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Spirometra infection in a captive Samar cobra (*Naja samarensis*) in the United States: An imported case?

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ABSTRACT

We report a case of *Spirometra* infection in a Samar cobra (*Naja samarensis*) imported from the Philippines, belonging to a zoological collection in the southern United States. Under a poor post-surgical prognosis, the snake was euthanized, and at necropsy plerocercoids of a Diphyllobotriidea were found in its subcutaneous tissues and musculature. Molecular and phylogenetic analyses of the complete cytochrome oxidase c subunit I (*cox1*) gene of the mitochondrial DNA confirmed that the isolate belonged to the genus *Spirometra* and was closely related to *Spirometra mansoni* isolates from Asian countries (bootstrap support = 99.4%). Considering the origin and clinical history and handling of the animal, the snake probably arrived infected in America. We suggest the inclusion of diagnostic imaging in the investigation of sparganosis in research and disease surveillance protocols applied in the pre- and post-quarantine period to asymptomatic animals imported from endemic areas.

1. Introduction

The billion-dollar trade in wildlife, both legal and illegal, is one of the world's main obstacles to controlling the spread of exotic zoonotic pathogens, including helminths such as Diphyllobotriidea cestodes of the genus *Spirometra*. These intestinal cestodes infect mammals, amphibian, and reptile hosts, including snakes, among which the infection is commonly reported in specimens from Asian countries (Bezerra-Santos et al., 2021; Krishnasamy and Zavagli, 2020). In the USA, thousands of snakes have been imported by zoos in the last decades, mainly from Southeast Asia, potentiating the spread of zoonotic parasites (Rush et al., 2021; Sosnowski and Petrossian, 2020; Tow et al., 2021).

The life cycle of *Spirometra* begins with the elimination of eggs in the feces of the mammalian definitive hosts, mainly carnivores. The eggs embryonate and hatch in the aquatic environment, releasing ciliated coracidia, which are ingested by freshwater cyclopoid copepods. In the copepod, which are first intermediate hosts, coracidia develop into the procercoid larvae. The consumption of infected copepods by reptiles and amphibians, the second intermediate hosts, leads to the development of plerocercoid larvae in tissues, which are the infective stages to the mammal hosts. In intermediate or paratenic hosts (e. g., humans), plerocercoids can establish themselves in various organs and tissues, causing system-specific symptoms, a condition called sparganosis, which can lead to clinical disease or death (Mueller, 1938, 1974).

In a recent taxonomic review based on geographic distribution and molecular phylogeny, it was proposed to rearrange the species within the genus *Spirometra* into at least 6 distinct species, including *Spirometra mansoni*, *Spirometra folium*, *Spirometra erinaceieuropaei*, *Spirometra decipiens*, and an additional uncharacterized *Spirometra* species. (Kuchta et al., 2021). In the USA, *Spirometra* infections have been reported mainly in domestic carnivores, with cats (Hoggard et al., 2019; Mueller, 1935; Wyrosdick et al., 2017) being more frequently infected than dogs (Conboy, 2009; Drake et al., 2008; Nagamori et al., 2020; Sobotyk et al., 2021). It has also been reported from a variety of wild carnivore hosts, including raccoons (*Procyon lotor*) (Schaffer et al., 1981), gray foxes (*Urocyon cinereoargenteus*) (Conti, 1984), coyotes (*Canis latrans*) (Gompper et al., 2003), a lynx (*Lynx rufus*) (Harkema and Miller, 1964; Heidt et al., 1988; Kuchta et al., 2021), and a cougar (*Puma concolor*) (Foster et al., 2006).

