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Case reports

Cryptosporidiosis in a fire skink (*Lepidothyris fernandi*) and molecular identification of infecting species

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ABSTRACT. Cryptosporidiosis is an infectious protozoan disease that affects a wide range of animals including reptiles. This is the first report of cryptosporidiosis in a fire skink (*Lepidothyris fernandi*), an insectivorous reptile commonly found in tropical West Africa. Faecal sample was collected from a fire skink at necropsy for the detection of parasites by faecal sedimentation method, Ziehl-Neelsen (ZN) acid-fast staining, Nested Polymerase Chain Reaction (PCR) and Nucleotide sequencing. Sections of the intestines were also processed for histopathology. Light microscopy revealed the presence of *Ophidascarids* sp. eggs and *Cryptosporidium* oocysts. Amplification of the 18S rRNA gene and nucleotide sequencing confirmed *Cryptosporidium varanii* as the infecting species. Histopathology revealed cellular infiltration and disruption of the epithelial cells along the brush border characteristic of intestinal inflammation.

Key words: fire skink, *Cryptosporidium varanii*, *Lepidothyris fernandi*, reptiles

Background

Cryptosporidium infection is a zoonotic disease that affects a wide range of vertebrate hosts, including mammals, birds, fish and reptiles [1]. This apicomplexan protozoan parasite invades and multiplies in the gastrointestinal tracts of its hosts, thereby causing disruption of the integrity and function of the gastrointestinal tracts resulting in diarrhea [2], cryptosporidiosis is self-limiting in an infected host, but it is now known worldwide as an important opportunistic infection in children [3] and immuno-compromised humans with HIV infection [4]. Livestock, pets, rodents, reptiles and other animals are thought to be reservoirs or infection sources of *Cryptosporidium* species to humans [2].

Studies have been conducted on the role of reptiles in the epidemiology of cryptosporidiosis in several countries, with the isolation of *Cryptosporidium* oocysts from the faeces of some monitor lizards (*Varanus griseus*, *V. prasinus)* Schneider's skinks (*Eumeces schneideri*), adult skink (*Mabuya perrotetti*), frilled lizard (*Chiamydosaurus kingi*) and leopard geckos in Switzerland, Slovak Republic, Czech Republic, Egypt, Ghana, Australia and Czech Republic respectively [5–6], although, the infecting *Cryptosporidium* species in these reptiles were not determined. However, *Cryptosporidium serpentis* has also been reported in monitor lizards (*Varanus exanthematicus* and *Varanus niloticus*) and green iguana (*Iguana iguana*) in the USA [5], while *Cryptosporidium varanii* (syn. *C. saurophilum*) have also been isolated from leopard gecko (*Eublepharis macularius*) in Argentina [6].

Here we report a confirmed case of *Cryptosporidium varanii* infection in a fire skink, a pet reptile found in tropical West Africa [7].

Case presentation

On 5th November 2016, fresh faecal sample from a dead fire skink that was trapped in a neighbourhood at 7.452ºN, 3.895ºE in Ibadan, Nigeria was brought to the diagnostic unit of the Department of Veterinary Parasitology, University of Ibadan, for detection of parasites. Sections of the stomach and intestines were also sent for histopathology at the Department of Veterinary Pathology.

