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SHORT COMMUNICATION

Cutaneus myxosporidiasis in the Australian green tree frog (*Litoria caerulea)*

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Abstract This case is reported with the intention of highlighting the presentation of cutaneous myxosporidiasis in Australian tree frog (*Litoria caerulea*) caused by genus *Myxobolus*. The morphology and morphometric characteristic of the spores were determined using light microscopy and differential interference contrast microscopy. Spores were pyriform in shape in frontal view and oval in lateral view, and the average size was respectively 11.4 × 6.0 × 4.5 μm (12.1 − 9.5 × 6.3 − 5.4 × 5.0 − 4.1 μm). To the best of our knowledge, this is the second case of skin invasion caused by myxosporeans in amphibians.

Introduction

Myxosporidiasis are invasions caused by parasites *Myxosporea*. According to National Center for Biotechnology Information, they are currently classified as the *Myxozoa* and are closely related with phylum Cnidaria. These are intracellular organisms, which may be localized in the internal organs or on surface of the body. Over 2,180 species of *Myxosporea* belonging to approximately 60 genera have been described. Myxosporeans are common parasites of cold-blooded vertebrates, particularly fishes. Thus far, only 15 species were found in 83 representatives of the class amphibians, most of them in frogs and toads (Eiras 2005).

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Material and methods

A single adult *Litoria caerulea* was delivered to Sub-Department of Parasitology and Invasive Diseases, University of Life Sciences in Lublin in order to diagnose skin lesions observed by the owner. The animal was bought about 2 weeks earlier, in good condition, and without noticeable dermatological problems. According to medical history, the frog was in good condition all the time from purchase. It was the only animal in owner's collection. Detailed husbandry information such as cage temperature, humidity, water quality, and eating habits has been established. All these environmental parameters were generally appropriate for this species.

Microscopic examination material from the lesions revealed a myxosporidian plasmodium containing numerous mature spores. Morphometric characteristics of the spores (based on 30 mature spores from the skin lesions of the examined frog) were determined with the aid of a light microscope with Screen Measurement package. The morphological details were established using a differential interference contrast microscopy (DIC).

Results and discussion

The physical examination of the frog skin showed two types of lesions: a several-millimeter-sized, polymorphic, gray-white cyst with partially liquid-filled content and ulcers which, according to the owner, were the consequence of rupture of the primary cyst (Fig. 1). The lesions were located on the head, back, and legs of the frog. During routine examinations, other signs of the disease were not observed.

Spores were pyriform in shape in frontal view, and oval in lateral view, and the average size was respectively 11.4×6.0×

Fig. 1 Photgraph showing the macroscopically visible lesions in the skin of *Litoria caerulea*. **a**, **b** Primary lesions (*black arrowhead*). **c**, **d** Ulcers on the skin (*black arrowhead*)