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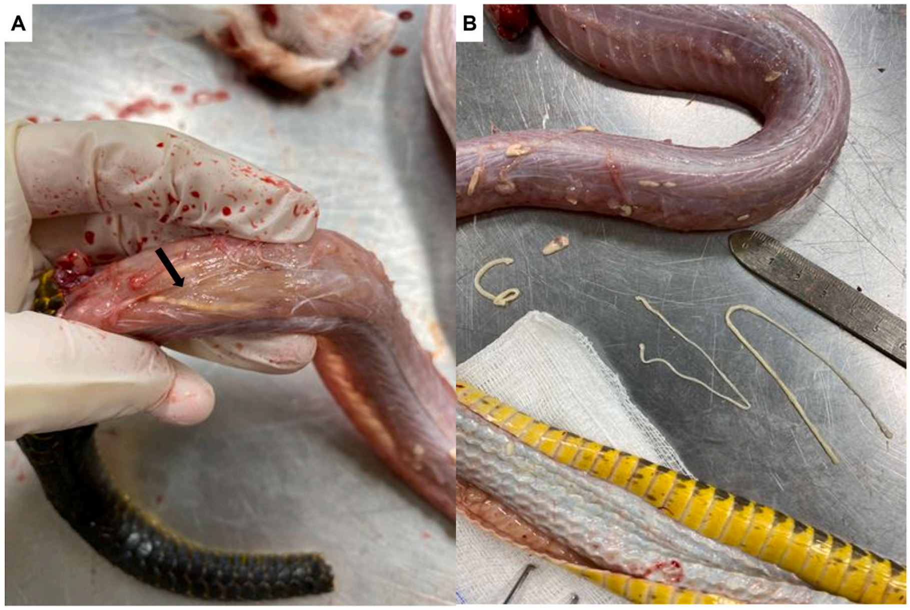

Fig. 1. A) Presence of *Spirometra* plerocercoid in subcutaneous tissue of a male Samar cobra (*Naja samarensis*) from a zoological facility in the USA; B) Plerocercoids isolated from de subcutaneous tissue of the same specimen.

Until recently, all *Spirometra* isolates circulating in the American continent are phylogenetically grouped into two genetic lineages, *S. decipiens* complex 1 and 2 (Kuchta et al., 2021). In USA snakes, there are reports of infection in the black rat snake (*Pantherophis obsoletus*) and eastern racer (*Coluber constrictor*) (Kuchta et al., 2021; Waeschenbach et al., 2017) serving as intermediate or paratenic hosts (McHale et al., 2020). However, *S. mansoni,* which is commonly found in the Old World, has been recently reported from a wild crab-eating fox (*Cerdocyon thous*) from Colombia, suggesting introduction to host species of the New World (Brabec et al., 2022).

We report a case of infection by *Spirometra* plerocercoids in an imported Samar cobra (*Naja samarensis*) from a zoological collection in the Texas, USA. Molecular analysis suggests that this isolate belongs to *S. mansoni,* which possibly was acquired prior to importation.

2. Materials and methods

2.1. Case

Plerocercoids of *Spirometra* sp. were collected from subcutaneous and skeletal muscles of a deceased 16-year-old, captive, male, Samar cobra (*Naja samarensis*) from a zoological collection in Texas, USA. The snake was purchased from a zoo in the Philippines and had been in the United States for 15 years. At the time of importation (2006), the animal was approximately 1 year old, and arrived in quarantine apparently healthy and in good nutritional body condition. Routine parasitological examinations detected cestode, coccidian, and oxyurid infections in 2006, 2008 and 2009. After this period, no enteric infections were detected in fecal samples. Between 2010 and 2020, palpable coelomic masses were observed, accompanied or not by inappetence and regurgitation, in addition to the presence of xanthoma in the tail. Subsequent radiographic examination indicated the presence of a mass in the splenopancreas region. In March 2021, the snake was submitted to an exploratory celiotomy, and was euthanized due to a poor prognosis and suspected poor quality of life following mass removal and recovery. The cobra was in good body and post-mortem conditions. Two small masses were present on the distal tail, and a coelomic mass was present in the mid coelomic cavity. Approximately 10 white flat endoparasites resembling plerocercoids were present in between the skin and the underlying musculature, up to 12 cm in length (Fig. 1). Approximately 30–50 white cysts were present, elliptical in shape, approximately 0.75 cm in length and 0.3 mm in width (Fig. 1). The carcass was incinerated.

