Network for wildlife health surveillance in Europe

Diagnosis Card

Batrachochytrium salamandrivorans

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Etiology

Batrachochytrium salamandrivorans is a chytridiomycete fungus belonging to the order Rhizophydiales. It is an intracellular pathogen that develops inside epidermal cells and produces two types of spores: motile and non-motile encysted spores and causes the lethal skin disease chytridiomycosis. Phylogenetic analyses show that *B. salamandrivorans* forms a clade with its sister species *B. dendrobatidis*. The genome size of the type-strain AMFP13/01 was determined at 32.6 Mb with 10,138 protein-coding genes predicted. The contribution of these proteins to virulence is currently not clear.

Affected species (wildlife, domestic animals, humans)

B. salamandrivorans mainly affects urodeles. Evidence from experimental infections and disease outbreaks in the wild and in captivity show that at least most species of the family Salamandridae, as well as several species of the family Hynobiidae are susceptible when exposed to *B. salamandrivorans*. For the Ambystomatidae and the large family of lungless salamanders (Plethodontidae), little information is currently available, but several species can be experimentally infected. Susceptibility of the family of Cryptobranchidae is not clear, with a single infection found in a farmed Chinese giant salamander (*Andrias davidianus*). The olm (*Proteus anguinus*, Proteidae) can be persistently infected at low infection intensity. No or few information is available on the urodele families Rhyacotritonidae and Amphiumidae. *B. salamandrivorans* infection in anurans has only been detected, mainly sublinically, in a limited number of species i.e., the wild small-webbed fire-bellied toads (*Bombina microdeladigitora)* and midwife toads (*Alytes obstetricans)* in captivity, the wild and in lab trials.
Thus far, infections with *B. salamandrivorans* have been demonstrated only in amphibians post-metamorphosis.

Epidemiological characteristics and disease course

Disease outbreaks have been described in wild salamander populations in the Netherlands, Belgium, Germany and Spain and in captive populations in Belgium, the Netherlands, Germany, the UK and Spain. The fungus produces two types of spores (motile and non-motile encysted spores). Transmission is likely to occur during animal-to-animal contact (e.g. during courtship, territorial interactions) and indirectly by encysted spores floating on water, motile zoospores or by contaminated forest soil. Adherence of encysted spores to inert matrices (e.g. scales on bird feet) may promote large distance spread. In susceptible animals, *B. salamandrivorans* causes epidermal necrosis, resulting in loss of the epidermal barrier and subsequent overgrowth and invasion by opportunistic bacteria, bacterial septicemia and death. The extent of epidermal infection correlates with the severity of the disease, which varies strongly between, and even within, amphibian host species. The outcome of infection depends on complex host, pathogen and environmental interactions and can vary from absence of clinical signs to rapid death.

Clinical signs

Chytridiomycosis caused by *B. salamandrivorans* may be accompanied by a combination of the following clinical signs: epidermal ulcerations (ranging from discrete, pin-point to extensive), excessive skin shedding, skin haemorrhages and/or fluid loss, anorexia, apathy, abnormal body postures and convulsions and death. These clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

Gross lesions

Skin changes (haemorrhages, ulcerations, sloughed skin remnants) are the main pathological findings.

Histological lesions

In a haematoxylin/eosin-stained skin sections, multifocal epidermal necrosis with loss of demarcation between keratinocytes layers in associated with myriad of both intracellular and extracellular chytrid-type fungal thalli is noticed.

Differential diagnosis

Similar clinical signs may be observed in any pathology that causes skin changes, including infections with *B. dendrobatidis*, Ranavirus, *Amhibiichlamydia* or opportunistic bacteria along with hypovitaminosis A.

