Infection with Batracho-chytrium dendrobatidis

PATHOGEN INFORMATION

1. CAUSATIVE AGENT

1.1. Pathogen type

Fungus.

1.2. Disease name and synonyms

Infection with *Batrachochytrium dendrobatidis.* Chytridiomycosis or cutaneous chytridiomycosis.

1.3. Pathogen common name and synonyms

Amphibian chytrid fungus, frog chytrid fungus, frog chytrid.

1.4. Taxonomic affiliation

1.4.1. Pathogen scientific name (Genus, species, sub-species or type)

Batrachochytrium dendrobatidis (*B. dendrobatidis*, Bd). There is evidence that strains of varying virulence do occur, but these have not been formally described (Berger *et al.* 2005, Retallick and Miera 2007).

1.4.2. Phylum, class, family, etc.

Fungi, Chytridiomycota, Rhizophydiales, *incertae sedis*.

1.5. Description of the pathogen

The fungus thallus is a roughly spherical zoosporangium (diameter 5-40 µm) with one or more discharge tubes through which zoospores are released to the external environment. Zoosporangia extrude small "root-like" structures (rhizoids) when growing. Zoospores are roughly spherical (diameter 3-5 µm) with a single anterior flagellum and are motile in fresh water.

1.6. Authority (first scientific description, reference)

LONGCORE JE, PESSIER AP, NICHOLS DK. (1999) *Batrachochytrium dendrobatidis gen. et sp. nov.*, a chytrid pathogenic to amphibians. *Mycologia*, **91**, 219-227.

1.7. Pathogen environment (fresh, brackish, marine waters)

Fresh water.

2. MODES OF TRANSMISSION

2.1. Routes of transmission (horizontal, vertical, direct, indirect)

Horizontal transmission via zoospores in fresh water. Possibly also by direct skin to skin contact between individual amphibians, but no experimental confirmation. Vertical transmission via eggs is unlikely. The pathogen infects only keratinised tissues (i.e. skin of metamorphosed amphibians or the mouthparts of anuran larvae (tadpoles)).

2.2. Life cycle

2.2.1. Life cycle on amphibian host

Zoospores penetrate cells of the *stratum corneum* and *stratum granulosum* of metamorphosed amphibians or into the cells of the keratinised mouthparts of anuran larvae. Once intracellular, the zoospore develops into a zoosporangium within the cytoplasm of the invaded cell. The zoosporangium releases new zoospores into the environment via discharge tubes which project through the host cell membrane. These zoospores then invade adjacent keratinised cells to produce a cluster of infected cells in the original host or are released into the fresh water environment of the host. Time from zoospore penetration to zoospore release is approximately 4 days.

2.2.2. Life cycle in environment

B. dendrobatidis has the same life cycle in culture as in the skin of the amphibian host except the zoosporangium attaches to an inert substrate. Some authors consider that the organism can live saprophytically in the environment, but this has not been proved. Zoospores are motile, but in still fresh water they settle within several hours and rarely move more than 3 cm from point of release (Piotrowski *et al.* 2004).

2.3. Associated factors (temperature salinity, etc.)

Temperature affects the survival and growth of *B. dendrobatidis*, maximum growth occurs between 17°C and 25°C (Piotrowski *et al.* 2004). Above 29°C growth ceases and death occurs within 4 days at 32°C, within 4 hours at 37°C , within 30 minutes at 47°C, and within 5 minutes at 60°C (Johnson *et al.* 2003). Salinity of 5% and above kills *B. dendrobatidis* within 30 min. Mortality of amphibians due to *B. dendrobatidis* increases experimentally with ambient temperatures below 25°C and in natural infections in the cooler months of the year in tropical and subtropical areas.

2.4. Additional comments

None.

3. HOST RANGE

3.1. Host type

Amphibians, including members of all orders Anura (frogs and toads), Caudata (salamanders, newts and sirens) and Gymnophiona (caecilians).

3.2. Host scientific names

All amphibians.

3.3. Other known or suspected hosts

None known or suspected.

3.4. Affected life stage

Tadpoles (larvae), metamorphs and post-metamorphs (juveniles, sub-adults and adults).

3.5. Additional comments

No evidence of occurrence of *B. dendrobatidis* in or on eggs.

4. Geographical Distribution

4.1. Region

Africa, Americas, Australia, and Europe. No evidence from Asia although this may be due to inadequate surveillance.

