DOI 10.2478/s11687-014-0223-x

HELMINTHOLOGIA, 51, 2: 158 – 161, 2014

Research Note

Serological survey for sparganum infection in people of central China

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Summary

During 2006 – 2008, twenty cases with sparganosis caused by eating live tadpoles emerged in Henan province, central China. To determine seroprevalence of anti-sparganum antibodies and obtain information about habits of eating live tadpoles and risks for sparganum infection, a serological survey was carried out in one village of Henan. Anti-sparganum IgG in 298 serum samples were assayed by ELISA using excretory and secretory (ES) antigens of *Spirometra mansoni* spargana. The results showed 56.71 % (169/298) of inhabitants had the history of eating live tadpoles. The overall seroprevalence was 5.7 % (17/298). The seroprevalence of the inhabitants who had the habit of eating tadpoles (9.47 %) was obviously higher than those who did not (0.78 %) ($P < 0.01$). Eating live tadpoles had become the most common risk behavior for sparganum infection. Hence, the comprehensive public health education should be carried out in endemic areas, and the habit of eating live tadpoles must be discouraged.

Keywords: Sparganosis; prevalence; ELISA; tadpole; Central China

Introduction

Sparganosis is a zoonotic disease caused by infection with spargana, the plerocercoid larvae of various diphyllobothroid tapeworms belonging to the genus *Spirometra* (Nithiuthai *et al*., 2004). The most important species of the genus *Spirometra* with plerocercoids that can produce human sparganosis include *S. mansoni* (syn. *S. erinaceieuropae* or *S. erinacei*) and *S. mansonoides*. The former is most frequently found in Asia, and the later is mainly distributed in North America (Holodniy *et al*., 1991). Adult worms dwell in the intestine of carnivores such as cats and dogs (final hosts). The freshwater copepods (cyclops) and frogs (or tadpoles) are respectively the first and second intermediate hosts of *Spirometra* spp., Snakes, pigs, and mice become infected as paratenic hosts by ingesting procercoids in cyclops or plerocercoids in frogs or tadpoles (Fukushima & Yamane, 1999). Human is an accidental host. Sparganosis poses a serious threat to human health. Clinically, one or more lesions are found in different parts of the body. The larvae usually lodge in the subcutaneous tissues and sometimes invade the abdominal cavity, eye and central nervous system causing blindness, epilepsy, paralysis, and even death (Kim *et al*., 1996; Wiwanitkit, 2005).

Although human sparganosis is cosmopolitan, it is more frequently found in East and Southeast Asia. In China, sparganosis is an important zoonosis, with more than 1000 human cases reported in 27 out of 34 provinces, autonomous regions, or municipal districts during 1927–2009, most of cases were in the southern China, where humans infection were mainly acquired by eating raw or insufficiently cooked meat of frogs and snakes, and by placing frog or snake flesh on open wound for treatment of skin ulcers or on eyes to treat inflammation (Qiu & Qiu, 2009). Sparganosis is rarely seen in central and northern China; only three imported cases from southern China were reported in Henan province in central China before 2006 (Wang, 1996). However, 20 autochthonous cases caused by ingesting live tadpoles emerged in Henan since 2006, and are often neglected and misdiagnosed. Our previous studies showed that the plerocercoids were found in 11.93 % (163/1,366) of frog tadpoles in Henan, and the adult worms and eggs recovered from the cat experimentally infected with plerocercoids from tadpoles were morphologically identified as that of *S. mansoni* (Cui *et al*., 2011a). The present study was therefore carried out to determine seroprevalence of anti-sparganum antibodies in one village of Henan province, central China, and obtain information about habits of eating live tadpoles and risks for sparganum infection.

Materials and methods

Study Field

The study was conducted in Kangwa village at northeast of Luohe city of Henan province, with a total population of 400, because sparganosis cases had been reported from the local communal medical centre. Of the total population, 298 inhabitants were involved in this study. Their age ranged from 5 to 76 years. In the village, there are a number of rivers, ponds and ditches in which some wild frogs (mainly *Rana limmocharis* and *R. nigromaculata*) live. The local temperature, humidity, and plants are suitable for multiplying of frogs. Stray dogs and cats are often found in the village.