2.2. Molecular analysis

Genomic DNA was extracted from a plerocercoid fragment using DNeasy Blood & Tissue Kit (Qiagen, CA, USA) according to the manufacturer's recommendations. To amplify the complete cytochrome oxidase c subunit I (*cox1*) gene of the mitochondrial DNA we utilized the primers Cox1Forward (5′-TATCAAATTAAGTTAAGTAGACTA-3′) and Cox1Reverse (5-CCAAATAGCATGATGCAAAAG-3′), according to Wicht et al. (2010). Nuclease-free water was used as negative control, and a fragment of an adult *Spirometra* sp. recovered from the feces of a cat treated at the Small Animal Veterinary Hospital of Texas A&M University, USA, was used as positive control. PCR products were purified using E.Z.N.A.® Cycle Pure Kit (Omega Bio-tek, USA), then sequenced in both directions using the original PCR primers in a 3730xl DNA Analyzer at Eurofins Genomics (Louisville, KY, United States). The positive control amplicon was also sequenced.

2.3. Phylogenetic analysis

Sequence alignment and condensed phylogenetic tree were constructed with the sequences available in the nucleotide sequence database at National Center of Biotechnology Information (NCBI) using MEGA X 10.1 software (Kumar et al., 2018). The phylogenetic analysis was inferred from the *cox1* gene sequence using the maximum-likelihood method with 1000 bootstrap (BS). The best fit nucleotide substitution model for the data set was Hasegawa-Kishino-Yano model using a discrete gamma distribution with 5 rate categories and by assuming that a certain fraction of sites is evolutionarily invariable (HKY + G + I).

3. Results

The complete *cox1* gene was amplified, confirming the presence of *Spirometra* sp. DNA in both the Samar cobra (1566 bp; OP093552), and a fragment amplified from domestic cat (983 bp; OP093553). BLAST search showed that the sequence obtained from the snake was most like *Spirometra* plerocercoid isolates from frogs in China, MK085779 (95.7%;