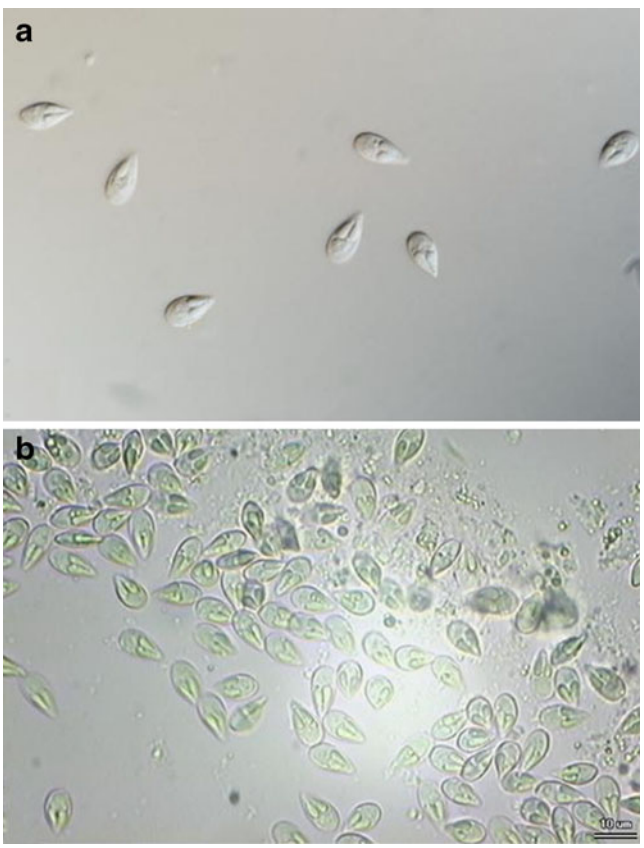

Fig. 2 The spores of *Myxobolu*s sp. isolated from the skin of *Litoria caerulea*. **a** Differential interference contrast microscopy (DIC). **b** Fresh isolated spores from skin lesions in light microscope

4.5 μm (12.1−9.5×6.3−5.4×5.0−4,1 μm). Each spore contains two unequal polar capsule. The average size of the large polar capsule was 6.04×2.05 μm (6.8−5.4×2.5−1.6 μm), while the smaller one was 4.66×2.04 μm (5.4−4.0×2.7−1.4 μm). Inside the spore body, a sporoplasm with two nuclei and vacuoles were seen (Figs. 2 and 3). Based on the morphology of spores isolated from the examined *L. caerulea*, the lesions on the skin were the consequence of myxosporean invasion from the genus *Myxobolus*.

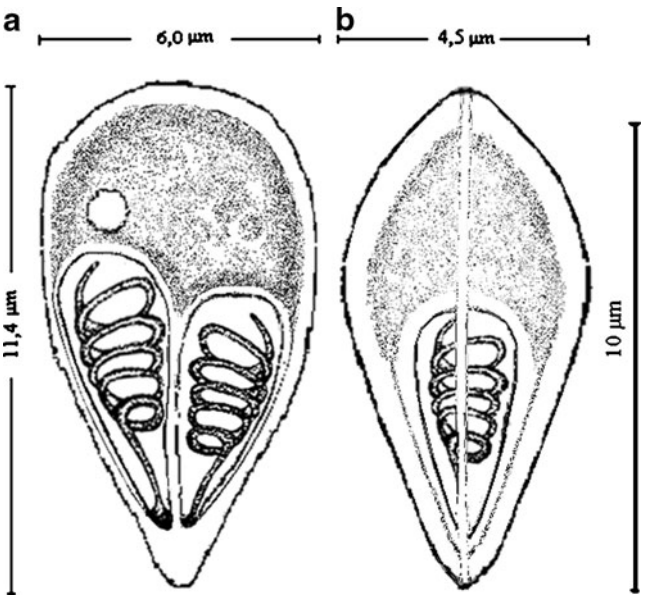

Fig. 3 Line diagram of *Myxobolus* sp. spores isolated from *Litoria caerulea* skin according to a differential interference contrast microscopy (DIC). **a** Frontal view, **b** lateral view

Six genera of *Myxosporea* have been described from amphibians (*Myxobolus, Myxidium, Hoferellus, Chloromyxum, Caudomyxum,* and *Spherospora*) which were mainly observed in the gonads, deferent ducts, oviducts, kidneys, urinary bladder, ureter, gallbladder, and liver. Only two genera have been described in genus *Litoria. Myxidium* and *Myxobolus* species cause many pathological changes in the liver, gallbladder, bile ducts, and in the gonads (Eiras 2005). There are three reports on the occurrence of *Myxosporea* in the Australian tree frog (*L. caerulea*). Delvinquier et al. (1992) described *Myxidium immersum* in the gallbladder in 32 species of tree frogs belonging to genus *Litoria* including *L. caerulea*. Hill et al. (1997) found trophozoites of *Myxidium* sp. in the bile ducts of several farmed Australian tree frogs with associated hepatitis. According to these authors, pathological changes in the liver and bile ducts could lead to the death of the host. Zwart has observed pathological effects of *Myxobolus* sp. in *L. caerulea* ovary (Sitjà-Bobadilla 2009). So far, only one report has described cutaneus myxosporidiasis in amphibians. Guyénot and Naville (1922) described species of *Myxobolus ranae* infecting the skin of the anuran *Rana temporaria* in Switzerland. Skin diseases caused by *Myxosporea* are common in fish. Cutaneus myxosporidiasis in fish are usually caused by species belonging to genera *Myxobolus* (*Myxobolus squamae, Myxobolus ellipsoides, Myxobolus sandrae,*and *Myxobolus cotlani*), *Spherospora (Spherospora carassii* and *Spherospora molnari*), *Thelohanellus* (*Thelohanellus dogieli*), and *Henneguya* (*Henneguya wolinensis).* It seems that most myxosporeans occurring in amphibians are characterized by little host specificity. Several species of *Myxosporea* were observed infecting several host species often living in separate geographical land (Eiras 2005; Sitjà-Bobadilla 2009). However, there are no reports about the presence in amphibian species of myxosporeans occurring in fish or reptiles.