Questionnaire investigation

People, 298, were interviewed about their life style habits. Major content of the questionnaire included eating live tadpoles, eating raw meat of frogs or snakes, drinking raw water in rivers or ponds, using raw flesh of frog or snake as poultices. The clinical manifestations of sparganosis (such as fever, eruption, abdominal pain, subcutaneous nodules) were also investigated.

Collection of serum samples

The serum samples of 298 inhabitants were collected, and name, sex and age were recorded.

Positive control sera were obtained from 20 patients with sparganosis confirmed by finding the plerocerocoids in biopsy of the subcutaneous nodule. Negative control sera were obtained from 22 healthy persons who had no parasite eggs in their stools and no serum antibodies for cysticercosis, echinococcosis, paragonimiosis, clonorchiosis, and schistosomiasis. All of the serum samples had been stored at -80°C until used.

Detection of anti-sparganum antibodies in serum samples

Anti-sparganum IgG were detected by ELISA using excretory and secretory (ES) antigens of *Spirometra mansoni* spargana, as previously described (Cui *et al*., 2011b). In brief, 96-well ELISA plates (Corning, USA) were coated with 2.5 μg of ES antigens in 100 μl of bicarbonate buffer (pH 9.6) overnight at 4 ºC, were washed three times with 0.1 % Tween-20 in PBS (PBS-T), and blocked in 3 % skimmed milk in PBS-T for 2 h at 37 ºC. After washing three times, the following reagents were sequentially added and incubated for 1 h at 37 ºC: (1) sera diluted at 1:100 in PBS-T, and (2) HRP-conjugated antihuman IgG (Sigma, USA) diluted at 1:5,000. After the final wash, color was developed by incubating with a 50-μl aliquot of orthophenylene diamine (5 mg/10 ml of citrate–phosphate buffer) and 5 μl of 30 % H_2O_2 for 30 min. The reaction was stopped by adding 50 μl of 2M H_2SO_4. Optical density at 492 nm was measured with a microplate reader (TECAN, Austria). All samples were run in duplicate. The mean OD values of positive and negative controls were 0.60 and 0.23, respectively. The ratio <2.1 of samples to be tested/negative sample (OD values of the samples to be tested divided by OD of the negative, S/N < 2.1) were regarded as negative and S/N ≥2.1 as positive (Liu *et al*., 2013). The cut-off values of ELISA were 0.48. The specificity and sensitivity of ELISA used in this study was 100 % and 97 %, respectively.

Statistical analysis

All statistical analyses of data were done with SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL). The seroprevalence in different populations was compared with Chi-square test. All statistical tests were considered significant at P value of 0.05.

Results

The results of epidemiological investigations showed that 56.71 % (169/298) of inhabitants had the history of eating live tadpoles. Out of 298 inhabitants, 19 had the history of drinking raw water in rivers or ponds. All 298 persons did not have the past history of eating raw or undercooked meat of frogs and snakes, or using raw flesh of frog or snake as poultices.

The results of serological test were shown in Table 1. The overall seroprevalence was 5.70 % (17/298). The seroprevalence of the inhabitants who had history of eating tadpoles (9.47 %) was obviously higher than those who did not (0.78 %) ($\chi^2 = 10.276$, $P < 0.01$). The seroprevalence between males and females was not statistically different (4.82 % vs. 6.82 %, respectively; $\chi^2 = 0.546$, $P > 0.05$). The seroprevalence between school-age children (5 – 18 years) and adults (>18 years) was also not statistically different (6.36 % vs. 5.00 %, respectively; $\chi^2 = 0.239$, $P > 0.05$).

Out of 17 seropositive persons, 13 cases had different clinical manifestations of sparganosis, such as fever (13 cases), eruption (5 cases), abdominal pain (5 cases), subcutaneous nodules (13 cases), and eosinophilia (13 cases). Biopsy of the subcutaneous nodule was performed with the permission of 3 patients and the larvae were found in all of the 3 patients. The larvae removed from lesions were still alive; they were wrinkled, whitish and ribbon-shaped worms, and continuously moved while in normal saline. These larvae were 12 – 54 cm long, 1 – 2.5 mm wide, and morphologically identified as plerocercoids. Their scoles was finger shaped and had dorsal and ventral bothria (Roberts *et al*., 2009).

