BATRACHOCHYTRIUM SALAMANDRIVORANS

INFORMATION ON THE PATHOGENIC AGENT

1. CAUSATIVE AGENT

1.1. Pathogenic agent type

Fungus.

1.2. Disease name and synonyms

Infection with *Batrachochytrium salamandrivorans*.

1.3. The pathogen agent's common names and synonyms

B. salamandrivorans, Bsal

1.4. Taxonomic affiliation

Batrachochytrium salamandrivorans (Kingdom Fungi, Phylum Chytridiomycota, Order Rhizophydiales, Genus *Batrachochytrium*).

1.5. Authority (first scientific description, reference)

B. salamandrivorans was first identified in 2013 following dramatic declines among populations of European fire salamanders (*Salamandra salamandra*) in the Netherlands (Martel *et al.*, 2013).

1.6. Pathogenic agent environment (fresh, brackish, marine waters)

Fresh water.

2. MODES OF TRANSMISSION

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

The pathogenic agent transmits horizontally. It can persist in the environment as non-motile or motile spores (Stegen *et al.*, 2017). It is not known whether direct animal to animal contact is epidemiologically important.

2.2. Reservoir

Based on characteristics of *B. dendrobatidis*, it is expected that resistant non-motile spores can survive in water and soil (Johnson & Speare, 2003). Individuals surviving disease can be persistently infected (Martel *et al.*, 2013; Martel *et al.*, 2014).

2.3 Risk factors (temperature, salinity, etc.)

B. salamandrivorans thrives best at temperatures around 15°C (Martel *et al.*, 2013). Salamanders have been shown experimentally not to be colonised by *B. salamandrivorans* at temperatures above 25°C (Blooi *et al.*, 2015a).

The zoospores actively swim in water, the fungus is dependent on water, and desiccation is fatal to all life stages (EFSA, 2017).

3 HOST RANGE

3.1. Susceptible species

B. salamandrivorans has a wide and as yet not fully characterised host range. It has been found to be susceptible in 14 amphibian species (EFSA, 2017).

Infection with *B. salamandrivorans* has also been reported in several species of wild, captive or museum specimens (Martel *et al.*, 2014; Spitzen van der Sluijs *et al.*, 2016). A survey in China found *B. salamandrivorans* to be present across a wide taxonomic range (Yuan et al., 2018).

3.2. Affected life stage

No published work has investigated susceptibility of different life stages; however there are no reports of resistant life stages.

4. GEOGRAPHICAL DISTRIBUTION

B. salamandrivorans was first detected in the Netherlands in 2013, and has later been found on several locations in neighbouring regions of Belgium in 2013 and 2014 (Martel *et al.*, 2014). The fungus has also been identified in captive populations of salamanders and newts in Germany (Spitzen-van der Sluijs *et al.*, 2016) and in the United Kingdom (Cunningham *et al.*, 2015).

The origin of *B. salamandrivorans* is thought to lie in south-east Asia and it has been identified in Japan, Thailand and Vietnam (Laking *et al.*, 2017; Martel *et al.*, 2014) and most recently China (Yuan et al., 2018). However, a survey in South Korea of 200 individuals over 2 years found no evidence of infection (Samantha, Mi-Sook, & Bruce, 2018).

Outside of Western Europe, *B. salamandrivorans* is known to infect a number of species of newts native to Asia, but does not appear to cause significant disease or mortality in those species. Current evidence strongly suggests that *B. salamandrivorans* is endemic to Asia and that species within this region may act as a disease reservoir (Laking *et al.*, 2017).

5. CLINICAL SIGNS AND CASE DESCRIPTION

5.1. Host tissues and infected organs

The main organ where pathology is observed is the skin (Martel *et al.*, 2013; Gray *et al.*, 2015).

5.2. Gross observations and macroscopic lesions

B. salamandrivorans parasitises epidermal cells of salamanders, causing skin ulcerations with significant degradation of the epidermis, impairment of vital skin functions, and subsequent death of susceptible species within two to three weeks (Martel *et al.*, 2013; Gray *et al.*, 2015; Laking *et al.*, 2017). Clinical signs of disease caused by fungi of the Genus *Batrachochytrium* are in general variable and not pathognomonic, although the lesions linked to *B. salamandrivorans* are characterised by marked skin ulcerations, in contrast to those caused by *B. dendrobatidis*, which typically induces epidermal hyperplasia and hyperkeratosis (Martel *et al.*, 2013 and 2014). As a consequence, clinical signs alone are not a suitable means for diagnosis.

5.3. Microscopic lesions and tissue abnormality

Histopathological lesions consist of focal epidermal ulcerations with high numbers of collonial thalli of *B. salamandrivorans* (Martel *et al.*, 2013).

