AN OUTBREAK OF ANGIOSTRONGYLIASIS IN GUANGING, PEOPLE'S REPUBLIC OF CHINA: MIGRANTS VULNERABLE TO AN EMERGING DISEASE

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Abstract. Angiostrongyliasis has been frequently reported from the People's Republic of China during the last decade. An outbreak of angiostrongyliasis among migrant laborers in Guangning, Guangdong Province is described here. A questionnaire was developed to collect epidemiological and clinical information about 17 migrant laborers from the Bai ethnic group in Dali, Yunnan Province. Serum samples were collected and tested by enzyme-linked immunosorbent assay. Rats and mollusks from the same area where patients had collected *Pomacea canaliculata* were examined for presence of *Angiostrongylus cantonensis*. All 17 Bai migrant laborers consumed *P. canaliculata* and six had meningitis 3-19 days after consumption of *P. canaliculata*. Headache, myalgia and fatigue were the most common symptoms. Blood samples from 5 patients were positive for antibodies to *A. cantonensis*. The places where the migrant laborers collected *P. canaliculata* were identified as endemic areas for *A. cantonensis*. This outbreak highlights the vulnerability of migrants to angiostrongyliasis.

Keywords: *Angiostrongylus cantonensis*, outbreak, epidemiology, migrant, vulnerability

INTRODUCTION

Angiostrongylus cantonensis, the rat lungworm, is the most common cause of eosinophilic meningitis (angiostrongyliasis) in humans (Graeff-Teixeira *et al*, 2009). The nematode was first described as a parasite of the Norwegian rat (*Rattus norvegicus*) and the black rat (*R. rattus*) in Guangzhou (formerly called Canton), China in 1935 (Chen, 1935). The life cycle was not clarified until 1955. Mollusks, such as terrestrial slugs and snails, serve as intermediate hosts (Mackerras and Sandars, 1955). Humans acquire infection primarily via consumption of raw snails harboring third-stage (L_3) larvae (Lv *et al*, 2010). Contaminated vegetables and paratenic hosts (*eg*, freshwater prawns, crabs, frogs and lizards) have also been implicated in transmission (Slom *et al*, 2002; Panackel *et al*, 2006; Lai *et al*, 2007). Humans are non-permissive definitive hosts. *A. cantonensis* rarely develops to the sexually mature stage in the pulmonary arteries or heart (Lv *et al*, 2010).

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Most of these larvae, which may develop to the fourth or fifth stage, infect the human central nervous system causing meningitis characterized by an elevated eosinophilic count in cerebrospinal fluid (Lv *et al*, 2010).

An estimate of the current global disease burden is approximately 2,800 cases (Wang *et al*, 2008). Human infections with *A. cantonensis* are typically found in East Asia, the Pacific islands and the Caribbean. Approximately 77% are located in Southeast Asia, the People's Republic of China (PR China) and Japan. International travel has become a risk factor for infection. Sporadic cases and even outbreaks have been reported in non-endemic countries (Lo Re 3rd and Gluckman, 2001; Slom *et al*, 2002; Leone *et al*, 2007; Ali *et al*, 2008; Malvy *et al*, 2008). To date, few cases have been reported in other countries due to acquiring infection in China, partially because of the lower likelihood of consumption of undercooked snails. Consumption of raw snails, particularly *Pomacea canaliculata*, is the most common route of infection in China (Lv *et al*, 2008). Diversification of food items and convenient transportation facilitate the transmission of angiostrongyliasis to non-endemic areas of China, as illustrated by an outbreak in Beijing in 2006 (He *et al*, 2007).

Special eating habits were implicated in an outbreak in Dali, in early 2008 (Lv *et al*, 2009a). Interestingly, a previous outbreak occurred in Guangning County of Guangdong Province in the spring of 2007 involving several people of the same ethnic group, the Bai. In this paper, we describe the outbreak in order to highlight emerging angiostrongyliasis in China and the vulnerability of migrants to this disease.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Ethics Committee of Guangdong Provincial Center for Disease Control and Prevention (Guangdong CDC), and verbal informed consent was obtained from each participant.

