Kaohsiung Journal of Medical Sciences (2011) 27, 184–189

available at www.sciencedirect.com

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journal homepage: http://www.kjms-online.com

ORIGINAL ARTICLE

Eosinophilic meningitis risk associated with raw *Ampullarium canaliculatus* snails consumption
生吃福壽螺罹患嗜伊紅性腦膜炎的風險

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Received 16 September 2010; accepted 13 December 2010
Available online 21 March 2011

KEYWORDS
Angiostrongylus cantonensis;
Larvae infectivity;
Larvae viability;
Raw snail

關鍵詞
廣東住血線蟲;
蟲體感染力;
蟲體活性;
生福壽螺

Abstract In Taiwan, *Angiostrongylus cantonensis* infection has been reported in foreign laborers who had consumed raw *Ampullarium canaliculatus* snails. This study analyzed three foreign laborers who had contracted enzyme-linked immunosorbent assay-confirmed *A cantonensis* infection while working in Taiwan. All three workers had consumed either roasted snails or raw snails flavored with seasoning while drinking wine. This study investigated possible risk factors for *A cantonensis*, including naturally occurring *A cantonensis* in *A canaliculatus* snails, viability of third-stage *A cantonensis* larvae in raw seasoned snails and in roasted snails, infectivity of larvae, and effects of alcohol while consuming snails. Positive infection rates in snails from five different irrigation canals in south Taiwan ranged from 12.3% to 29.4% and the average number of motile larvae per infected snail ranged from 36 to 65. The number of motile and coiled larvae in snail meat after 120 minutes seasoning was 93 (27.7%) and 233 (69.3%), respectively. After 20 minutes of roasting, most larvae in the snail meat were dead. The infectivities of motile and coiled larvae from snail meat after 60 minutes seasoning were 53.2% and 33.2%, respectively, and those from snail meat after 5 minutes roasting were 33.2% and 7.0%, respectively. Eating Taiwan *A canaliculatus* snails raw is extremely risky given their high infection rates and infection intensities. Even after 120 minutes seasoning or after 20 minutes roasting, snail meat should be considered unsafe for human consumption. Finally, experimental rodent studies indicated that consuming alcohol while ingesting larvae does not significantly reduced infectivity.

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doi:10.1016/j.kjms.2010.12.013

摘要 生吃福壽螺肉會感染廣東住血線蟲，經訪視血清抗體陽性的外勞，瞭解他們都是喝酒時吃烤螺肉或吃浸在辣椒醬、醋及魚醬等量混合的調味料的生螺肉，因此本研究分析田野福壽螺感染率及帶蟲數，在浸調味料的生螺肉或螺烤後的螺肉內第三期幼蟲的活性和感染力，以及酒精對蟲體感染的影響，來探討感染廣東住血線蟲的危險因子。結果在台灣南部5條灌溉渠道的福壽螺感染率介於12.3%至29.4%，每個螺帶蟲數介於36至65，螺肉切成0.5公分厚浸於調味料120分鐘分別有27.7%及69.3%第三期幼蟲呈活動及捲曲狀，螺烤20分鐘後尚有少部份蟲體成捲曲狀，螺肉浸調味料60分鐘後活動及捲曲蟲體的感染力分別為53.2%及26.1%，而螺烤5分鐘蟲體的感染力分別為33.2%及7.0%，因為福壽螺在台灣高感染率及高帶蟲數，因此吃浸調味料120分鐘及烤20分鐘的福壽螺肉均不安全，而且在鼷鼠的感染實驗顯示酒精並不會降低感染風險。

Introduction

Angiostrongylus cantonensis, a nematode parasite that inhabits the lung of rats, is the major cause of eosinophilic meningitis or meningoencephalitis in humans [1,2]. The parasite is endemic to the Pacific islands, Taiwan, Thailand, and Japan [3–5] but has recently been identified in Africa, India, Australia, North America, Brazil, Germany, and China [6–9]. Most patients are infected after eating raw or undercooked molluscan intermediate hosts and paratenic hosts that contain the infective third-stage larvae of the worm. Consuming uncooked or contaminated vegetables may also cause infection. Thus, *A cantonensis* is a food-borne zoonotic parasite of considerable public health importance in many countries throughout the world [10,11].

