies, zoo and wildlife parks, wildlife carers, universities and members of the public. Coordinators in each of Australia’s States and Territories report monthly on significant wildlife cases identified in their jurisdictions. NOTE: access to information contained within the National Wildlife Health Information System dataset is by application. Please contact admin@wildlifehealthaustralia.com.au.

There is no threat abatement plan currently in existence for mucormycosis in the platypus. Mucormycosis in the platypus is not included in AUSVETPLAN. Platypus mucormycosis has been listed on the OIE Wildlife Diseases List. Surveillance and risk analysis for mucormycosis in the platypus, especially in currently disease-free zones, is highly recommended in this fact sheet. Little or no coordinated monitoring of Tasmanian platypuses for this disease has been carried out since 1995. Ongoing Tasmanian platypus surveys inviting public reporting of sightings are continuing in 2006.

**Research**

Ongoing Tasmanian platypus surveys are continuing in 2006. Confirming and sampling new cases and assessing the prevalence of the disease at sites previously studied are required. Further work to identify the niche of *Mucor amphibiorum* in the environment, the means of transmission and risk factors for platypus infection is essential. As part of the environmental sampling, a frog survey for mucormycosis should be carried out. Future work should include the development of diagnostic tools such as a PCR to detect free-living forms of *M. amphibiorum* in water or the environment as well as from platypus and/or frog lesions. Isolates of *M. amphibiorum* from diseased Tasmanian platypuses and mainland frogs will be compared to determine if the frogs were responsible for the introduction of this disease into Tasmanian platypus populations. The outcome will be an improved understanding of the source of the fungus and how it is spread in Tasmania. This should lead to control measures to prevent the further spread of this disease.

**Key research questions**

- What is the niche for *Mucor amphibiorum* in sites where platypuses are diseased?
- Does *M. amphibiorum* exist as a free-living organism in suitable habitats, particularly natural water bodies and moist substrate?
- What areas in Tasmania are *M. amphibiorum*-free?
- How do environmental characteristics of natural water bodies (pH, pO2, ion content, nitrate, organic content) and weather (temperature, rainfall) affect the biology and survival of *M. amphibiorum*?
- How does *M. amphibiorum* spread between water bodies?
- Are Tasmanian frogs infected or acting as vectors of *M. amphibiorum*?
- Are there any other vectors of *M. amphibiorum*?
- Can *M. amphibiorum* be eradicated from contaminated water bodies and/or soil?
• In populations where mucormycosis is endemic, what determines the impact on the platypus population?
• Can resistance to infection or disease caused by *M. amphibiorum* be selected for?

**Research activities**

• Mapping the distribution of *M. amphibiorum*-infected platypuses.
• Identifying the source (niche and/or vectors) of *M. amphibiorum*.
• Monitoring of platypus populations that have survived initial epidemic wave to look for evidence of survival of individuals and recovery in numbers.
• Evaluation of impact of mucormycosis on platypus populations.
• Developing rapid diagnostic tools for detection of *M. amphibiorum* in the environment and in infected animals.

**Human health implications**

Nil. *M. amphibiorum* will not grow above 36°C. Homeotherms are considered unsuitable hosts because of this.

**Conclusions**

The distribution of Tasmanian platypus populations with mucormycosis is expanding, with mucormycosis observed in at least eleven catchments since the index cases were seen in the Elizabeth River at Campbell Town in the South Esk River catchment in 1982. The environmental niche of *Mucor amphibiorum* in Tasmania has not yet been identified, although it probably entered the state via an infected frog with fruit from Queensland. Further surveillance of Tasmanian platypus and frog populations is required, as is investigation into the risk factors for the disease. More information is required before control and/or eradication strategies can be planned.

**References and other information**


Acknowledgments

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To provide feedback on this fact sheet

We are interested in hearing from anyone with information on this condition in Australia, including laboratory reports, historical datasets or survey results that could be added to the National Wildlife Health Information System. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

Wildlife Health Australia would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to admin@wildlifehealthaustralia.com.au. We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

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