Description of the outbreak

On April 13, 2007, a 37-year old woman was admitted to Guangzhou Eighth People's Hospital with a 16-day history of severe headache (lasting 2-3 hours early every morning) and frequent vomiting. She had an elevated eosinophil count of 22.4% in the peripheral blood and an immunoserological assay was positive for *A. cantonensis* antibodies. This woman, the index case, was one of a group of Bai migrant laborers from Dali, Yunnan Province. Five more migrant laborers from the same group reported similar symptoms from March 27 to April 12, 2007.

Questionnaire and individual interview

A questionnaire was developed for individual interviews, which included demographical information, symptoms of meningitis and food history (suspected food items, frequencies and dates). Eating habits and cooking methods were also recorded. All 17 Bai migrants from Dali, Yunnan Province were individually interviewed. Eleven local residents and 4 migrants from Fujian Province were interviewed and used as controls, they were either from the same road building team or from a local village. Individual interviews were performed by the authors on April 18-19, 2007. Cases of angiostrongyliasis were identified using tentative diagnostic criteria set by the Ministry of Health, China in 2006 (Lv *et al*, 2009a). There were no follow-ups, since all the

Bai migrants returned to Dali after the outbreak of *A. cantonensis*.

Serum test

Peripheral blood samples from five patients and two asymptomatic migrant laborers were collected. The other individuals refused blood collection. The blood samples were tested for the antibodies (IgG) to *A. cantonensis* by enzyme-linked immunosorbent assay (ELISA). All serum samples were also tested for cysticercosis and schistosomiasis by ELISA and dot immunogold filtration assay (DIGFA), respectively. These diseases are common causes of central nervous system infections and are endemic in Dali, Yunnan Province.

Supplementary field survey

Rats were trapped around the residence of migrant laborers for 2 consecutive nights. All captured rats were euthanized and dissected to determine the presence of adult *A. cantonensis* in their heart and lung arteries. Fresh feces of wild rats were collected in the houses of migrants and villagers. The feces were examined under the microscope after water sedimentation for the presence of first-stage larvae of *A. cantonensis* (Lv *et al*, 2009c).

Samples of the freshwater snail, *P. canaliculata*, were collected from fields and pools where migrant laborers collected their snails and areas near those places. Samples of *Achatina fulica* (African land snails), were captured around garbage dumps, shrubs, and in the hills of the village. The collected snails were categorized by weight, then crushed. The soft tissue of each snail was ground and put in a cone-shaped flask with dechlorinated water. The suspension was kept still to precipitate for 20 to 30 minutes, then the supernatant was poured out. The same procedure was repeated 2 to 3 times. The precipitate was examined under the microscope for the presence of *A. cantonensis* larvae. The larvae were identified by standard keys (Lv *et al*, 2009c).

Data were analyzed using R software (version 2.9.0). A p-value < 0.05 was considered significant.

RESULTS

All 17 Bai workers from Dali, Yunnan Province were in the same road building group and shared the same residence rented from a local village. All the migrants consumed snails (*P. canaliculata*, collected from fields close to their residence) on March 24, 2007. The snails were prepared in two ways: 1) undercooked (snails in their shell were put in boiling water for a few minutes and the foot parts were then removed. The foot muscle was prepared with condiments and ingested); 2) sufficiently cooked (the foot muscles of some snails were fried in a pan with vegetables). Fourteen migrants ate undercooked or mixed snails and the other three migrants only consumed sufficiently cooked food. The participants in the control group did not consume snails during that month.

