Amphibian disease and declines: Chytridiomycosis

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Chytridiomycosis is caused by *Batrachochytrium dendrobatidis,* a unique fungal organism of the phylum *Chytridiomycota,* a large group of ubiquitous fungi better known as silent digesters of organic waste or as parasites of nematodes and unicellular organisms such as pollen or algae. *Chytr* is the Greek root for earthen pot, *Batracho* – frog and *dendrobates* the genus of frogs from which it was first formally described in 1999 [1]. When viewed with a scanning electron microscope (Figure 1) it is easy to understand the origins of the name chytrid given to this group of fungi.

Epidemiology

Using histological examination of toe web skin, the earliest museum record for amphibian chytrid is a specimen of *Xenopus laevis* (African clawed frog) from Cape Horn Museum [2]. This has led to the hypothesis that the fungal pathogen originated in South Africa and was introduced to naïve populations of frogs by the widespread use of *Xenopus* as a laboratory and pregnancy test animal. Hopping from one species to another it managed to spread throughout the Americas, Australia, New Zealand and Europe. It may have arrived in Australia somewhere in the Southeast Queensland region in the late 1970s. The geographical range in Australia is mainly along the eastern and south eastern coastlines extending inland through the ranges, with smaller separate areas in the states of Western Australia and South Australia [3]. Disease investigation and field surveys show that only the Northern Territory and central regions appear to be free of the organism. Amphibian chytrid is not the only infectious organism causally linked to amphibian declines, but, in Australia, chytrid appears to be highly endemic and can infect a wide range of frog species. The extinction of one Australian species, the northern tinker frog, *Taudactylus acutirostris*, has been directly correlated to chytridiomycosis [4]. It also appears that, post epidemic, many frog species have recovered in number and are surviving with the chytrid endemic in their populations [5].

Figure 1. Scanning Electron Microscopy picture of B. dendrobatidis. Photo: Courtesy R Speare Amphibian diseases group JCU (bar 10 microns)

Laboratory transmission studies have demonstrated that *B. dendrobatidis* is fatal to adult and subadult frogs [1,6]. In natural or field infections a number of mature adults in good condition are often found dead, usually between the winter months of June to August. There are a number of reports of high – up to 100% – mortality in captive colonies, usually of the younger age group, but sometimes with subclinically infected mature adults and tadpoles in the same population [6,7]. In natural outbreaks other frogs species living and breeding in the same habitat can remain unaffected. In the adult and subadult frog, chytrid has a predilection for the toes, inside the hind legs/ inguinal region and ventral abdomen. There can be a high prevalence in tadpoles and they carry the fungus through metamorphosis leading to death usually at about 30-40 days post emergence. Tadpoles remain unaffected as the infection is localised to the keratinised parts of the oral disc. For most wild populations of frogs, subadults are rarely seen once they leave the water and therefore the impact on these populations remains unknown

Biology

Amphibian chytrid is only found in the skin of adult frogs and the mouthparts of tadpoles. This specificity in host cell type suggests that chytrid has an affiliation for keratin as a substrate. Parasitic chytrid that have been studied demonstrate a specific attraction, known as chemotaxis, for their substrate of choice. Amphibian chytrid will grow on boiled snake skin [1] but digestion of keratin or a specific attraction to frog keratin has not been researched. It is an intracellular organism with a simple life cycle, the infectious stage – a zoospore (1-2 μm) – possesses a single flagellum. It is free living but appears to move only an average of two centimetres, and most have encysted by 24 hrs [8]. No hardy resting phase or alternative host other than a range of amphibian species has been found [1].

The host dependent stage is the sporangium. Chytrid zoospores attach to host cells and evert their contents into a maturing keratinocyte via a transmembranous tube or rhizoid. Here a sporangium (6-15 μm) forms. The sporangium or thallus produces a number of zoospores which are released via a characteristic discharge papilla (Figure 2).

Amphibian chytrid, like many of its relatives, favours a moist cool environment. It grows best in a TgHL[1] agar or tryptone broth[1] at temperatures of 13-23˚C. This explains the geographical distribution in Australia. It may survive prolonged periods in freezing conditions, but temperatures above 25˚C will kill all sporangia and zoospores in culture. *B. dendrobatidis* produces proteases that

can digest casein and gelatin and it can tolerate a pH range of 4- 8. The pH does not change during growth in culture and therefore the growth medium does not require a buffer. *B. dendrobatidis* can be very fastidious to grow and difficult to isolate so addition of antibiotics to growth media is necessary. Once established, isolates can be maintained in a tryptone broth with twice weekly refreshing, or cryopreserved in liquid nitrogen.

