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Spirometra erinaceieuropaei severely infect frogs and snakes from food markets in Guangdong, China: implications a highly risk for zoonotic sparganosis

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Abstract. Sparganosis is a parasitic disease caused by plerocercoid larvae of the genus *Spirometra*. In China, the main source of sparganosis is from Guangdong, 16.1% of the country's human sparganosis cases occur in this province. Frequent international trade of amphibians and reptiles in Guangdong may introduce new species of *Spirometra* into the local market. In this study, a large-scale, high-intensity sampling survey was conducted to find out the causative species and epidemic situation of Sparganosis in Guangdong. The prevalence of sparganum infection in five species of frogs (*Boulengerana guentheri*, *Fejervarya multistriata*, *Hoplobatrachus chinensis*, *Pelophylax nigromaculatus* and *Quasipaa spinosa*) and nine species of snakes (*Elaphe carinata*, *Lycodon rufozonatum*, *Hypsiscopus plumbea*, *Ptyas dhumnades*, *P. korros*, *P. mucosa*, *Naja atra*, *Sinonatrix annularis* and *Xenochrophis piscator*) was investigated in Guangdong, Southern China from May 2014 to August 2015. The results showed that 9.8% (50/511) of the frogs and 40.8% (141/346) of snakes were found to be infected by plerocercoids (spargana). To identify the species of the collected spargana, a partial sequence of the mitochondrial cytochrome *c* oxidase subunit1 gene (*cox1*) was amplified and sequenced. Phylogenetic analysis identified all the spargana specimens as *Spirometra erinaceieuropaei*. Our study indicated that *S. erinaceieuropaei*, a highly pathogenic parasite, is the only causative agent of sparganosis in Guangdong, China. This study suggests that the large numbers of frogs and snakes in food markets in Guangdong may impact public health in China by transmitting *S. erinaceieuropaei* sparganum. Additional steps should be considered by the governments and public health agencies to prevent the risk of food-associated *Spirometra* infections in humans in China.

INTRODUCTION

Sparganosis is a parasitic disease caused by plerocercoid larvae of the genus *Spirometra*. There are three intermediate hosts in the life cycle of *Spirometra*. The first is small crustaceans (*Cyclops* genus), the second consists primarily of tadpoles and frogs, and carnivores such as dogs and cats are the final hosts (Yin *et al.*, 2006). Other vertebrates, includes birds and snakes that can also become infected with *Spirometra* by participating in the food chain, and can infect humans (Magnino *et al.*, 2009). In China, human sparganosis has been reported in 25 provinces, and Guangdong, is the main source of sparganosis, and human sparganosis cases account for 16.1% of the total in China (Lu *et al.*, 2014; Liu *et al.*, 2015). The high prevalence of sparganosis in Guangdong may be related to the consumption of frogs and snakes in the local diet (Xu *et al.*, 2007; Meng *et al.*, 2009; Wang *et al.*, 2014).

Several species of the genus *Spirometra* can cause the disease, including *S. erinaceieuropaei*, *S. mansonoides*, *S. proliferum* and *S. ranarum* (Gray *et al.*, 1999; Zhu *et al.*, 2002; Lescano *et al.*, 2013). Of these, *S. erinaceieuropaei* and *S. mansonoides* are the most common causative agents, with *S. erinaceieuropaei* mainly found in East and South Asia, while *S. mansonoides* primarily occur in North America (Anantaphruti *et al.*, 2011). In previous studies, the prevalence of spargana was surveyed; in small-scale investigations in some parts of Guangdong Province that showed that the most common species was *S. erinaceieuropaei*, but proper species identification was not done (Wang *et al.*, 2011; Wang *et al.*, 2014). Guangdong is one of the hotspots for international trade for wild animals (Li *et al.*, 2004). The International trade of amphibians and reptiles is frequently done in Guangdong (Li *et al.*, 2004; Gong *et al.*, 2009), so it is possible to introduce new species of *Spirometra* into the local market. In this study, a large-scale and high-intensity sampling survey was conducted to determine the causative agents (species) and the epidemic situation of sparganosis in Guangdong.