The faecal sample obtained was subjected to

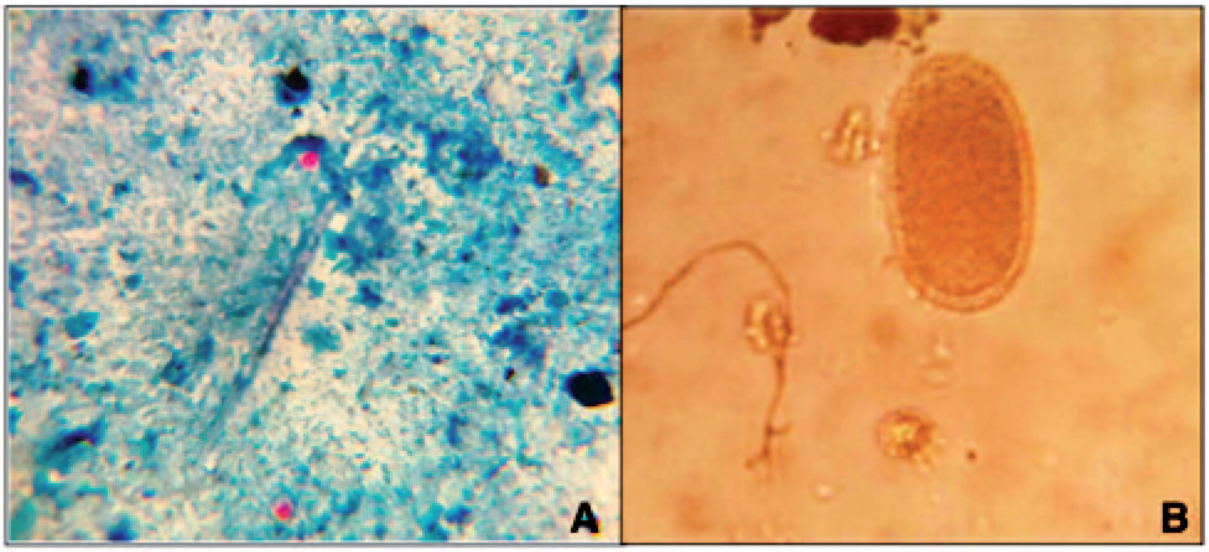

Fig. 1. Microscopic examination of processed faecal sample obtained from fire skink. A. Pinkish round oocysts characteristics of *Cryptosporidium* sp. with Ziehl-Neelsen acid fast stain (×1000). B. Egg of *Ophidascaris* sp. in unstained smear from faecal sedementation (×400).

faecal sedimentation and flotation technique to determine the presence of egg/larvae and oocysts of helminths and protozoa respectively [8]. *Cryptosporidium* oocysts were detected from faecal smear using Ziehl-Neelsen acid fast staining method [9]. Smears from sedimentation and Ziehl-Neelsen

