

AMPHIBIAN CHYTRIDIOMYCOSIS

ANIMAL GROUP AFFECTED	AGE GROUP AFFECTED	TRANSMISSION	CLINICAL SIGNS	FATAL DISEASE?	TREATMENT	PREVENTION & CONTROL
Amphibians	Larval and post-metamorphic animals	Direct contact, through water, including damp substrate	Abnormal behaviour, skin lesions: hyperkeratosis, érosions...	May be epizootic with high mortality, dependant on amphibian species	Immersion baths with itraconazole or formaldehyde and malachite green	Isolate affected amphibians. Tanks should have separate water sources.

Fact sheet compiled by Norin Chai, Head of the Veterinary Dept. of the M�nagerie du Jardin des Plantes, Paris, France	Last update January 2009
Fact sheet reviewed by Andrew A. Cunningham BVMS PhD MRCVS, Reader & Head of Wildlife Epidemiology, Institute of Zoology Zoological Society of London, Regent's Park, London NW1 4RY, UK Rachel E. Marschang, Institut f�r Umwelt- und Tierhygiene, Hohenheim University, Stuttgart, Germany Gidona Goodman, R(D)SVS, University of Edinburgh, Scotland	
Susceptible animal groups Mostly described in anurans (frogs and toads) and caudates (salamanders and newts). The evidence that <i>B. dendrobatidis</i> can infect all species of amphibian is increasing as the number of amphibian species examined for the chytrid increases. Many species have been shown experimentally to be highly susceptible to amphibian chytridiomycosis. A case reported in a caecilian (<i>P. kaupii</i>) was suspected but not confirmed.	
Susceptible age groups Tadpoles can carry infection on their keratinized mouthparts but usually mortality rates are not high. Affected larvae can show reduced growth rate, smaller size at metamorphosis and oral abnormalities, particularly jaw sheath depigmentation. In post-metamorphic animals, morbidity and mortality rates vary greatly among species but can be up to 100%. Generally, recently-metamorphosed animals are more susceptible to chytridiomycosis than adult animals, although all post-metamorphic stages can be susceptible, depending on the host species and the environment.	
Causative organism <i>Batrachochytrium dendrobatidis</i> (Fungi, Chytridiomycota, Rhizophydiales). A low level of genetic variation was shown for 35 strains of <i>B. dendrobatidis</i> , suggesting a recently emerged clone.	
Zoonotic potential No (not reported)	
Distribution Global distribution highly linked with international movement of amphibians for research facilities and pet trade. Africa has been proposed by some authors as the likely origin of the amphibian chytrid. The international trade of <i>X.laevis</i> that began in the mid-1930s could represent the first means of dissemination. The American bullfrog (<i>Rana catesbeiana</i>) also has been proposed as an important vector (mainly through international trade).	
Transmission Horizontal transmission: Direct contact (movement of individual infected amphibians), through the water and via surface water during precipitation. Indirect contact: such as via water and moist substrate. The role of alternative (non-amphibian) hosts in disease transmission is not yet demonstrated.	
Incubation period Generally between 18 and 70 days, but may be shorter (14 days). Some species (in the wild or in captivity) may carry light infections in the absence of disease. For example, a small number of species, e.g. <i>X. laevis</i> and <i>R. catesbeiana</i> , appear to show no apparent clinical effects even in the face of heavy experimental challenge. If affected populations survive, the pathogen can persist and become endemic, sometimes with reduced mortality rates, but the recovery of populations to pre-outbreak levels has not yet been reported.	
Clinical signs Sudden death with no clinical signs has been described for most affected species, but some frogs with <i>B. dendrobatidis</i> infection can appear clinically normal (see above). Typically, infected post-metamorphic frogs	

are found dead, but, if clinical signs are apparent, these might include weight loss, abnormal behaviour (spending prolonged periods in the vivarium's water dish, failure to seek shelter, reluctance to flee) and, *in extremis*, neurological signs, such as loss of the righting reflex. Skin lesions (sloughing of the superficial epidermis, roughening of the surface, occasional small skin ulcers or necrosis of digits/feet) can occur in some species.

Visual inspection of anuran tadpole mouthparts may be an indicator of *B. dendrobatidis* infection, but other conditions (e.g. over-wintering loss of oral keratin) can mimic the signs of *B. dendrobatidis* infection of larval mouthparts.