Due to similar characteristics of the parasites makes it difficult to identify the species morphologically. Fortunately, "DNA barcoding" as a rapid and accurate method for species identification, has been used in many animals. Mitochondrial cytochrome *c* oxidase subunit1 (*cox1*) is a "DNA barcoding" gene, which is a powerful marker for species identification. With regards to species causing sparganosis in Guangdong, no molecular identification has been done thus far. Detailed investigations and accurate identification of the parasite species will be helpful in developing targeted prevention and control measures for this disease. In this study, we investigated the prevalence of sparganum infection in five frog species and nine snake species, which are the most common species sold in the food market in Guangdong of Southern China, and we identified the parasite species using molecular method.

MATERIALS AND METHODS

Spargana investigation

From May 2014 to August 2015, frogs and snakes were collected from the major food markets in Guangdong, China, including northern Guangdong (Shaoguan), western Guangdong (Zhaoqing), eastern and central Guangdong (Guangzhou, Huizhou and Shenzhen). Host samples included five frog species (*Boulengerana guentheri*, *Fejervarya multistriata*, *Hoplobatrachus chinensis*, *Pelophylax nigromaculatus* and *Quasipaa spinosa*) and nine snake species (*Elaphe carinata*, *Lycodon rufozonatum*, *Hypsiscopus plumbea*, *Ptyas dhumnades*, *P. korros*, *P. mucosa*, *Naja atra*, *Sinonatrix annularis* and *Xenochrophis piscator*). The presence of spargana in frogs and snakes was determined according to the methods of Wang (Wang *et al.*, 2014). Briefly, the frogs and snakes were euthanized using ethyl-ether anesthesia, weighed, and skinned. The muscles and subcutaneous tissues were visually assessed for the presence of spargana. Then, the spargana were removed from the muscles or subcutaneous tissues and put in a Petri dish containing physiological saline to observe for movement.

Species identification

The spargana were observed under a microscope and then preliminarily identified as belonging to the genus *Spirometra* according to their morphological features. Further identification was then conducted using a molecular marker. The total genomic DNA was extracted from each individual using the phenol-chloroform extraction method. Partial sequences of the *cox1* gene were amplified using primers pr-a (5'-TGGTTTTTTGTGCATCCTGAGGTTTA-3') and pr-b (5'-AGAAAGAACGTAATGAAAATGAGCAAC-3') (Bessho *et al.*, 1992). The PCR products were sequenced from both ends and phylogenetic analysis was conducted in order to identify the species of these samples.

RESULTS

A total of 511 frogs and 346 snakes were collected from northern Guangdong (Shaoguan), western Guangdong (Zhaoqing), eastern and central Guangdong (Guangzhou, Huizhou and Shenzhen) (Table 1). *Spirometra* spargana were found in 9.8% (50/511) of the frogs and 40.8% (141/346) of the snakes, respectively (Fig. 1). These spargana were 1–30 cm long and 1–2.5 mm wide. The prevalence of sparganum infection ranged from 2.9% to 93.8%.

Most host samples were infected by several spargana, and 1-3 sparganum was selected from each host sample. In most cases, the spargana from the same host species were mixed into one parasite sample. Finally, a total of 17 parasite samples were used for molecular identification. Partial sequences of the *cox1* gene were successfully amplified and sequenced for each sample. Phylogenetic analysis identified all specimens as belonging to the species *S. erinaceieuropaei*. This study therefore indicated that *S. erinaceieuropaei* is the major causative agent of sparganosis in Guangdong, China.

DISCUSSION

Spargana of *Spirometra* can parasitize humans and result in sparganosis, which is an important foodborne parasitic zoonosis. Sparganosis mainly occurs in east and south Asia, but has been reported in 39 countries worldwide (Li *et al.*, 2011), including Europe, America, Africa and Australia (Wu *et al.*, 2005).