The *A cantonensis* is among the most important zoonotic parasites in Taiwan [12]. Hundreds of infections associated with ingestion of the *Achatina fulica* and *Pila* species of snail have been reported in indigenous populations of Taiwan and Thailand, respectively [4,13–17]. So far, few reports indicate that patients were infected with *A cantonensis* by the transmission of *Ampullarium canaliculatus*. In recent years, three outbreaks of *A cantonensis* infection have occurred among foreign laborers who had consumed raw *A canaliculatus* (golden apple snail) [18–20]. The present study analyzed three foreign laborers with eosinophilic meningitis associated with *A cantonensis* infection contracted after consuming *A canaliculatus* while drinking wine. The workers indicated that they had prepared the snails by roasting over open flame or had eaten them raw after seasoning. This study evaluated the risk factors for eosinophilic meningitis caused by *A canaliculatus* by investigating the rates and intensities of *A canaliculatus* infection caused by *A cantonensis* in several areas of Taiwan. The viability of third-stage larvae was tested in snail meat prepared by the process typically used by foreign laborers. The infectivity of the larvae and the effect of consuming alcohol when ingesting larvae were studied in a mouse infection model.

Methods

Patients

This study analyzed three male foreign laborers who had been hospitalized in a southern Taiwan medical center after contracting clinically defined eosinophilic meningitis in November 2009. All three patients indicated that they had consumed wine with *A canaliculatus* snails gathered from an irrigation canal. Patient 1 and 2 had eaten sliced raw snail meat mixed with seasoning. Patient 3 had eaten meat of snails roasted for 5–10 minutes over an open fire. Spinal taps were performed on all patients, and 5 mL specimens of cerebrospinal fluid (CSF) from each patient were sent to the authors for laboratory analysis by enzyme-linked immunosorbent assay (ELISA) to detect antibodies specific to *A cantonensis* and *Toxocara canis*.

Detection of CSF antibodies

Antibodies to *A cantonensis* and *T canis* in the CSF of patients and control subjects were assayed by ELISA [21]. Briefly, CSF specimens were added to the wells of a microplate precoated with somatic antigens of *A cantonensis* or *T canis* and incubated at room temperature for 1 hour. After removing unbound components by washing, antihuman immunoglobulin (G + A + M)-horseradish peroxidase conjugate was added and incubated for 1 hour. After washing the wells, tetramethylbenzidine substrate solution was added and incubated for 20 minutes at room temperature. In each well, absorbance was read at 450 nm after adding 2N sulfuric acid to stop the reaction.

Field survey of *A canaliculatus* infection

Live field snails were captured from five randomly selected irrigation canals in Kaohsiung County in South Taiwan. About 100 snails larger than 4 cm were randomly collected from each canal during the last week of November 2009. Third-stage *A cantonensis* larvae were obtained using a method described previously [22]. Briefly, after crushing the snails and removing the shells, each snail was minced individually and digested in artificial gastric juice with magnetic stirring at room temperature for 1 hour. Larvae in the sediments were counted under a dissecting microscope.

Viability of third-stage larvae in seasoned snail meat

Twenty-five infected snails larger than 4 cm were sliced into specimens 0.5 cm thick. The specimens were randomly divided into five groups. Specimens were then immersed for 5, 30, 60, or 120 minutes in the seasoning mixture typically used by foreign laborers, that is, equal volumes of chili

Table 1 Results for CSF specimens examined for young adult *Angiostrongylus cantonensis* by dissecting microscope and examined for antibodies to *A cantonensis* and *Toxocara canis* by ELISA

Patients	Specific antibodies to		Number of *A cantonensis* young-adult worm
	A cantonensis	*T canis*	
1	1.07	0.19	2
2	1.09	0.20	1
3	0.91	0.16	0
(+) control	1.13	0.98	2
(−) control	0.18	0.15	0

ELISA = enzyme-linked immunosorbent assay.

sauce, vinegar (4.5% acidity), and fish sauce. One unseasoned group of specimens was used as the control group. After digestion with artificial gastric juice, the total third-stage larvae in each preparing group were examined to determine the numbers of motile, coiled, and dead larvae.

Viability of third-stage larvae in roasted snail

Infected *A canaliculatus* snails were placed on a typical netted aluminum roasting plate with a handle and roasted over a fire fueled with dry tree branches. Five snails of each group were roasted for 5, 10, or 20 minutes. All snails were turned over every 1—2 minutes during roasting and were treated with artificial gastric juice after roasting. All third-stage larvae in each group of snail meat were analyzed to determine the numbers of motile, coiled, and dead larvae remaining after the digestion process.

