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Prevalence of Paragonimus and *Angiostrongylus cantonensis* Infections in Snails in Southeastern China

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Abstract: Paragonimiasis and angiostrongyliasis are important food-borne parasitic diseases in a number of countries including China. Both diseases are transmitted by freshwater and terrestrial intermediate snail hosts. In the present investigation, we examined the prevalence of Paragonimus and *Angiostrongylus cantonensis* in the intermediate host snails in southeastern China's Fujian province where paragonimiasis and angiostrongyliasis are endemic. The prevalence of *P. westermani* cercariae in the stream-type snail *Semisulcospira libertine* found in the breeding grounds in Shouning county was 0.09% in the upstream and 0.56% in the downstream, respectively. For pit-ditch type, the prevalence of *P. westermani* cercariae was 0.19% and 0.82% in upstream and downstream, respectively. The snail *Tricula xiaoqiaoensis* was infected with *Paragonimus skrjabini* cercariae with prevalence of 0.33% in the pit-ditch type and 1.52% in the seepage type in Zhenghe county. Prevalence of *A. cantonensis* larvae in *Achatina fulica* in Nan'an county in garbage stack, vegetable plot and crop land were 83.54, 39.53 and 19.23%, respectively. In Lianjiang county, prevalence of *A. cantonensis* larvae in snails *Pila polita* in trench, paddy field, residential ditch, vegetable irrigation ditch and pond in the same river system were 4.08, 8.82, 75.34, 34.04 and 5.56%, respectively. The snail *Bellamya lithophaga* found in the western suburb of Fuzhou city was identified as the new intermediated host for *A. cantonensis* with a prevalence of 14.38%. Demonstration of prevalence of Paragonimus and *A. cantonensis* in wild snails in Fujian province poses substantial risk for future outbreaks of the two food-borne parasitic diseases.

Key words: Paragonimiasis, angiostrongyliasis, *Semisulcospira libertine*, *Tricula xiaoqiaoensis*, *Paragonimus skrjabini*, *Achatina fulica*, *Bellamya lithophaga*

INTRODUCTION

Paragonimiasis and angiostrongyliasis are important food-borne parasitic diseases in a number of countries including China. Paragonimus infection in human can cause pulmonary, neurologic and abdominal diseases (Velez *et al*., 2003; Liu *et al*., 2008; Yahiro *et al*., 2008; Kim *et al*., 2009; Lane *et al*., 2009; Chen *et al*., 2010; Diao *et al*., 2010; He *et al*., 2009) while symptom of *Angiostrongylus cantonensis* infection in human includes eosinophilic meningitis (Sawabe and Makiya, 1995; Lindo *et al*., 2002; Wang *et al*., 2005; Hochberg *et al*., 2007; Zhang *et al*., 2008). Interestingly, both of the parasites are transmitted by freshwater and terrestrial intermediate snail hosts. With the increase of people's living standards and the pursuit of exotic and delicate foods, both paragonimosis and angiostrongyliasis are emerging as important foodborne parasitic zoonoses (Waugh *et al*., 2005; Lv *et al*., 2008; Sohn *et al*., 2009), causing public health concern worldwide.

Fujian province is situated in the southeast coast of China and has a subtropical humid monsoon climate. The natural eco-environment there is well suitable for breeding and multiplying of various parasites and their intermediate/vector hosts. To assess the risk for snail hosts with infection of Paragonimus and *A. cantonensis*

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in this province and to strengthen public food safety awareness, we conducted a comprehensive investigation of Paragonimus and *A. cantonensis* infection in their intermediate snail hosts following standard procedures (Kim *et al*., 2009; Lindo *et al*., 2002; Zhang *et al*., 2008).

MATERIALS AND METHODS

Snail samples: Both freshwater snails and terrestrial snails were shown in Table 1 and 2 of the investigation. The on the spot snail samples were collected to study the relationship between different types of ecological environments and different snail hosts for which the geography was marked by GPS.

Testing methods: All specimens were taken back to the lab. The shells of *Semisulcospira libertine* and *Tricula xiaoqiaoensis* were broken and the livers were taken out to detected Paragonimus cercariae under microscope. Shells of *Achatina fulica*, *Pila polita* and Bellamya were removed to smash the muscle and viscera individually. After washing by tissue homogenization, the filtrate was placed in the refrigerator (4°C) for about 30 min. Then, the precipitation in lower layer was removed out to detected *Angiostrongylus cantonensis* larvae under microscope and the infection rate were observed as well as the infection degree. Statistical analysis on data diversity about infection rate and infection degree was conducted among different mini breeding grounds in the same investigation spot for one species of snail. The relationships between snail host infection and eco-environment of Paragonimus and *Angiostrongylus cantonensis* were analyzed. Of 80 *Angiostrongylus cantonensis* third stage larvae were isolated from *Bellamya lithophaga* and put into abdominal cavities of 2 rats. At the same time, 500,000 of penicillin were also injected to prevent bacterial infection. Since, day 35, *Angiostrongylus cantonensis* first stage larvae in rats' feces have been checked day by day. Rats were provided by experimental animal yard of Fujian province.