There are several species in the genus *Spirometra* that can cause the disease, and the spargana of these species are often found in frogs and snakes. In previous studies in Guangdong, the prevalence of spargana was surveyed, but accurate species identification work was not done (Wang *et al.*, 2011; Wang *et al.*, 2014). The present study accurately identified the spargana from frogs and snakes in Guangdong that showed that *S. erinaceieuropaei* is the major causative agent of sparganosis in this area. There have been more than 1,700 global reports of sparganosis to date (Liu *et al.*, 2015). Sparganosis caused by *S. erinaceieuropaei* is dominant in South and East Asia, so our results are consistent with previous studies.

Table 1. S*pirometra* and host samples from food markets in Guangdong Province, China

Host Species	Source of samples	Infected host sample/ Host sample size	Prevalence (%)
Boulengerana guentheri	Shaoguan, Zhaoqing	4/112	3.6
Fejervarya multistriata	Zhaoqing	3/97	3.1
Hoplobatrachus chinensis	Shaoguan, Zhaoqing	26/99	26.3
Pelophylax nigromaculatus	Shaoguan	14/101	13.9
Quasipaa spinosa	Shaoguan	3/102	2.9
In total (frogs)		50/511	9.8
Elaphe carinata	Guangzhou	16/25	64.0
Lycodon rufozonatum	Huizhou	15/30	50.0
Hypsiscopus plumbea	Huizhou	7/49	14.3
Ptyas dhumnades	Guangzhou	30/32	93.8
Ptyas korros	Guangzhou	12/30	40.0
Ptyas mucosa	Guangzhou, Shenzhen	40/50	80.0
Naja atra	Guangzhou, Shenzhen	7/50	14.0
Sinonatrix annularis	Huizhou	12/30	40.0
Xenochrophis piscator	Zhaoqing	2/50	4.0
In total (snakes)		141/346	40.8

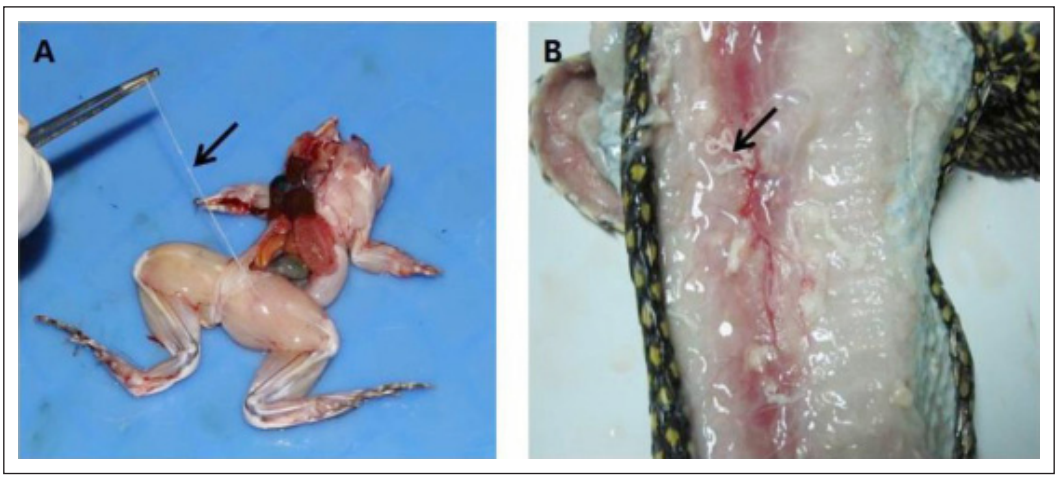

Figure 1. *Spirometra* spargana in frogs (A) and snakes (B).

Based on the results of this study, we propose the following control measures for sparganosis: 1) Periodic inspection of *S. erinaceieuropaei* infection in frogs and snakes in markets and farms is necessary; 2) Scientific propaganda should be carried out to inform the public not to eat wild-caught snakes and frogs; 3) Specific drugs for *S. erinaceieuropaei* should be developed and injected to human and animals infected with the disease.

CONCLUSIONS

In summary, the frogs and snakes sold at the food markets in Guangdong were severely infected by spargana. All the spargana specimens were identified as *S. erinaceieuropaei*. Our study indicated that eating frogs and snakes pose a great health risk and improper cooking methods may increase the risk of infection. The result of this study is helpful in developing targeted prevention and control measures for sparganosis.

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