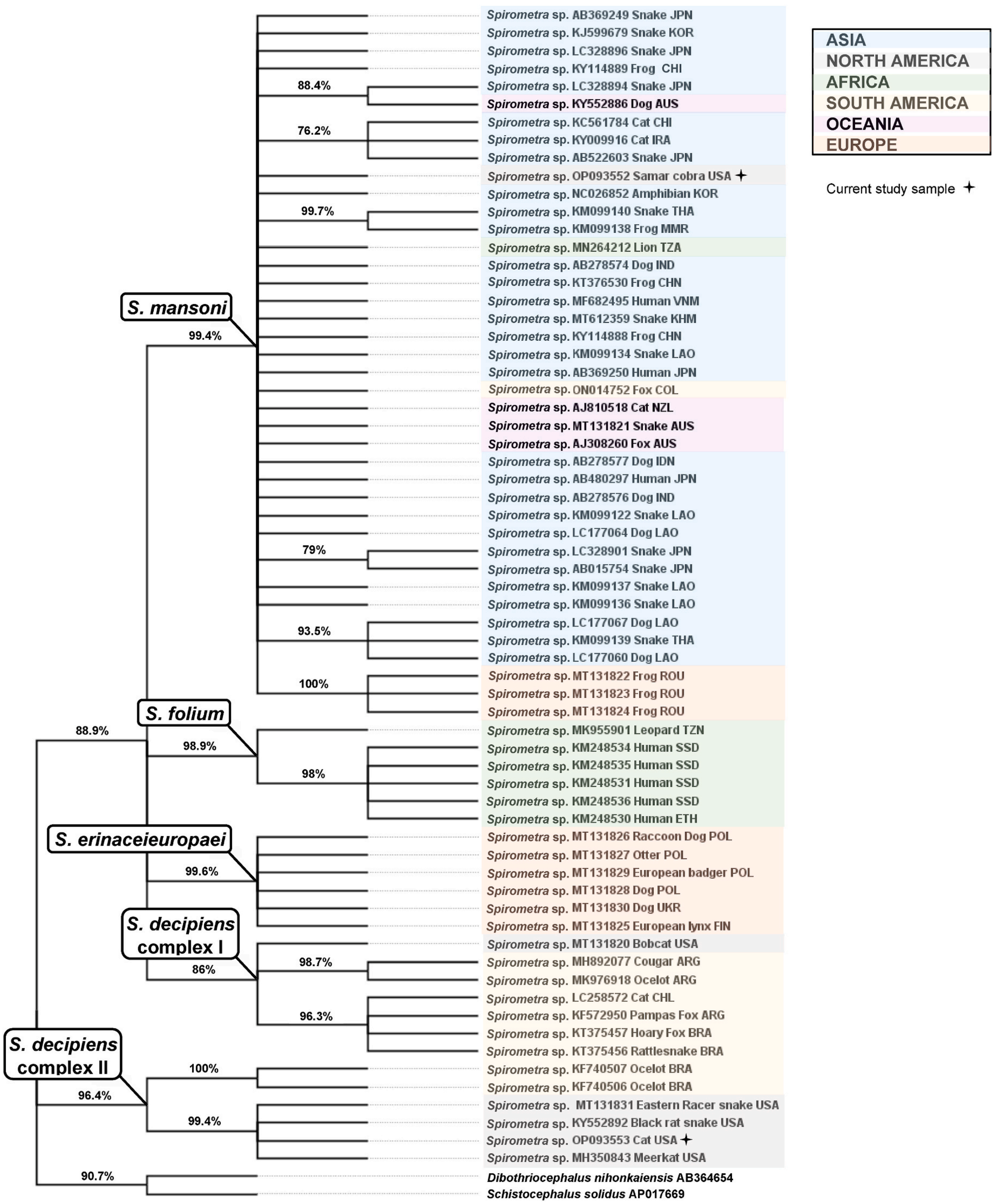

Fig. 2. Maximum likelihood tree constructed from partial *cox1* gene sequences of *Spirometra* samples and related taxa. HKY + G + I was used as the best substitution model. *Schistocephalus solidus* and *Dibothriocephalus nihonkaiensis* were used as outgroups.
(JPN – Japan; KOR – South Korea; CHI and CHN – China; AUS – Australia; IRA – Iran; USA – United States; THA – Thailand; MMR – Myanmar; TZA – Tanzania; IND – India; VNM – Vietnam; KHM – Cambodia; LAO – Laos; COL – Colombia; NZL – New Zealand; IDN – Indonesia; ROU – Romania; SSD – South Sudan; ETH – Ethiopia; POL – Poland; UKR – Ukraine; FIN – Finland; CHL – Chile; BRA – Brazil).

1566 bp) and KM605278 (95.6%; 1566 bp). On the other hand, the domestic cat sample was most like isolates from other snake hosts in the USA, KY55892 (99.6%; 555 bp) and MT131831 (99.2%; 1566 bp). In addition, pairwise analysis (Supplementary Table 1) showed that the Samar cobra sample, when compared to all other *S. mansoni* sequences with 1500 bp, had an average pairwise relationship of 92.3% (89.4–96.5%). The next closest *Spirometra* species was *S. folium* at 83.6% (82.2–84.6%).

Subsequent phylogenetic analysis (Fig. 2) revealed that the *Spirometra* from the Samar cobra has a close relationship with other *S. mansoni* species, with 99.4% BS support in its respective clade. In contrast, the sequence obtained from the domestic cat aligns most closely with the *S. decipiens* species complex 2 proposed by Kuchta et al. (2021), with 93% BS support. This clade comprises isolates from the USA but from different hosts including the Eastern racer snake (*Coluber constrictor*) (MT131831; 99%; 1566 bp) and meerkat (*Suricata suricatta*)

(MH350843; 409 bp), both found in southeastern states Mississippi and South Carolina, respectively.

4. Discussion

This seems to be the first report of *S. mansoni* from North America. This is strongly supported by our phylogenetic analysis, which demonstrated that the Samar cobra isolate is closely related to other *S. mansoni* from the Old World, including southeastern Asian countries, and is clearly distinct from other isolates previously reported in the USA (Kuchta et al., 2021; McHale et al., 2020; Waeschenbach et al., 2017). The presence of an exotic species of *Spirometra* in a snake in an American zoo brings to light the role of herpetofauna importation for Public Health, as a potential contributor to the dissemination of allochthonous pathogens in the USA.