Discussion

Human sparganosis caused by eating raw or undercooked meat, or using raw flesh as poultices is usually found in parts of the Orient, especially in southern China. For example, the persons in Guangdong province enjoy eating meat of wild frog and snake, many frogs and snakes have been sold in the market (Li *et al*., 2009). The mode of human sparganum infection in Henan province was different from that in southern China, since the inhabitants in Henan province did not have the traditional habit of eating

Table 1. Seroprevalence of anti-sparganum antibodies by ELISA in 298 inhabitants of Kangwa village of Henan province, central China

	Class	No. of tested	No. of positive	% positive
Gender	Male	166	8	4.82
	Female	132	9	6.82
Age (years)	5 – 18	173	11	6.36
	>18	125	6	4.80
Eating live tadpoles	Yes	169	16	9.47
	No	129	1	0.78
Eating raw meat	Yes	0	0	0
	No	298	17	5.70
Drinking raw water	Yes	19	1	5.26
	No	279	16	5.73
Using raw flesh as poultices	Yes	0	0	0
	No	298	17	5.70
Clinical manifestations of sparganosis	Yes	13	13	100.00
	No	285	4	1.40

raw or undercooked flesh of frogs or snakes and using raw flesh as poultices. Although some inhabitants in cities of Henan eat flesh of frogs or snakes, it is eaten cooked. However, in recent years, eating live tadpoles have become a self-treatment method in some rural areas of Henan since some villagers believe the live tadpoles to have the medicinal role for treating diseases. Our results showed that 56.71 % (169/298) of inhabitants had the history of eating live tadpoles. The seroprevalence of the inhabitants who had the habit of ingesting live tadpoles (9.47 %) was significantly higher than those who did not (0.79 %) ($P < 0.01$). Hence, eating live tadpoles had become the most common risk behavior for sparganum infection in humans.

The specific anti-sparganum antibodies were detected in one serum samples of 129 inhabitants without the history of eating live tadpoles. By further inquiring, the inhabitant with seropositivity had the habit of drinking untreated raw water from rivers or ponds, which may be the cause of sparganum infection. The drinking of untreated water, which can contained the infected cyclops, was also risk behavior (Norman *et al*., 1980). The problem of impure water can be seen in remote rural areas where running water is not available. Although this problem has been seen in the USA in the past (Taylor, 1976), it still occurs in China and Thailand today. In Henan, procercoids were microscopically found in the hemocele of 3.53 % (3/85) of cyclops (Cui *et al*., 2011a). Thus, drinking untreated raw water also poses a small risk for sparganosis.

The clinical manifestations of sparganosis in Henna province were different from that in southern China and in Thailand, where the patients usually had only one local lesion (mainly the ocular, oromaxillo-facial or subcutaneous lesions), because the local inhabitants had the unusual practice of placing poultices of frog or snake flesh on gum or eye to treat toothache or inflammation (Anantaphruti *et al*., 2011). For example, 50 % (17/34) of patients with sparganosis had ocular lesions in Thailand (Wiwanitkit, 2005). In southern China, 44.74 % (217/488) and 21.03 % (102/488) of patients had ocular and oromaxillo-facial lesions, respectively (Liu, 1990). Nevertheless, the patients in Henan usually had the multiple lesions and general symptoms and signs, the prominent clinical manifestations were fever, abdominal pain, eruption, subcutaneous nodules, and eosinophilia. The different manifestations may be related to the mode and severity of infection. An investigation found that the plerocercoids (1 – 13 cm long and 1 – 2.5 mm wide) in frogs are usually larger than that (1 – 8 mm long and 0.2 – 0.5 mm wide) in tadpoles (Cui *et al*., 2011a). The plerocercoids in frogs and snakes were often seen by naked eye; the larvae can be picked up and discarded during processing of flesh of frogs and snakes or before using as poultices. Thus, the patients in southern China usually get the light infection, and have local manifestations; the patients in Henan often get the severe infection and have general manifestations. So, sparganosis is an emerging zoonosis with an important health impact in Henan province.

To prevent human infection with spargana, the government, public health officials and medical practitioners should be aware of this zoonotic sparganosis. The comprehensive public health education should be carried out; the people in endemic areas must be advised of the risk of eating live tadpoles for sparganum infection and the habit must be discouraged.

Acknowledgments

The study was financially supported by grants from National Natural Science Foundation of China (No. 81172612) and Henan Medical Science & Technology (No. 200903151 and No. 201003006).

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RECEIVED JANUARY 17, 2014

ACCEPTED APRIL 22, 2014