Table 1: Detection results of snail hosts infected with Paragonimus in different breeding grounds

Breeding ground	Snail species	Detected no.	Infected no.	Infection rate (%)
Stream (Upstream)	*Semisulcospira libertine*	1,082	1	0.09
Stream (Downstream)	*Semisulcospira libertine*	892	5	0.56
Pit-ditch (Upstream)	*Semisulcospira libertine*	1,035	2	0.19
Pit-ditch (Downstream)	*Semisulcospira libertine*	1,219	10	0.82
Pit ditch	*Tricula xiaoqiaoensis*	2,116	7	0.33
Seepage	*Tricula xiaoqiaoensis*	1,834	28	1.52

Table 2: Detection results of snail hosts infected with *Angiostrongylus cantonensis* in different breeding grounds

Breeding ground	Snail species	Detected no.	Infected no.	Infection rate (%)
Garbage stacks	*Achatina fulica*	79	66	83.54
Vegetable plots	*Achatina fulica*	43	17	39.53
Crop lands	*Achatina fulica*	52	10	19.23
Trenches	*Pila polita*	49	2	4.08
Rice fields	*Pila polita*	68	6	8.82
Residential ditches	*Pila polita*	73	55	75.34
Vegetable irrigation ditches	*Pila polita*	47	16	34.04
Ponds	*Pila polita*	36	2	34.04
Vegetable irrigation ditches	*Bellamya lithophaga*	292	42	14.38
Ponds	*Bellamya aeruginosa*	211	5	2.37

RESULTS AND DISCUSSION

The prevalence of *P. westermani* cercariae in the stream-type snail *Semisulcospira libertine* found in the breeding grounds of Yangmeixi village in Shouning county was 0.09% (1/1,082) in the upstream and 0.56% (5/892) (χ^2 = 4.658, $p<0.05$) in the downstream, respectively. For pit-ditch type, the prevalence of *P. westermani* cercariae were 0.19% (2/1,035) and 0.82% (10/1,219) (χ^2 = 4.15, $p<0.05$) in upstream and downstream, respectively (Table 1).

The snail *Tricula xiaoqiaoensis* was found to be infected with *Paragonimus skrjabini* cercariae with prevalence in the pit-ditch type (0.33%, 7/2,116) and seepage type (1.52%, (28/1,834) (χ^2 = 15.99, $p<0.05$) in Xibiao village of Zhenghe county. This result indicated that seepage type breeding ground may be more suitable for the growth of *T. xiaoqiaoensis* than the pit-ditch type, possibly due to that the former was able to keep a moist circumstance persistently (Table 1).

A. cantonensis larvae were found in the snail *Achatina fulica* at the Humei village in Nan'an county and in the snail *Pila polita* at the Xiaowan village in Lianjiang county. In the Humei village, prevalence of *A. cantonensis* larvae in *A. fulica* in garbage stack, vegetable plot and crop land were 83.54% (66/79), 39.53% (17/43) and 19.23% (10/52) (χ^2 = 24.797-53.251, $p<0.05$), respectively (Table 2). The highest prevalence of *A. cantonensis* in *A. fulica* in garbage stack is possibly because garbage stack is close to the residential area and interconnected with kitchens of households. As the water for washing food or dishes is always spilled on garbage stack, the breeding ground is wet all year round.

At Xiaowan village of Lianjiang county in the east central Fujian province, prevalence of *A. cantonensis* in snails *Pila polita* in trench, paddy field, residential ditch, vegetable irrigation ditch and pond in the same river system were 4.08, 8.82, 75.34, 34.04 and 5.56%,

Fig. 1: The snail *Bellamya lithophaga*, the new intermediated host for *Angiostrongylus cantonensis*

respectively (Table 2) (χ^2 = 24.79-53.25, p<0.05). The highest prevalence was at the residential ditch which suggested that environment contamination play an important role in the infection of *A. cantonensis* in host snails (Table 2). More interestingly, it was found that the snail *Bellamya lithophaga* (Fig. 1) found at Xixia village in the western suburbs of Fuzhou city was the new intermediated host for *A. cantonensis* with a prevalence of 14.38% (42/292) in vegetable irrigation ditch (Table 2).

Snail infection with Paragonimus and *A. cantonensis* is not only related to the macro-environmental factors such as geographical features, vegetation, elevation and wildlife but also related to the mini eco-environment of the snails' breeding grounds. The favorable natural foci for Paragonimus are the mountainous streams, little ditches or seepage places with gently terrain and sluggish flow. However, the natural foci of *A. cantonensis* are depends on the species, population size and distribution of rodent reservoir hosts and snail intermediate hosts. The favorable foci of *A. cantonensis* are the wet breeding grounds which are close to the residential districts with rubbish dumps and corrosive. Moreover, as a new host for *A. cantonensis*, the snail Bellamya should be concerned as the new possible source of human infection with *A. cantonensis*.

CONCLUSION

The present investigation demonstrated the prevalence of Paragonimus and *A. cantonensis* infections in wild snails in Fujian province, southeastern China which poses substantial risk for future outbreaks of the two food-borne parasitic diseases. Therefore, integrated strategies should be taken to reduce or eliminate such risks.

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