To the best of our knowledge, this case is only the second reported case of cutaneus myxosporidiasis in amphibians. Location and morphometric characteristics of the spores isolated from the skin lesions of described Australian tree frog do not fit into any of the known species of the genus *Myxobolus* found so far in amphibians (Table 1). We can conclude that the present material represents a species not yet described.

We do not know what really had happened to the invasion in the described Australian tree frog because little is known about the life cycles of myxosporeans in amphibians. Most of the available knowledge about the details of the development of these parasites is based on the disease caused in fishes. It is possible that anurans may become infected in aquatic environment (as tadpoles or as adults) in a similar way as the fishes, either by oral route or through the skin. This mode of infection by *Myxobolus* sp. in the Australian tree frog seems to be possible because this species spends most of its adult life in the treetops outside the water environment.

There are various reports on the pathology of myxosporean invasions in amphibians. Some species seem to be not pathogenic like *Chloromyxum salamandrae* which has been isolated from the gallbladder from healthy salamanders (*Eurycea multiplicata griseogaster*, *Eurycea multiplicata*

Table 1 Members of the genus *Myxobolus* isolated from amphibians (Eiras 2005; Sitjà-Bobadilla 2009)

Species	Host	Geographic location	Parasite location	The shape of the spores	The size of the spores (μm) length, width, and thickness	The size of polar capsule (μm)
Myxobolus bufonis	*Bufo regularis* *Bufo maculatus*	Egypt Cameron	Testes, kidneys liver, gallbladder	Disk-shaped	9.2×8.9×4.0	4.1×3.2
Myxobolus chimbuensis	*Litoria darlington* *Litoria thesaurensis*	New Guinea	Testes	Ovoid	11.9×8.4×6.2	4.7×2.2
Myxobolus fallax	*Litoria fallax*	Australia	Testes	Ovoid	13.4×9.5×6.8	4.2×2.4
Myxobolus hylae	*Hyla aurea*	Australia	Testes, oviduct, kidneys	Oval, egg-shaped	13.9×9.1×7.2	4.2×2.6
Myxobolus sp.	*Bufo regularis*	Togo	Testes	–	–	–
Myxobolus sp.	*Ptychadena maccarthyensis*	Togo	Testes	–	–	–
Myxobolus sp.	*Litoria caerulea*	Australia	Ovaries	–	–	–
Myxobolus sp.	*Litoria infrafrenata*	Indonesia	Testes, ovaries	–	–	–
Myxobolus sp.	*Litoria leuseri*	Indonesia	Ovaries	–	–	–
Myxobolus ranae	*Rana temporaria*	Switzerland	Skin	Ovoid	11.1×8.1	4.5×2.5
Myxobolus sp.	*Litoria caerulea*	Poland	Skin	Pyriform	11.4×6.0×4.5	4.66×2.04 6.04×2.05

and *Eurycea neotenes*) (Upton et al. 1995). Myxozoan infection associated with host reproductive system and gonads reduce fertility (Browne et al. 2002; Mubarak and Abed 2001; Upton et al. 1992). The most serious symptoms and pathological changes, which could lead to the death of the host, were associated with hepatic and renal myxosporidiasis (Duncan et al. 2004; Mutschmann 1999). Lesions of the skin caused by myxospreans seem to have relatively the lowest health consequences for amphibians. During this invasion, we have observed only the skin lesions with no clear impact on the general condition of the animal.

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