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Original Communication

Chytrid fungus *Batrachochytrium dendrobatidis* in common toad *Bufo bufo* in northeastern Poland

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ABSTRACT

Dead and live specimens of the common toad, *Bufo bufo*, were collected in northeastern Poland. The histological diagnosis for chytridiomycosis caused by *Batrachochytrium dendrobatidis* was positive in two of the seven specimens. Different stages–immature, mature, mature with zoospores, empty, and collapsed–were identified. This paper is the second report of chytridiomycosis in *Bufo bufo* in Europe, and the first in Poland.

KEYWORDS: chytridiomycosis, *Bufo bufo*, common toad, Poland

INTRODUCTION

Bufo bufo, is a common toad species in Europe [1], including the northeastern part of Poland [2]. In recent years, this species has often been attacked by a chytrid fungal pathogen, *Batrachochytrium dendrobatidis* (hereafter *B. dendrobatidis*), which has been implicated in the decline in amphibian populations around the world [3]. *B. dendrobatidis* has already infected 14 families and 365 species of amphibians on six continents [4]. According to the International Union of Conservation Nature Red List, 32.5% of amphibians have been assessed as "globally threatened", more than mammals (23%) and birds (12%) [5]. Approximately 12 toad species of the *Bufo* genus are hosts for *B. dendrobatidis*. The first documented infections of wild specimens of *Bufo haematiticus* (Panama) and *Bufo marinus* (Australia) caused by the above-mentioned pathogen were reported by Berger *et al.* [3] in 1998. Later, this chytrid pathogen was reported in the USA, in Californian specimens of *Bufo canorus* [6] and in *Bufo baxteri* (Wyoming) [7]. In USA, *B. dendrobatidis* has also been found in the wild populations of *Bufo americanus* (Indiana), *Bufo boreas* (Colorado), *Bufo guttatus* (Washington) [8], and *Bufo californicus* (California) [9]. In 2007, *B. dendrobatidis* was found in *Bufo longinasus dunni* in Cuba [10], and in 2009, in wild populations of *Bufo gargarizans* in South Korea [11].

In European *Bufo* genus toads, the first documented infection in the wild population of *Bufo bufo* was made in Spain in 2006 [12, 13]. Adams *et al.* [14] described the presence of this pathogen in specimens of *Bufo viridis* in Italy. Because the presence of *B. dendrobatidis* has been mainly described in Western Europe, we decided to publish this study on the presence of chytridiomycosis in specimens of *Bufo bufo* in Poland, as the first site in Eastern Europe.

MATERIAL AND METHODS

Two dead (killed in road traffic) and five live specimens of the common toad, *Bufo bufo* (Linnaeus, 1758), were collected in spring 2010 from a site near Lake Blizno, located at 53° 57.6'N–23° 04'E, altitude 133.2 m, in Augustow Forest, Suwalki Lake District, during the reproduction period. The ecological characterisation of this environment was described in our previous paper [15]. The live specimens were euthanised and fixed in the field in 10% neutral buffered

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formaldehyde. Strips of skin (approximately 5 x 10 mm) from five sites on the ventral part of the body (thighs and toe clips) were collected for the examination. A calibrated eyepiece micrometer was used to make a variety of measurements on 25-mm long sections in the centre of each strip, including the measurements of the number of sporangia [16]. Standard histological techniques for light microscopy were applied, the samples were sectioned at 6 µm, stained with haematoxylin and eosin, and examined at 60x and 100x magnification [17]. The presence of sporangia in this section was noted. We followed the procedures described by Berger *et al.* [18] and Pessier *et al.* [19] for chytrid identification.

RESULTS

Two of the seven specimens of the common toad species were positive for the presence of *B. dendrobatidis* in the samples from the ventral parts of the skin (Table 1). In the stratum corneum and stratum granulosum, different stages of zoosporangia (immature, mature with zoospores, empty, and collapsed) were observed. In addition, immature zoosporangia containing granular basophilic and eosinophilic materials were found. Mature zoosporangia ranged 5.8-9.7 µm in diameter, with a thin wall. The diameters of the zoospores ranged 1- 2 µm. Each zoospore had a single flagellum (19-20 µm). The most frequently observed sporangia in the stratum corneum had empty post-discharge sporangia. Characteristic septa were also observed in some sporangia. The similarity of the zoosporangial morphology of *B. dendrobatidis* has also been observed by other investigators [16, 17]. The mean number of sporangia of this chytrid fungus ranged from 38.2 (toe clips) to 58.4/mm skin section (thighs).