Post mortem findings

Gross lesions are generally uncommon and, when present, are limited to the skin in post-metamorphic amphibians and to the keratinized mouthparts in larval anurans. Histopathological changes are characterized by multifocal hyperplastic and hyperkeratotic dermatitis with sloughing of the prominent keratin layer, within which, numerous fungal zoosporangia can be observed. The skin is not affected evenly: the skin of the ventral pelvic area and of the digits, webbing and hind-legs is most commonly affected, while the skin of the head and dorsum is least commonly affected.

Diagnosis

- Cytological (direct microscopical) examination of stained or unstained skin smears for the detection of zoosporangia. This method provides rapid diagnosis, but experience is required in order to provide reliable results.
- Histopathology : from both clinical (toe clip) and necropsy samples
- Electronmicroscopical feature and immunohistochemistry using polyclonal antibodies: as an aid in histologic diagnosis
- PCR: clinical or *post mortem* diagnosis of infection; high degree of sensitivity and specificity
- qPCR: clinical or *post mortem* diagnosis of infection; high degree of specificity and highest degree of sensitivity (more sensitive than standard PCR); recommended for screening healthy frogs for *B. dendrobatidis* infection.

Material required for laboratory analysis

Cytology: shed skin fragments, skin scraping

Histopathology: mouthparts of anuran larvae; for post-metamorphic amphibians: fixed fragments of shedding skin in formalin, multiple skin section, whole-body sections (small animals); toe clips from live animals.

PCR or qPCR: skin swabs from the ventral pelvic patch, hindlegs and feet of live post-metamorphic animals, skin samples from these regions of freshly dead, frozen or ethanol-preserved post-metamorphic amphibians.

Mouthpart swabs from live anuran larvae; mouthparts or mouthpart swabs from dead anuran larvae.

Relevant diagnostic laboratories

- Histology: any specialized laboratories
- qPCR: Institute of Zoology: Zoological Society of London, Regent's Park, London NW1 4RY, UK; matthew.perkins@ioz.ac.uk.
- PCR : Exomed, Erich-Kurz-Str. 7, 10319 Berlin, Germany, mutschmann@exomed.de
- PCR : Tobias Eisenberg, Landesbetrieb Hessisches Landeslabor, Schubert Str. 60 - Haus 13, 35392, Giessen, Germany

Contact before sample submission.

Treatment

Topical treatment with 5 min immersion baths daily for 11 days of itraconazole at 0.01% in water seem successful in many cases. Systemic treatment (associated with baths) has been successfully reported: itraconazole, 0.1 mg/kg body weight orally for five days in three cycles.

Topical treatment with overnight immersion baths every other day for 3 to 5 treatments of 25 ppm formaldehyde and 0,1 mg/L of malachite green. This translates to 0.8ml (of 30 %) formaldehyde plus 0,1 ml (of 1%) malachite green per 10 L of water.

Elevations of environmental temperature, supportive care, control of secondary bacterial infection are warranted. Additionally, stress should be minimized.

Prevention and control in zoos

Newly acquired animals should be maintained in quarantine as individuals in separate containers for at least 2 months. Individuals should undergo thorough physical examinations both before and after quarantine. A thorough necropsy including PCR (ideally qPCR) on the appropriate tissues must be performed on any animals that die.

Collect skin swabs (and tadpoles' mouthpart swabs) for PCR assay on arrival and 7 weeks post-arrival.

Enclosures and all equipment should be disinfected regularly.

Important hygiene practices include wearing disposable gloves and changing gloves between enclosures, disinfection of equipment between uses.

Suggested disinfectant for housing facilities

Virkon STM: broad-spectrum disinfectant effective against viruses, bacteria and fungi, manufactured by Antec



International Ltd, Sudbury, Suffolk CO10 2XD, UK.