5.4. OIE status

Infection with *B. salamandrivorans* was listed by the OIE in 2017 (see Chapter 1.3. of the *Aquatic Animal Health Code)* (OIE, 2017).

6. SOCIAL, ECONOMIC AND ENVIRONMENTAL SIGNIFICANCE

Amphibians are the most commonly traded animal across many regions of the world (Smith *et al.*, 2009; Nijman, 2010), though much of this trade has been reported to remain unregulated and unrecorded (Rowley *et al.*, 2016).

Investigations by Martel *et al.* (2013) provide very solid evidence that *B. salamandrivorans* is causing disease in fire salamanders in the Netherlands. Field observations and experimental studies indicate that case fatality approaches 100%. Between 2010 and 2013 the fire salamander in affected populations in the Netherlands was reduced by 96%.

The disease has the potential to negatively impact many amphibian populations. Yap *et al.* (2015) modelled the likely impact of *B. salamandrivorans* in North America and concluded that it is a serious threat to biodiversity there if introduced.

B. salamandrivorans could cause significant negative population level effects (including extinction) in many amphibian species (Yap *et al.*, 2015).

7. ZOONOTIC IMPORTANCE

None

8. DIAGNOSTIC METHODS

8.1. Definition of suspicion

High levels of mortality in amphibian populations with or without erosive skin disease.

8.2. Presumptive test methods

A presumptive diagnosis can be made on the basis of histology and the identification of characteristic skin lesions and colonial thalli (Martel *et al.*, 2013). Thus this method is only suitable for diagnosis in clinical cases.

8.3. Confirmatory test methods

Diagnosis can be confirmed by PCR or culture.

Currently the most reliable and widely used method for *B. salamandrivorans* detection is quantitative PCR (qPCR) (Blooi *et al.*, 2013).

Blooi *et al.* (2013) also described a PCR protocol (Duplex Real-Time PCR) which allows for the simultaneous detection and quantification of both *B. dendrobatidis* and *B. salamandrivorans* in amphibian samples.

Immunohistochemistry can be used to detect *B. salamandrivorans* in samples stored in formaldehyde (Thomas *et al.*, n.d.).

Isolation by culture methods has a low sensitivity (Martel *et al.*, 2014).

9. CONTROL METHODS

A protocol for treating infected salamanders has been developed (Blooi *et al.*, 2015a; Blooi *et al.*, 2015b). Infected salamanders exposed to temperatures of 25°C for 10 days were demonstrated to be cleared of infection. However, the margin between the temperature required to eliminate *B. salamandrivorans* and the upper thermal limit tolerated by most urodelans is narrow (Blooi *et al.*, 2015a). Alternatively, combinations of antibiotics have been shown to be similarly effective, but only kept or captured animals can be treated.

Trade in salamanders is considered to be the most likely pathway for entry of *B. salamandrivorans* into new geographical regions (Yap *et al.*, 2015; Grant *et al.*, 2016). Movement restrictions to limit pathogen introduction and early detection through surveillance of high-risk areas should be implemented to control pathogen invasion.

10. TRANSMISSION RISK

As *B. salamandrivorans* has been horizontally transmitted through cohabitation, disease transmission is likely with movement of aquatic animals.

The principal route for the global spread in *B. salamandrivorans* is considered to be the international trade in salamanders and newts (Martel *et al.*, 2014. Stephen *et al.*, 2015; Cooper, 2016; RAVON Reptielen Amfibieën Onderzoek Nederland 2016; U.S. Fish & Wildlife Service 2016). In Europe hobbyists are known to transport salamanders across borders to attend shows. This presents a potential risk for the spread of the pathogenic agent that is difficult to control.

B. salamandrivorans is also more likely to spread within crowded conditions, which create stress and weaken individuals' natural immune systems (Rachowicz *et al.*, 2005; Rowley *et al.*, 2007; Rollins-Smith *et al.*, 2011 in: U.S. Fish & Wildlife Service, 2016). *B. salamandrivorans* may also be introduced to the natural environment through improper disposal of contaminated water or material to transport salamanders (Stephen *et al.*, 2015; U.S. Fish & Wildlife Service, 2016). The intentional release of non-native salamanders (often as fishing baits) or unintentional escapes from enclosures may also result in introduction and establishment of *B. salamandrivorans* in wild populations (Picco and Collins, 2008; Krysko *et al.*, 2011 in: U.S. Fish & Wildlife Service, 2016).

The fact that salamanders can originate either from wildlife or from captivity (farms, hobby breeders, pet traders, etc.) is an aspect that should be considered when assessing the feasibility of a trade restriction.

For *B. dendrobatidis* all water and transport materials (*e.g.*, moist soil, packaging) used to import amphibians should be treated as contaminated for quarantine purposes (Johnson & Speare, 2003). It must be assumed that this also applies to *B. salamandrivorans*.

11. REFERENCES

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