Criteria for diagnosis

Real-time PCR positivity; histopathological changes consistent with the presence of the pathogen or consistent clinical signs; the presence of motile spores, compatible with chytrid zoospores, in wet mount of urodele skin; positive antigen detection by LFA.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

Samples:
Skin tissue: both invasive (skin biopsies) and non-invasive (cotton tipped swabs) samples sampling are appropriate. In dead animals, dorsal skin is the preferred tissue, given its slower post-mortem decay.
Cotton tipped swabs should be rubbed firmly over the abdomen (10 times), the underside of a foot (10 times) and the ventral aspect of the tail (10 times) using the tip of the swab. The use of disposable gloves for manipulating amphibians is highly recommended. Pooling of up to four five skin swab samples can be done in clinically affected animals. Given low pathogen load in subclinically infected animals, sampling of individual animals is recommended.
Preservation:
Molecular detection: tissue samples for qPCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1. If the material cannot be fixed it may be frozen. Skin swabs should be stored dry and preferably frozen.
Histopathology, immunohistochemistry: skin samples should be fixed immediately after collection. The recommended ratio of formalin (10%) to tissue is 10:1.
Wet mount: immediate examination at magnification 10× using light microscopy. The presence of motile spores of approximately 5 µm in diameter are indicative of amphibian chytrid infection.
Diagnosis:
qPCR: Samples are preferably run in duplicate. A sample is considered positive based on the combination of (1) the shape of the amplification curves (2) positive result of both duplicates, (3) returning GE values above the detection threshold (1 GE per reaction) (4) low variability between duplicates (< 0.3 Ct value).
Histopathology: in haematoxylin/eosin-stained skin sections, histopathological changes consistent with *B. salamandrivorans* infections include (i) multifocal epidermal necrosis with loss of demarcation between keratinocytes layers, (ii) associated with myriad of both intracellular and extracellular chytrid-type fungal thalli. Immunohistochemistry facilitates especially low-level infections with chytrid fungi, however, currently it is not *B. salamandrivorans* specific, because of the lack of anti-*B. salamandrivorans* specific antibodies.
Light microscopy: Wet mounts of skin scrapings or pieces of shed skin can be examined at 10x magnification. The presence of motile spores of approximately 5 µm in diameter are indicative of amphibian chytrid infection.
LFA: A lateral flow assay (LFA) using an IgM monoclonal antibody (MAb), which binds to a glycoprotein antigen present on the surface of zoospores, sporangia and zoosporangia, was developed to detect infection in amphibian skin samples. This MAb does not discriminate between *B. salamandrivorans, B. dendrobatidis* and *Homolaphlyctis polyrhiza*. The sensitivity of this test is likely to be lower than that of real-time qPCR. This would make this technique particularly suitable in animals with high infection loads. Such techniques may be useful for point-of-care testing if specificity is increased and provided thorough validation.
Culture methods: given the difficulties to isolate *B. salamandrivorans* from infected animals and the high uncertainty to obtain a viable culture, this method is not recommended as first diagnostic approach a routine diagnostic method

EWDA proposed harmonized protocol (for harmonization at large scale)

For diagnosing a new case (novel locality, novel host species), the combination of qPCR (on skin swab or skin tissue) with histopathology (skin) is required. For follow up purposes, qPCR on skin swabs is considered appropriate.

Laboratories that can be contacted for diagnostic support

Laboratories offering *B. salamandrivorans* diagnostics are listed on the Bsal Europe website

(http://bsaleurope.com).

Recommended literature

Thomas V, Blooi M, Van Rooij P, Van Praet S, Verbrugghe E, Grasselli E, Lukac M, Smith S, Pasmans F, Martel A. 2018. Recommendations on diagnostic tools for Batrachochytrium salamandrivorans. (2018) Transboundary and Emerging Diseases 65(2): e478-e488

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WOAH Manual of Diagnostic Tests for Aquatic Animals, eleventh edition 2024, Chapter 2.1.2. Infection with Batrachochytrium salamandrivorans (version adopted in May 2021) (https://www.woah.org/fileadmin/Home/eng/Health_standards/aahm/current/2.1.02_Bsal.pdf)