The migrant workers were informed by the local people the snails were not safe when they were collecting the snails. The interview indicated a good awareness among the local villagers of the dangers of consuming the snails. The eating habits survey showed that freshwater snails are a common food item in Dali, Yunnan. An important local incentive for consumption of snails is the "Snail Festival" ("*Luoshi Jie*" in Chinese) which is held in March every year, when people catch and eat raw/undercooked snails, and released some snails in new places. This tradition has resulted in many people from Dali to eating raw

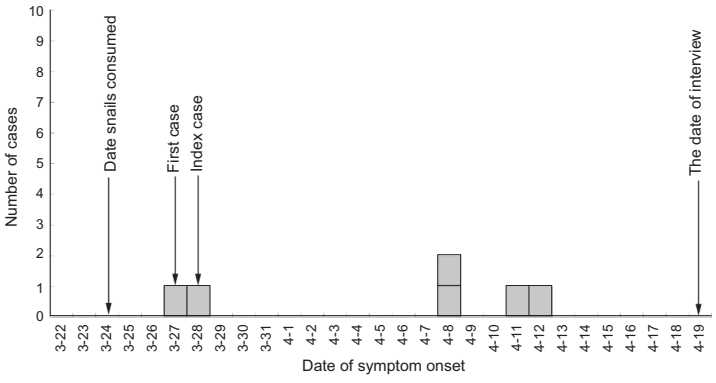

Fig 1–Date of onset of symptoms among 6 migrant laborers with *A. cantonensis* infection who ate raw snails on March 24, 2007. Each box represents one patient.

or undercooked snails. The snails which led to the outbreak were prepared for the special festival, which is the only time snails are eaten in that location.

Clinical manifestations

Following the tentative diagnostic criteria (Lv *et al*, 2009a), 6 of the 17 migrant workers (35.3%) were diagnosed as suspected cases of angiostrongyliasis, but only one patient was hospitalized. The median incubation period was 13 days (range 3 to 19 days) (Fig 1). The most common symptoms were headaches, myalgia, and fatigue (Table 1). Four cases had fever with a self-measured axillary temperature > 38.0ºC. Four patients complained of hyperesthesias or paresthesias of the trunk, arms and/or legs. Two patients complained of intermittent drowsiness. Two patients had nausea and vomiting.

A 30-year-old man was the first worker to have symptoms, which were intermittent headaches beginning March 27, accompanied by nausea, vomiting, skin pain and discomfort; he did not receive treatment. The second patient was a 37-year-old woman who experienced moderate fever (38.5ºC), severe headaches, and frequent vomiting (lasting for two weeks) beginning March 28. She was admitted to Guangzhou Eighth People's Hospital on April 13 and examination showed her muscular tension was normal and there were no visual disturbances. A second ELISA test was positive for *A. cantonensis* antibodies. Her sister had the same clinical symptoms and went to a clinic as an outpatient and only received albendazole treatment.

Serum testing

Serum samples from five cases and two asymptomatic laborers were obtained. Only 2 cases (the hospitalized patients and her sister) were positive for *A. cantonensis* antibodies. Testing for cysticercosis and schistosomiasis on all samples was negative.

Field survey for *A. cantonensis* and its hosts

Only one rat (*R. flavipectus*) was captured. No adult worms were found in the heart or pulmonary arteries. Eight fresh rat feces specimens were collected; 6 were positive for *A. cantonensis* (infection rate 75.0%).

A total of 189 *P. canaliculata* snails were collected and stratified into 3 groups by size: large (≥30g), medium (10-30g) and small (<10g). The overall infection rate was 2.1% (4/189) and the worm burden among the infected snails was 37 larvae per snail. The infection rate in the large snail group was higher, with a rate of 21.4% (3/14); the infection rate of the snails collected in the pond was higher than in the fields ($p<0.01$), with rates of 13.6% and 0.6%, respectively.

Table 1
Epidemiological and clinical characteristics of 17 migrant laborers from Dali, Yunnan Province.

Characteristics	Patients (*n*=6)	Laborers who did not become ill (*n* =11)
Male, no.(%)	4 (67)	9 (82)
Age, yrs (range)	27 (22-37)	28 (22-38)
Snails eatern, no. (raw)	6 (6)	11 (8)
Symptoms, no.(%)		
Headache	5 (83)	0
Myalgia	5 (83)	0
Fatigue	5 (83)	0
Hyperesthesias	4 (67)	0
Fever[a]	4 (67)	0
Vomiting	2 (33)	0
Intermittent drowsiness	2 (33)	0
Incubation period (range)	13 (3-19)	

[a]Axillary temperature self measured with a thermometer.