Figure 2. Life cycle of *Batrachochytrium dendrobatidis.*

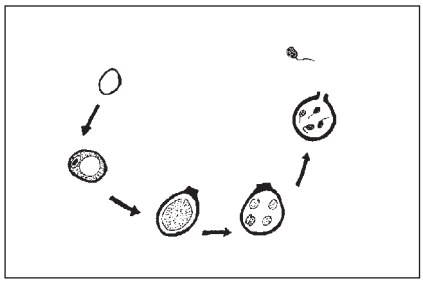


Figure 3. Typical H & E histological lesion in the skin of a Green Tree frog Litoria caerulea. The stratum corneum has sloughed away, there is acanthosis and evidence of sporangia in the outer stratum spinosum layer (arrows). This case has a particularly reactive lesion where there is loss of basement membrane integrity and vacuolation of the epidermis.

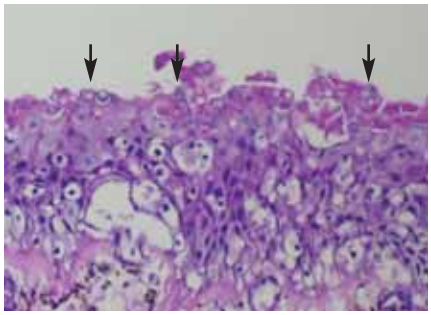
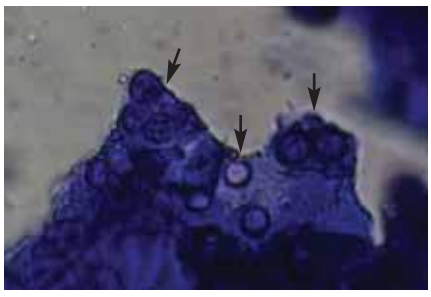


Figure 4 Gentian violet and safranin staining of skin scraping from same specimen as figure 2. Arrows show chytrid sporangia.



Pathogenesis

The characteristic histological lesion in frog skin (Figure 3) is a separation between the thin layer of the stratum corneum that is usually only 2-3 cell layers thick, with a thickening of the stratum spinosum or acanthosis. Hyperplasia of the epidermis is a typical host response. There is little if any cellular immune response observed and in field infections often there will be secondary bacterial invaders inside the empty sporangia[9].

A frog infected by chytrid may have any number of the following clinical signs. Their behaviour changes, they are less cryptic and reluctant to flee. Skin lesions, ulcers and skin tags are common, and moribund animals are unable to slough and ingest their skin normally. Hyperemia indicates a septicemia and this is often sequelae of the disease. Some North Queensland tree frog species were reported as having neurological signs before death such as tetany and spasming of limbs[3].

Diagnosis

The physical examination of a sick frog is limited; there are no pathognomonic signs that diagnose a chytrid infection. The best option for rapid inhouse diagnosis in a live animal is use of a skin scraping to identify the organism microscopically (Figure 4). Plain wet mounts or stains such as cotton blue with KOH[7] and Wrights or Diff Quik[10] can be used. Diagnosis can be made via histology, and has a high specificity; the size shape and position of the chytrid thallus in the epidermis are characteristic in an H & E stain or PAS[9]. This can be performed post mortem or in live animals using a toe clip or a skin biopsy, the skin of the toe webbing is preferred. There is an immunoperoxidase antibody available from AAHL[11], for light or immature infections that are difficult to diagnose. A quantitative PCR test is currently under development by Alex Hyatt from CSIRO AAHL[12], but it is not commercially available. PCR has a high sensitivity and as few as 10^{-1} zoospores can be detected.

B. dendrobatidis is an excellent example of how little we know about the species of microscopic organisms that surround us. Conservative estimates suggest that there are likely to be over 1.5 million species of fungi, of which less than 5% have been formally described[13]. Were it not for the global decline of amphibians, brought to the attention of the world by the First International Congress of Herpetologists in 1987, an organism such as this may have remained undiscovered.

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