4.2. Countries

Known presence in Australia, New Zealand, Canada, USA, Mexico, Costa Rica, El Salvador, Panama, Venezuela, Brazil, Argentina, Uruguay, Ecuador, Puerto Rico, Cuba, UK, Spain, Germany, Switzerland, France, Italy, South Africa, Swaziland, Tanzania, Kenya, .

DISEASE INFORMATION

5. Clinical Signs and Case Description

5.1. Host tissues and infected organs

B. dendrobatidis infects the skin of metamorphosed amphibians and the mouthparts of anuran larvae. Within the skin, the pathogen is confined to the superficial layers of the epidermis, the *stratum corneum* and *stratum granulosum*.

5.2. Gross observations and macroscopic lesions

Macroscopic lesions of chytridiomycosis vary with the species of host, but in general the skin lesions are not severe and are non-specific. Skin lesions vary from no macroscopic changes to sloughing (but as flakes of skin, not as sheets). Some species show erythema (redness) of the posterior ventral surface. The most obvious changes in severely affected amphibians are nervous signs, manifested as behavioural changes, ataxia, loss of flee response, loss of righting reflex, coma and death. Behavioural changes include abduction of thighs, nocturnal species coming out during daylight, and fossorial (burrowing) species remaining on the surface.

Infected tadpoles show loss of pigmented jaw sheaths in their mouths.

5.3. Microscopic lesions and tissue abnormality

Hyperkeratosis of the epidermis in areas where zoosporangia occur with disruption of normal microscopic architecture and sloughing of surface layers. Zoosporangia are easily seen in haematoxylin and eosin stained sections and by use of fungal stains such as PAS and silver stains (Berger *et al.* 2000). Suspect zoosporangia can be confirmed by polyvalent immunoperoxidase staining (Berger *et al.* 2003). The density of zoosporangia is highest on the feet and ventral surface of amphibians. None to mild inflammatory infiltrate into dermis underlying epidermal lesions.

In tadpoles zoosporangia can be seen in the superficial epidermis of jaw sheaths and denticles, usually with only mild degree of hyperkeratosis, and in advanced tadpoles in the epidermis of feet and resorbing tail.

5.4. OIE status

Listed under Article 1.2.3 of the Aquatic Code

6. Social and Economic Significance

Of economic importance due to its impact on the commercial amphibian trade, particularly the pet and scientific trade, and on the harvesting of wild amphibians for food trade in some areas. Social impact is predominantly through deaths in pet amphibians and socio-economic consequences for harvesters of wild amphibians.

Significant impact on diversity of wild amphibian populations and has resulted in extinctions of species, species becoming threatened or increasing the severity of their threatened status.

7. Zoonotic Importance

None

8. Diagnostic Methods

8.1. Surveillance methods

Diagnostic tests available include microscopic examination of unstained or stained sheets of superficial epidermis, routine histological examination of skin (epecially toe clips) or tadpole mouthparts, immunohistochemistry, PCR (standard or real time) and isolation and culture (Hyatt *et al.* 2007, Speare *et al.* 2005). Surveillance is through investigation of amphibian mortality and targeted surveys if no mortality has occurred (Speare *et al.* 2005). All tests can be performed without sacrifice of test animals, but histological tests of the live animal require removal of a toe tip. Real-time PCR is recommended for surveillance as this test has a high degree of sensitivity and specificity (Hyatt *et al.* 2007).

8.2. Presumptive methods

None. However, epidemic mortality occurring in amphibians, particularly during cooler periods of the year in tropical or subtropical areas, should make chytridiomycosis a diagnosis to be excluded (Berger *et al.* 2005; Speare *et al.* 2005). Some species appear to be most susceptible within a few weeks post-metamorphosis, so large-scale mortality of newly metamorphosed amphibians could indicate *B. dendrobatidis* infection.

8.3. Confirmatory methods

RT-PCR (Hyatt *et al.* 2007), standard PCR (Annis *et al.* 2004), immunohistology (Berger *et al.*

2003), and histology (Berger *et al.* 2000, Pessier *et al.* 1999) are methods with high specificity and with levels of sensitivity in decreasing order.

9. CONTROL METHODS

Disinfection of water and fomites through use of chemical disinfectants (bleach, quaternary ammonium compounds, ethanol, Virkon, Trigene, F10) or physical methods (heat, drying) (Johnson *et al.* 2003, Webb *et al.* 2007). Treatment of infected animals with antifungal compounds or heat has variable success, but cure of individuals can be confirmed using PCR. National control strategies for Australia have been proposed (Department of Environment 2006) and could serve as a model for other countries (Fisher and Garner 2007).

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