In the USA, millions of wild animals have been imported legally or illegally in the last decades, mainly from Southeast Asia, with zoological collections as a common destination. Herptile import comprises a large portion of this market, most of which are traded as live species, enhancing the spread of invasive and zoonotic parasites (Rosen and Smith, 2010; Smith et al., 2017; Tow et al., 2021). The zoonotic transmission of *Spirometra* is dependent on the consumption of reptile intermediate or paratenic hosts by a susceptible carnivore or omnivore host. (Mendoza-Roldan et al., 2020). Fortunately, consumption of this captive Samar cobra would be unlikely in North America due to the fact it was maintained in captivity and properly disposed. However, our aim is to highlight that even legally imported animals could serve as a vehicle for introduction of zoonotic pathogens. It is necessary to keep in mind that, despite the implementation of quarantine in the legal introductory process of animals, there is a lack of specific measures and protocols for tracking and investigating the introduction of zoonotic pathogens, including preventive protocols for all actors involved in this market (Bezerra-Santos et al., 2021). This health perception deficit is well represented in a study by Rush et al. (2021) who investigated infections by zoonotic pathogens in illegally traded wild animals, demonstrating that the USA and Brazil received most zoonotic pathogens between 1990 and 2020.

Still within the aspect of pathogen globalization, we must emphasize the importance of evaluating the commercial profiles of the places of origin or acquisition of wild animals, and their level of commitment to the legal and sanitary requirements for legal wildlife trade and importation. The Samar cobra specimen was acquired from the Philippines, directly from the largest reptile collection in Southeast Asia, a hub for exporting snakes to several countries on all continents. According to Krishnasamy and Zavagli (2020), gaps in the implementation of environmental laws and the flawed enforcement of the laws perpetuate the inadequate procedures of the import and export legal processes, while disinhibiting illegal activities within the country.

As highlighted before, snakes are common second intermediate or paratenic hosts of *Spirometra* across Asian countries (Kuchta et al., 2021; Yamasaki et al., 2021).Undoubtedly, we should not rule out other transmission routes for this snake's infection that are intrinsic to its food management. However, the fact that the animal was fed rats and mice, usually frozen, and sometimes freshly killed, acquired from animal facilities suggests that this is not the most likely route of infection. This condition, added to the phylogenetic profile of the isolate, reinforces the suspicion that the snake arrived in the American territory already infected. On the other hand, the consumption of water contaminated with infected copepods, the primary hosts, may also be a possibility, if we consider the possible presence of definitive hosts in the zoo's enclosure, which may facilitate the contamination of water sources (Lee et al., 1990).

Apparently, the proliferative presence of *Spirometra* plerocercoids in the subcutaneous tissues and musculature did not cause clinical disease in the snake. In fact, we found no reports of clinical sparganosis of snakes in the scientific literature. Instead, studies focused on prevalence or molecular identification to understand a broader objective of host species conservation or parasite biogeographic distribution (Kuchta et al., 2021; Wang et al., 2011, 2014; Yamasaki et al., 2021). However, we note that there are reports of sparganosis resulting in severe illness or death in other vertebrates (Buergelt et al., 1984; Drake et al., 2008; Hwang et al., 2019; Kikuchi and Maruyama, 2020).

Asymptomatic parasitic diseases, regardless of their zoonotic potential can also lead to deleterious impacts to susceptible hosts and environment in which they are introduced. As often these may go undiagnosed, they represent a silent threat, and possibly favor the cosmopolitan spread of exotic diseases between humans and animals. With this context, we emphasize two important points for zoo/exotic clinical veterinarians and other professionals in the herptile industry to consider. Firstly, to define and implement the most effective disease surveillance protocols for the tracking of diseases in imported animals, including those apparently healthy. In this situation, we suggest the inclusion of diagnostic imaging, an underutilized tool, in the investigation of *Spirometra* infections, especially in specimens from endemic areas (Hwang et al., 2019; Kazemi et al., 2018; Mitchell, 2009). Secondly, we understand that sanitary strategies that prevent the establishment of exotic pathogens and the transmission of zoonotic diseases between humans and animals in the zoo environment must be rigorously studied and applied. Thus, we urge that this care should not be limited to the quarantine period, which should last at least two months (Mitchell, 2009), and use of incineration for the disposal of dead animals.

5. Conclusions

Our data support that the Samar cobra from a Texan zoo in the United States, imported from the Philippines, was infected with *Spirometra mansoni*, an exotic, zoonotic cestode.

Declarations of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2023.02.001.

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