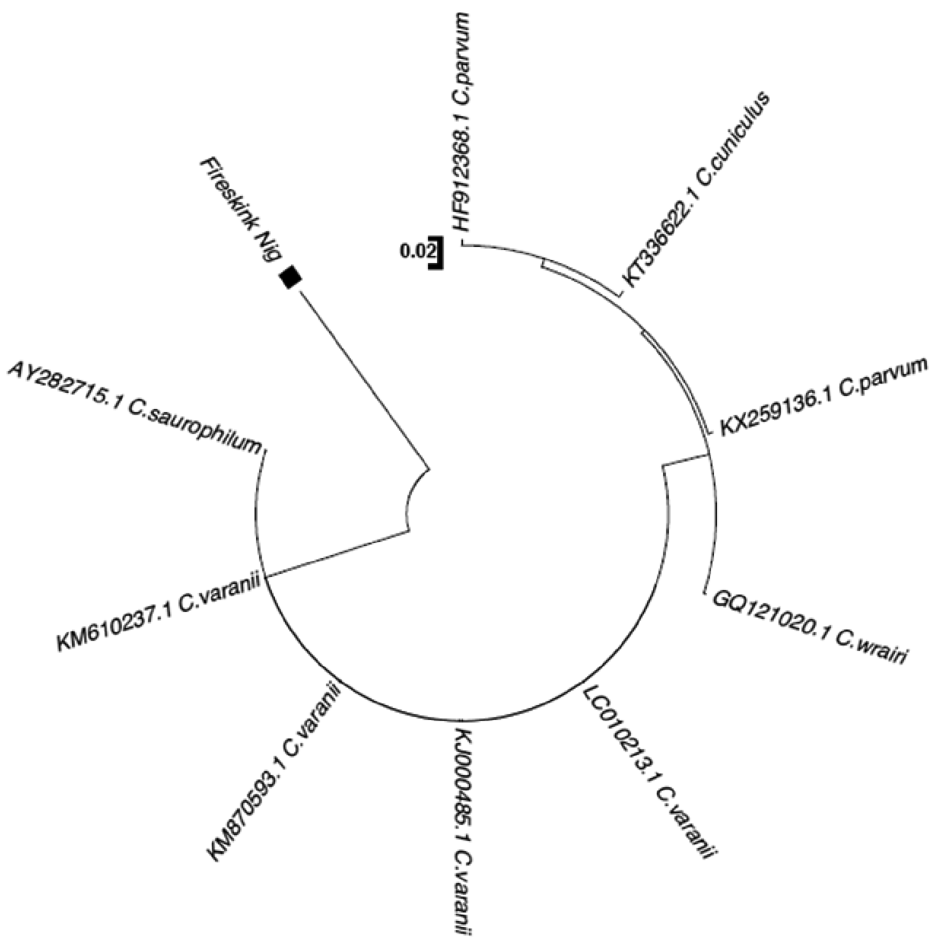

Fig. 2. Genetic relationship between *Cryptosporidium* isolate from the fire skink and those detected in reptiles inferred by a neighbor-joining analysis of the partial SSU rRNA gene sequences

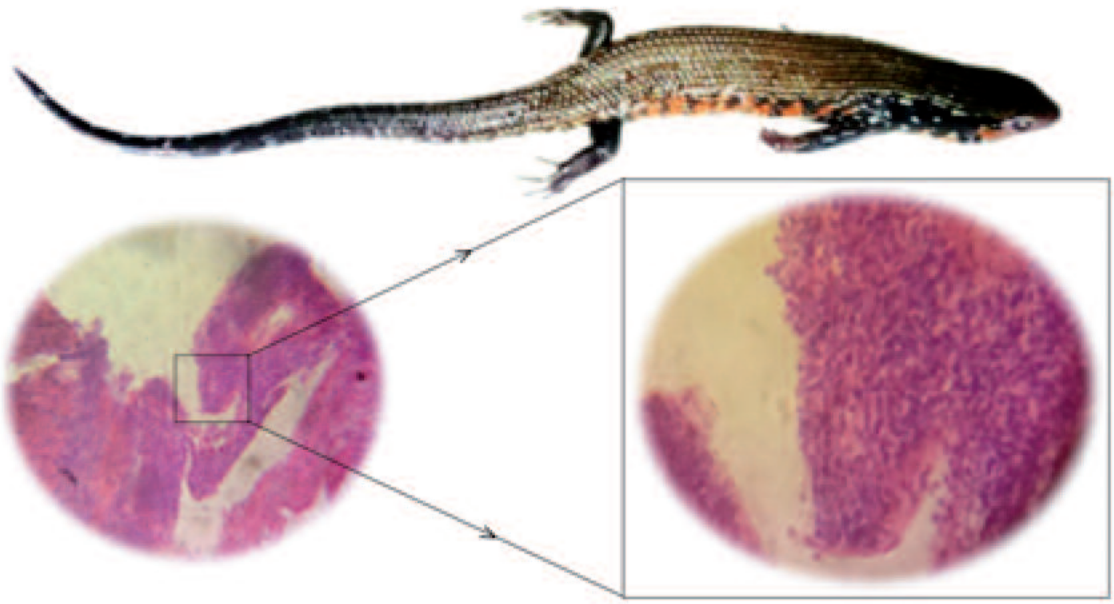

Fig. 3. The picture of the fire skink before post mortem (PM) and sections of the small intestine infected with *Cryptosporidium* showing altered mucosal architecture, with shortening, blunting, widening of the intestinal villi and meront at the brush border (haematoxylin and eosin ×1000).

method were examined under ×40 and ×100 objectives, respectively. Smears from sedimentation and Ziehl-Neelsen method were examined under ×40 and ×100 objectives, respectively while the magnification of the eyepiece was ×10.