Notification

Listed as notifiable by OIE see: http://www.oie.int/Eng/normes/fcode/en_chapitre_2.4.1.htm

Recommendations on importation of amphibians from a country, zone or compartment not declared free from *B. dendrobatidis*:

- identify stock of interest (cultured or wild) in its current location;
- evaluate stock health/disease history;
- take and test samples for *B. dendrobatidis*, evaluate general health/disease status;
- import and quarantine in a secure facility a founder (F-0) population;
- produce F-1 generation from the F-0 stock in quarantine;
- culture F-1 stock and at critical times in its development (life cycle) sample and test for *B. dendrobatidis* and perform general examinations for pests and general health/disease status;
- if *B. dendrobatidis* is not detected, pests are not present, and the general health/disease status of the stock is considered to meet the basic biosecurity conditions of the importing country, zone or compartment, the F-1 stock may be defined as *B. dendrobatidis* free or specific pathogen free (SPF) for *B. dendrobatidis*;
- release SPF F-1 stock from quarantine for aquaculture or stocking purposes in the country, zone or compartment.

Guarantees required under EU Legislation

None for amphibians

Guarantees required by EAZA Zoos**Measures required under the Animal Disease Surveillance Plan**

None currently. See: http://www.oie.int/Eng/normes/fcode/A_summry.htm

Measures required for introducing animals from non-approved sources

Suggested measures see notification.

Measures to be taken in case of disease outbreak or positive laboratory findings

Dead animals should be submitted for necropsy. Morbid animals should be immediately isolated and tested. If infection with *B. dendrobatidis* is diagnosed, the affected aquarium/terrarium should be quarantined.

Enclosures should be thoroughly disinfected; all organic material in the enclosures removed and burned or autoclaved (the fungus is highly sensitive to elevated temperatures, dying in 4 hours at 37°C and may be unable to persist outside the host when soil and water temperatures exceed 25°C for an extended period of time). A footbath with Virkon S should be placed at the entrance/exit of the quarantine room and disposable coveralls, boots, and gloves worn, with gloves change between enclosures.

Conditions for restoring disease-free status after an outbreak

A collection can be considered free of the disease when no further cases of chytridiomycosis appear 60 days after the end of the treatment. However, additional testing is required before a collection can be considered to be free of *B. dendrobatidis* infection. For this, the remaining animals should be tested via PCR (ideally qPCR) assay to confirm the absence of the fungus. There currently are no hard and fast rules for the amount of testing required as some animals can give positive, then negative, then positive results. It is recommended, therefore, that an apparently-negative collection is tested regularly (say, every three months) for up to one year before it is declared *B. dendrobatidis* free.

Contacts for further information

For detailed information about *B. dendrobatidis* and chytridiomycosis, including diagnosis and management, see the Amphibian Disease Home Page: <http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm>

References

1. Forzan MJ, Gunn H, Scott P. 2008. Chytridiomycosis in an aquarium collection of frogs: diagnosis, treatment and control. *Journal of Zoo and Wildlife Medicine* 39(3): 406–411
2. Hyatt AD, Boyle DG, Olsen V, Berger L, Obendorf D, Dalton A, et al. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Dis. Aquat. Org.* 73: 175–192.
3. Johnson M., Speare R. 2003. Survival of *Batrachochytrium dendrobatidis* in water: quarantine and disease control implications. *Emerg. Infect. Dis.* 9: 922–925.
4. Mazzoni R, Cunningham AA, Daszak P, Apolo A, Perdomo E, Speranza G. 2003. Emerging pathogen of wild amphibians in frogs (*Rana catesbeiana*) farmed for international trade. *Emerg. Infect. Dis.* 9: 995–998.
5. Moorehouse EA, James TY, Ganley ARD, Vilgalys R, Berger L, Murphy PJ, et al. 2003. Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Molec Ecol.* 12:395–403.
6. Rachowicz LJ, Vredenburg VT. 2004. Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. *Dis Aquat Organ.* 21;61(1-2):75-83.
7. Van Ellis T, Stanton J, Strieby A, Daszak P, Hyatt AD, Brown C. 2003. Use of immunohistochemistry to



- diagnose chytridiomycosis in dyeing poison dart frogs (*Dendrobates tinctorius*). *J. Wildl. Dis.* 39: 742–745.
8. Voyles J, Berger L, Young S, Speare R, Webb R, Warner J et al. 2007. Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. *Dis. Aquat. Org.* 77: 113–118.
 9. Weldon C, du Preez LH, Hyatt AD, Muller R, Speare R. 2004. Origin of the amphibian chytrid fungus. *Emerg. Infect. Dis.* 10: 2100–2105.
 10. Young S, Berger L, Speare R. 2007. Amphibian chytridiomycosis: strategies for captive management and conservation. *Int. Zoo Yb.* 41: 1–11