

Chytridiomycosis related mortality in a midwife toad (*Alytes obstetricans*) in Belgium

Sterfte door chytridiomycose bij een vroedmeesterpad (Alytes obstetricans) in België

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ABSTRACT

Chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis*, contributes to amphibian declines worldwide. Recently, the fungus has shown to be widely distributed in Belgium and the Netherlands, although no clinical cases of the disease have been diagnosed yet. This case report describes the first case of mortality due to chytridiomycosis in Belgium in a wild population of midwife toads (*Alytes obstetricans*). The presence of clinical chytridiomycosis, combined with the relatively high prevalence of the fungus in Belgium, emphasizes the urgent need for a thorough study on the impact of infection on the native amphibian populations in Belgium.

SAMENVATTING

Chytridiomycose wordt veroorzaakt door de schimmel *Batrachochytrium dendrobatidis* en draagt in belangrijke mate bij tot het uitsterven van amfibieën wereldwijd. Recent werd aangetoond dat deze schimmel wijd verspreid voorkomt in België en Nederland maar klinische gevallen van chytridiomycose werden tot nu toe niet vastgesteld. In deze casuïstiek wordt het eerste geval beschreven van chytridiomycose met dodelijke afloop bij een wilde vroedmeesterpad (*Alytes obstetricans*) in België. De ruime verspreiding van de schimmel in België en het potentieel letale verloop van een infectie bij inheemse amfibieën benadrukken de noodzaak van een grondige studie van de impact van de infectie op inheemse amfibieënpopulaties in België.

INTRODUCTION

Amphibian declines have been reported worldwide since the late eighties of the previous century. Several causes have been identified, such as habitat destruction, the use of pesticides and climate change (Houlahan *et al.*, 2000). The infectious disease chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis*, has caused a massive loss of amphibian biodiversity, notably in Central America and Australia, but also in Europe (Berger *et al.*, 1998; Bosch *et al.*, 2001; Bielby *et al.*, 2009; Walker *et al.*, 2010). The fungus is restricted to the keratinized body parts: the mouthparts in larvae and the skin in postmetamorphs. Clinical chytridiomycosis is characterized by lethargy, dysecdysis and death. Epidermal hyperplasia and hyperkeratosis are the only consistent histological lesions. Mortality is probably induced by the disturbance of the skin function, compromising for example electrolyte homeostasis (Voyles *et al.*, 2009). Recently, the presence of the fungus has been detected in Belgium and the Netherlands (Spitzen *et al.*, 2010) and clinical chytridiomycosis is not uncommon in captive amphibians in Belgium and the Netherlands (Pasmans *et al.*, 2004; Martel *et al.*, 2010). However, no adverse effects of *B. dendrobatidis* infections on indigenous amphibians have been reported yet.

The present paper describes the first case of mortality due to chytridiomycosis in native amphibians in Belgium.

CASE

A recently metamorphosed midwife toad (*Alytes obstetricans*) was found moribund under a stone (Figure 1) near Marche-en-Famenne (GPS coordinates: 50°08'16"N; 5°27'23"E). Necropsy did not reveal any macroscopic lesions, except for loose skin on the ventrum and dorsum. Samples of skin from the dorsum, pelvic patch, the middle digit of the left hindleg, liver and kidney were fixed in formalin 10%, wax embedded and 4 µm sections were stained using hematoxylin and eosin, periodic acid-Schiff (PAS) or Ziehl-Neelsen staining. To determine the presence of iridoviruses, the PCR described by Mao *et al.* (1997) was carried out on a liver sample. The presence and number of *B. dendrobatidis* organisms in a swab from the skin were determined using the real time PCR described by Boyle *et al.* (2004).

Skin lesions were similar at the ventral abdominal skin (pelvic patch), caudodorsal skin and middle digit of the left hindleg. There were diffuse epidermal hyperplasia and hyperkeratosis, with the presence of nu-



Figure 1. Midwife toadlet (*Alytes obstetricans*) with chytridiomycosis. Note the loose, shed skin layers on the animal's body. The black tail remnant is typical of recent metamorphosis.