Genomic DNA was extracted from the faecal sample using the ultra-pure® DNA Kit (Roche, Indianapolis, USA) according to the manufacturer's instruction. *Cryptosporidium* species was detected by the amplification of a 590 base pair fragment of the 18S rRNA gene using a nested PCR primer sets; 18SiCF2 (5'-GACATATCATTCAAGTTTCTGACC-3') and 18SiCR2 (5'-CTGAAGGAGTAAGGAACAACC-3'), followed by 18SiCF1 (5'-CCTATCAGCTTTAGACGGTAGG-3') and 18SiCR1 (5'-TCTAAGAATTTCACCTCTGACTG-3') as previously described [10]. The amplicons obtained were sequenced in both directions using the ABI Prism 3500 Genetic Analyser, the sequences obtained were aligned and analysed using program MEGA 5.2.2 (http://www.megasoftware.net) Consensus sequences obtained were compared with others published in GenBank by BLAST analysis.

The tissue specimens were dissected, fixed in 10% formalin, embedded in paraffin sections and stained with hematoxylin and eosin stain (H&E). The intestinal sections were examined for the detection of pathological changes.

Microscopic examination of sediments showed the presence of thick shelled *Ophidascaris* sp. egg with characteristic sub-globular shape and compact yolk in unstained wet mount, while faecal smear pink oval oocysts with Ziehl-Neelsen acid fast stain which is characteristic of *Cryptosporidium* species. (Fig. 1).

Nested PCR amplification of the 18S rRNA gene confirmed that sample was positive for *Cryptosporidium* sp. Nucleotide sequencing of the amplified gene confirmed the presence of *Cryptosporidium varanii* when compared with sequences *of C. varanii* (KM610237) from leopard geckos in Argentina [6] and *Cryptosporidium saurophilum* (AY282715) from Schneider's Skink in the Czech Republic [5] obtained in the GenBank (Fig. 2).

Histopathology of the small intestine revealed changes indicating the disruption of cellular architecture involving flattening of epithelial cells, the brush border, proliferation of gastric mucous cells and presence of ovoid cells suspected to be *Cryptosporidium* parasites at the luminal surface of the epithelium lining (Fig. 3).

Conclusions

Cryptosporidium infection has been reported in some breeds of skinks [5], lizards [6,11], snakes and other reptiles [11,12]. But this case to the best of our understanding is the first report of the infection in a fire skink in Nigeria. Although, the source of *Cryptosporidium* infection for the studied fire skink is not known, however, reptiles are thought to be infected through ingestion of contaminated objects as they feed on the ground. Ingested viable oocyst is thought to undergo both asexual and sexual lifecycle in the gastrointestinal tract of the reptile as in other mammals [11]. It has been reported that *Cryptosporidium* infection in reptiles is usually subclinical [11,12], though often symptomatic and fatal in snakes [11]. While it is not known if the presence of few oocysts could cause clinical illness in the fire skink, it is, however, worth acknowledging that the histological lesions are consistent with those found in reptiles infected with cryptosporidiosis [11,12].

Cryptosporidium varanii detected in this case has been suggested to be genetically identical at the 18S rRNA gene with the previously described *C. saurophilum* which has been isolated from leopard gecko, desert monitor lizards, green iguana and plated lizard [5,6]. Further studies are needed to investigate the clinical implications of *C. varanii (*syn. *C. saurophilum)* infection in the fire skink and also to determine their public health implications, more so, that there is yet to be confirmed report of potential zoonoses resulting from *C. saurophilum* infection [12]. Therefore, further investigations are required to understand the clinical and possible public health implications of cryptosporidiosis in the fire skink.

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