



# Concurrent ranavirus and *Batrachochytrium dendrobatidis* infection in captive frogs (*Phyllobates* and *Dendrobates* species), The Netherlands, 2012: A first report

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## Introduction

Ranavirus and *Batrachochytrium dendrobatidis* infections have been associated with amphibian mass mortalities and population decline worldwide.

The die-off began with two *Phyllobates bicolor* (blacklegged poison) frogs, followed by five *P. vittatus* (Golfo Dulce poison) frogs, and five *Dendrobates auratus* (green and black poison dart) frogs (12 animals in total).

Clinical signs in the frogs were dry, greyish skin. Animals spent more than 90% of their time in the water, showed anorexia, and then died.

On gross pathology, greyish skin, hepatomegaly, splenomegaly, and renomegaly were evident.

PCR for the MCP of ranavirus  
forward primer  
5'-GACTTGGCCACTTATGAC-3'  
reverse primer  
5'-GTCTCTGGAGAAGAAGAA-3'

PCR for the ITS-1–5.8S region of *B. dendrobatidis*.  
Forward primer  
CCTTGATATAATACAGTGTGCCATATGTC  
Reverse primer  
AGCCAAGAGATCCGTTGTCAA

## Histology and PCR

Epithelial hyperplasia and focal hyperkeratosis with multifocal intracellular sporangia (spore-containing bodies, arrows) of *B. dendrobatidis* in the stratum corneum (Fig. 1).

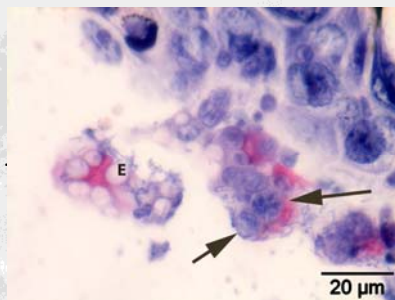


Fig. 1. E: empty sporangia.

Multifocal basophilic intracytoplasmic inclusion bodies (arrows), suggestive of viral infection, were evident in the erythrocytes, hepatocytes, villus enterocytes, and renal tubular epithelium (Fig. 2).

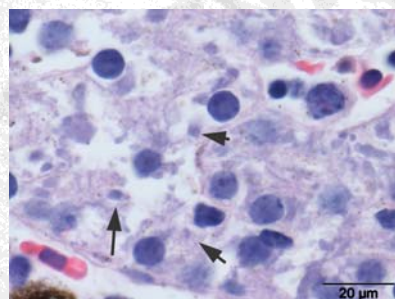


Fig. 2.

Sequencing of PCR products revealed high homology with respectively the common midwife toad ranavirus and *B. dendrobatidis*.

## Conclusion

The frogs died of a ranavirus infection, most likely the common midwife toad virus (CMTV), or a CMTV-like virus, combined with *B. dendrobatidis* infection.

It is difficult to determine which of the present pathogens contributed more to the deaths of the frogs.

The first time CMTV or a CMTV-like virus has been reported in these host species in captivity.

The findings highlight the importance of monitoring ranaviral and *B. dendrobatidis* infections in captive as well as wild amphibians.

Especially with the worldwide trade in amphibians, the risk of introducing these infections into wild populations with (un)intentionally released animals is high.

A spill-over of CMTV or CMTV-like ranavirus and *B. dendrobatidis* present in a captive population of frogs is a serious threat to wild populations of amphibia.