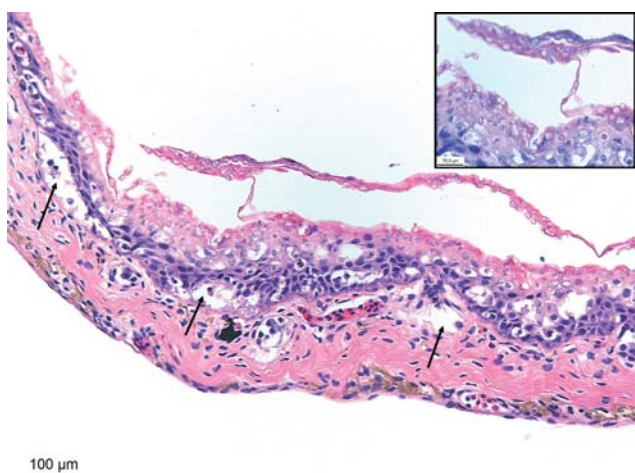


Figure 2. Hematoxylin and eosin stained formalin fixed section of ventral skin (drinking patch) of a midwife toad (*Alytes obstetricans*). Note epidermal hyperplasia and hyperkeratosis, with the presence of numerous zoosporangia in the keratinized epidermal layers. There are multifocal intra-epidermal vesicle formation (arrows), and scattered apoptosis and hydropic degeneration of keratinocytes. Inset: detail of zoosporangia at various stages of maturation.

merous PAS positive zoosporangia at various stages of maturation, confined to the keratinized epidermal layers (Figure 2). Numerous immature and mature 5–10 µm sized, thin-walled zoosporangia were invading the deeper portions of the corneal layers. Mature zoosporangia contained 1 µm sized basophilic, somewhat irregularly shaped spores. Superficial corneal layers were loaded with empty post-discharge zoosporangia which tended to collapse and were colonized by rod-shaped and coccoid bacteria. Discharge tubes were occasionally seen. There were severe epidermal alterations, especially in the ventral abdominal skin. Basal keratinocytes showed multifocal to coalescing vacuolar to hydropic degeneration and necrosis, with spongiosis and frequent intra-epidermal vesicle formation. This was sometimes accompanied by partial skin sloughing and erosion. Sloughed skin fragments were

heavily colonized by bacteria. Dermal blood vessels were congested. There was no clearly detectable difference in fungal load between the various locations.

In the liver, there was a diffuse microvacuolar degeneration of hepatocytes, and scattered single cell apoptosis. No histopathological lesions were noticed in the kidney. The Ziehl-Neelsen stains were negative for acid fast bacteria.

The PCR for the detection of iridoviruses was negative. In the skin swab, 1570 genomic equivalents of *B. dendrobatidis* were detected.

CONCLUSION

The presence of high numbers of *B. dendrobatidis* organisms, combined with the typical histological lesions and the absence of other obvious causes of death are conclusive evidence for clinical chytridiomycosis. Thus, this is the first published case of chytridiomycosis linked to mortality in indigenous amphibians in Belgium. Midwife toads (genus *Alytes*) are considered highly susceptible to chytridiomycosis (Bosch *et al.*, 2001; Martel *et al.*, 2010; Tobler and Schmidt, 2010). In 2009, 16% of all midwife toads examined in the Netherlands, were found positive for the presence of *B. dendrobatidis* (Spitzen-van der Sluijs *et al.*, 2010). The relatively high prevalence of the fungus combined with this clinical case sheds a new light on the potential risk of the presence of this emerging infectious disease for the native amphibians in Belgium. However, the short- and long-term effects of the presence of *B. dendrobatidis* at population level for the native amphibian communities are completely unclear at present. A thorough risk assessment, taking into account among others strain virulence, host susceptibility and factors that predispose to clinical disease, is urgently needed.

REFERENCES

- Berger L., Speare R., Daszak P., Green D.E., Cunningham A.A., Goggin C.L., Slocombe R., Ragan M.A., Hyatt A.D. Mc Donald K.R., Hines H.B., Lips K.R., Marantelli G., Parkess H. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. In: *Proceedings of the National Academy of Sciences of the USA* 95, 9031–9036.
- Bielby J., Bovero S., Sorgiu G., Tessa G., Favelli M., Angelini C., Doglio S., Clare F.C., Gazzaniga E., Lopieta F., Garner T.W.J. (2009). Fatal chytridiomycosis in the Thyrrhenian Painted Frog. *Ecohealth* 6, 27–32.
- Boyle D.G., Boyle D.B., Olsen V., Morgan J.A.T., Hyatt A.D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* 60, 133–139.
- Bosch J., Martinez-Solano I., Garcia-París M. (2001). Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biological Conservation* 97, 331–337.