DISCUSSION

Toad species is the most common amphibian in Europe [1], as well as in northeastern Poland [2, 20], as represented by the *Bufo* genus. The year-round life period, except mating, takes place on land. Mating of these toads in northeastern Poland occurs in natural ponds, lakes, and the floodwaters of rivers, which are rich in aqueous plants. Most of the mating occurs in April, depending on the weather conditions. We present evidence of *B. dendrobatidis*-infected native amphibian species (common toad) in northeastern Poland. This is the first report regarding a chytrid pathogen in common toads in Poland, and the second in Europe. The first record of *B. dendrobatidis* in a native population of the common toad was in Spain [12]. In our study, the morphological forms of *B. dendrobatidis*–sporangia, zoosporangia, and zoospores–were the same size as those observed by other researchers [3, 18, 19]. Only the average number of sporangia per mm^{-1} from ventral amphibian skin was different. In our investigations of *Bufo bufo*, the average number of sporangia was 41.2; however, according to Berger *et al.* [16], the number of sporangia from the ventral skin of the green tree frog (*Litoria caerulla*), from Queensland or northern New South Wales (Australia), was 94.3 mm^{-1}. Fisher *et al.* [13] showed that the genotype of

Table 1. *Batrachochytrium dendrobatidis* infecting *Bufo bufo*.

No	Stage	Sex	(+) positive (-) negative	Average no. of sporangia mm^{-1} ventral skin				
				forapart	middle	terminal	thighs	toes
1	Adult	Female	-					
2	Adult	Male	+	34.4	38.8	40.3	58.4	38.2
3	Adult	Female	-					
4	Adult	Female	-					
5	Adult	Female	+	36.2	39.3	41.8	44.7	40.0
6	Adult	Unknown	-					
7	Adult	Male	-					

B. dendrobatidis is linked to virulence. He demonstrated that the sporangia of five isolates of *B. dendrobatidis* from other species of amphibians from Mallorca, all with identical genotypes, were similar in size but differed significantly from isolates recovered from amphibians in central Spain and the UK. The virulence of Mallorcan and UK isolates of this chytrid pathogen was assayed in *Bufo bufo* [21].

The effects of *B. dendrobatidis* infection in amphibians are great. In some countries, mass mortality has been observed, and in others, there might be a very high prevalence of chytrid pathogen, but no pathological symptoms [22] or any apparent population decline [4]. Some authors call it "enigmatic declines" [5]. The pathogen may be present, but it does not cause death in the amphibians. This has also been observed in specimens of *Bufo bufo* [12, 23]. The skin of those specimens was found to be infected with *B. dendrobatidis*, but the animals were healthy, and it occurred only in a relatively small number of specimens. Those species are the reservoirs of *B. dendrobatidis* in particular areas. They transmit the pathogen on a local level and over a long distance [24]. Nowadays, about 30 amphibian species are known to be reservoirs of *B. dendrobatidis* [25], and the *Bufo bufo* species belongs to this group. As suggested by Rachowicz and Vredenburg [26], in species where the post-metamorphic amphibians are clinically healthy, this life stage could function as a reservoir or carrier, infecting members of sympatric species or more susceptible members of the same species. All specimens of *Bufo bufo* in our study were collected from a site near Lake Blizno, where this chytrid pathogen has been found in green water frogs. Daszak *et al.* [27, 28] suggested that tadpoles could be infected with fungal zoospores and could transmit the infection to each other and to post-metamorphic animals [29]. In tadpoles, *B. dendrobatidis* has been known to infect only the keratinized mouth parts [30]. The aggregation of tadpoles and post-metamorphic animals translocates along several lake shorelines [31]. The infection can be transmitted to different parts of the lake [26]; therefore, it is possible that common toad tadpoles have been infected by green frog tadpoles [32], or vice versa. In northeastern Poland, mass mortality of amphibians infected by *B. dendrobatidis* has not been observed. It is possible that this chytrid pathogen may simply be present, but does not cause the amphibians' death. The accumulation of the complexes of unfavourable effects of environmental changes modifies disease resistance, including *B. dendrobatidis* resistance. As is known, this chytrid fungus occurs only in the keratinized skin, and it can cause death by disturbing the respiration and osmoregulation processes of the organisms. However, not all of amphibian species are susceptible.

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