DISCUSSION

The present outbreak was attributed to *A. cantonensis*. All the Bai migrants from Dali, Yunnan consumed *P. canaliculata*, which has been confirmed as the leading vector of this parasite in China (Lv *et al*, 2009b). All 6 patients had similar symptoms, consistent with the clinical manifestations of eosinophilic meningitis caused by *A. cantonensis*. Despite the few samples, elevated eosinophilic cell counts and positive testing for antibodies against *A. cantonensis* on some samples provide evidence of infection with this parasite. The place where these patients collected *P. canaliculata* is endemic for *A. cantonensis*.

A. cantonensis is endemic in southern China (Lv *et al*, 2008). *P. canaliculata* is the primary intermediate host. However, the infection rate among *P. canaliculata* varies considerably by location. The place where the present outbreak occurred has a lower endemicity of *A. cantonensis* (Lv *et al*, 2009b). Only 2.1% of *P. canaliculata* snails had third stage larvae of *A. cantonensis* in our field survey. Larger snails had higher infection rates and larval burdens. These findings may explain why not all the 17 migrant laborers who consumed the raw snails became ill.

Like most travelers who are not aware of angiostrongyliasis, migrants are also vulnerable to *A. cantonensis* infection. Several outbreaks among migrants have been previously documented. In 1980, 21 of 24 Korean fishermen who worked in American Samoa consumed *A. fulica;* 16 developed eosinophilic meningitis (Kliks *et al*, 1982). Two more outbreaks occurred in Taiwan in 1998 and 1999 (Tsai *et al*, 2001). All 17 patients were Thai migrant laborers who consumed raw *P. canaliculata* snails. Snails are popular in the Far East (Lv *et al*, 2010). In the present outbreak, all 6 patients were from Dali, Yunnan Province, where the snail festival (*Luoshi*

Jie) shows the popularity of snails, which in part facilitated the outbreak of angiostrongyliasis in Dali in 2008 (Lv *et al*, 2009a). Other local villagers and those from Fujian Province did not ingest the snails because they do not have the same eating habits or knew the dangers of consuming *P. canaliculata*, although none of them knew the name of the disease.

Another challenge associated with vulnerability of migrants is low admission rates at hospitals. Although angiostrongyliasis is potentially fatal, many patients only experience mild clinical manifestations and finally recover without any sequelae. Patients may refuse hospitalization or treatment. This problem is more prominant among migrant laborers because of lower income or lack of access to health care. In the present outbreak, the first case was not admitted to the hospital. Three other migrants who became ill later did not seek medical care. Only the second patient with repeated severe headaches was hospitalized for treatment and her sister went to a clinic for albendazole.

Angiostrongyliasis is an emerging disease facilitated by biological invasion of *P. canaliculata* in China (Lv *et al*, 2008, 2009b). There are major challenges for controling angiostrongyliasis in China. The first is the need to develop a good diagnostic tool. The rate of parasitological diagnosis is low (Lv *et al*, 2010). Eosinophilia is not always present during the entire course of the illness (Lv *et al*, 2009a). An accurate immunoserological test would be a helpful tool for diagnosing angiostrongyliasis. To date, there are no commercially available assays (Wang *et al*, 2008). In our study 5 patients were examined with an immunoserological assay for IgG against *A. cantonensis* but only two samples were positive, which implies a low sensitivity for recent infections. Diagnostic criteria for angiostrongyliasis are needed. Tentative criteria were proposed by the Ministry of Health in 2006 but they have several considerable limitations (Lv *et al*, 2009a). A robust educational campaign is needed. Although health education has played an important role in food-borne diseases, a new plan should be developed to prevent angiostrongyliasis. Labor management agencies need to be aware of the major endemic diseases, including angiostrongyliasis in migrant areas, to help protect migrant laborers from infections.

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