- Houlahan J.E., Findlay C.S., Schmidt B.R., Meyer A.H., Kuzmin S.L. (2000). Quantitative evidence of global amphibian population declines. *Nature* 404, 752-755.
- Mao J.H., Hedrick R.P., Chinchar V.G. (1997). Molecular characterization, sequence analysis, and taxonomic position of newly isolated fish iridoviruses. *Virology* 229, 212-220.
- Martel A., Van Rooij P., Vercauteren G., Baert K., Van Waeyenberghe L., Debacker P., Garner T.W., Woeltjes T., Ducatelle R., Haesebrouck F. (2010). Developing a safe antifungal treatment protocol to eliminate *Batrachochytrium dendrobatidis* from amphibians. *Medical Mycology*, in press.
- Pasmans F., Zwart P., Hyatt A.D. (2004). Chytridiomycosis in the Central American bolitoglossine salamander (*Bolitoglossa dofleini*). *Veterinary Record* 154, 153.
- Spitzen-van der Sluijs A., Zollinger R., Bosman W., van Rooij P., Clare F., Martel A., Pasmans F. (2010). Short report: *Batrachochytrium dendrobatidis* in amphibians in the Netherlands and Flanders (Belgium). A report by RAVON for invasive Alien Species Team (TIE): Ministry of Agriculture, Nature and Food Quality.
- Tobler U., Schmidt B.R. (2010). Within- and among-population variation in chytridiomycosis-induced mortality in the toad *Alytes obstetricans*. *PLoS One* 5: e10927.
- Voyles J., Young S., Berger L., Campbell C., Voyles W.F., Dinudom A., Cook D., Webb R., Alford R.A., Skerratt L.F., Speare R. (2009). Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science* 326, 582-585.
- Walker S.F., Bosch J., Gomez V., Garner T.W.J., Cunningham A.A., Schmeller D.S., Ninyerola M., Henk D.A., Ginestet C., Arthur C-P. and Fischer M.C. (2010). Factors driving pathogenicity vs. prevalence of amphibian panzootic chytridiomycosis in Iberia. *Ecology letters* 13, 372-382.

Uit het verleden

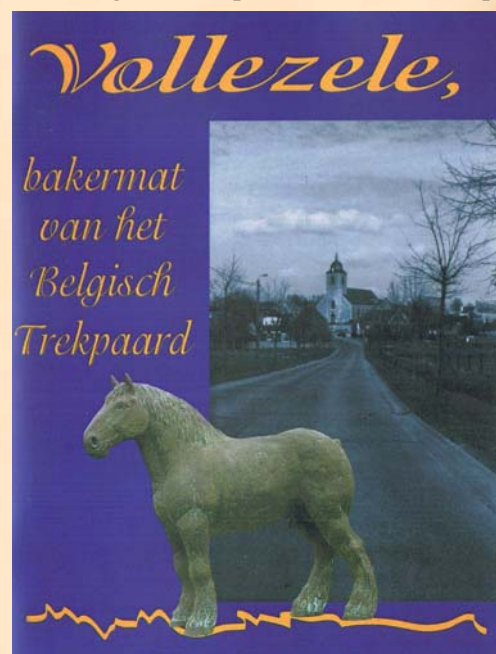
MUSEUM VAN HET BELGISCH TREKPAARD TE VOLLEZELE

Vollezele, nu deelgemeente van Galmaarden, in het Pajottenland niet zo ver van Henegouwen, herbergt een klein museum dat zonder twijfel een van de sympathiekste in het land is. In het vroegere gemeentehuis(je) wordt de geschiedenis geïllustreerd van de bloeitijd van het Belgisch Trekpaard. Het kleine dorp herbergde in de gloriejijd niet minder dan drie wereldvermaarde hengstenhouderijen. 'Wereldvermaard' is hier letterlijk te nemen. In 1910 werden 34.599 Belgische paarden uitgevoerd, waaronder 1600 naar Amerika. De uitvoer strekte van Argentinië tot Rusland.

Een belangrijk aantal van de beste en duurste hengsten was uit Vollezele afkomstig. Pionier Remi Van Der Schueren, gevestigd in dat dorp, had niet enkel fokkerstalent, hij begreep ook dat men het product goed moest promoten en dat gebeurde vooral op internationale tentoonstellingen en wedstrijden. Samen met een tweetal andere hengstenhouders stichtte hij in 1885 de vereniging van het Belgisch Trekpaard. In minder dan geen tijd vormde deze organisatie drie lokale strekkingen, de 'Kolossen uit de Mahaignestreek', de 'Grijzen uit het Nijvelse' en de 'Dikke uit de Denderstreek' om tot een succesvol uniform ras te komen. Van Der Schueren's Orange I en Brillant waren daarin voortrekkers. Het monsterachtig grote succes duurde echter niet veel langer dan een halve eeuw...

Een bezoekje is zonder meer aan te bevelen. Wel moet dat vooraf aangevraagd worden bij de vrijwilligers die het museumpje openhouden (dat kan via de site, gemakkelijk te vinden als je Vollezele en trekpaard intikt op Google).

De illustratie hierbij toont het voorplat van een museumboekje opgesteld door Philippe van Dixhoorn, telg uit een Zeeuws-Vlaams hengstenhoudersgeslacht gevestigd te Vollezele en verdienstelijke stichter van het museum.



Luc Devriese