Infectivity of each larvae group

Experiments were performed using male 6-week-old *BALB/C* mice that had been bred and maintained at the laboratory animal center at this institution. Each animal was infected with 30 third-stage larvae from snail meat subjected to one of the preparations via a stomach tube after slight ether anesthesia. In additional experimental groups, the mice were orally given 30 third-stage larvae from snail meat subjected to one of the treatments followed by 1.5 mL/kg of a local alcoholic drink (40% alcohol) via a stomach tube. The mice were sacrificed on Day 21 after infection, and young-adult worms were recovered from the brains. Larval infectivity was measured by the rate at which young-adult worms were recovered.

Statistics

Significant group differences in infection rates of *A canaliculatus* and viability of third-stage larvae were identified by chi-square test. Significant group differences in infection intensities of *A canaliculatus* and third-stage larvae infectivity were tested by analysis of variance. Significant group differences in third-stage larvae infectivity in mice with or without alcoholic drink were tested by Student *t* test. A p value less than 0.05 was considered significant.

Results

Examination of CSF antibodies and worms

Antibodies in the CSF of patients and control subjects were examined by ELISA using antigens of *A cantonensis* and *T canis*, respectively. The levels of CSF antibodies were expressed as ELISA values. Table 1 shows that, in three patients, the ELISA values increased for those observed in the *A cantonensis* positive control subject but did not significantly exceed for those observed in the *T canis* negative control subjects. Two and one young adult *A cantonensis* worms were found in CSF of Patient 1 and 2, respectively.

Table 2 Rates and intensities of infection with *Angiostrongylus cantonensis* in *Ampullarium canaliculatus* collected from five defined irrigation canals in south Taiwan

Defined irrigation	Number of examined canals	Positive, *n*(%)	Number of larvae per infected snail, mean ± SD
A	109	32 (29.4)	60 ± 8
B	112	20 (17.9)	49 ± 5
C	115	30 (26.1)	65 ± 9
D	98	12 (12.3)	36 ± 5
E	101	15 (14.9)	53 ± 6

Chi-square test of infected snails among five canals, $\chi^2 = 14.7$, $p < 0.01$.
Number of larvae per infected snail, one way analysis of variance, $F = 41.2$, $p < 0.01$.
SD = standard deviation.

Table 3 Viability of *Angiostrongylus cantonensis* third-stage larvae in meat of *Ampullarium canaliculatus* after four different durations of seasoning

Third-stage larvae activity	Numbers of larvae in snail meat seasoning for different durations				
	Untreated	5 min	30 min	60 min	120 min
Motile	323	319	307	165	93
Coiled	6	5	47	140	233
Dead	1	1	3	7	10
Total	330	325	357	312	336

Chi-square test of motile and coiled larvae from snail meat after four different seasoning treatments, $\chi^2 = 45.8$, $p < 0.001$ and $\chi^2 = 50.5$, $p < 0.001$, respectively.

Natural infection of *A canaliculatus*

As shown in Table 2, the positive rate of third-stage *A cantonensis* larvae in *A canaliculatus* collected from irrigation canals A—E were 12.3%—29.4%; the average number of motile larvae per infected snail ranged from 36 to 65. The foreign laborers with eosinophilic meningitis in this study had gathered *A canaliculatus* from Canal C.

Viability of third-stage larvae in seasoned snail meat

Table 3 compares the number of larvae and their activity in snail meat after different durations of seasoning. The number of motile larvae significantly decreased from 323 in unseasoned snail meat to 93 in snail meat seasoned for 120 minutes ($p < 0.001$). However, the longer the snail meat was immersed in seasoning, the more coiled larvae were found.

Viability of third-stage larvae in roasted snail meat

As shown in Table 4, motile larvae in the snails significantly decreased from 323 to 0 after 20 minutes roasting ($p < 0.0001$). The number of coiled larvae in the snails rapidly peaked (5 minutes after roasting) and then gradually decreased. However, the number of dead larvae in the snail meat was significantly and positively associated with snail roasting time ($p < 0.0001$). Roasting for 10 and 20 minutes resulted in 74.5% (228/306) and 87.5% (246/281) larvae death, respectively.

Infectivity of third-stage larvae and effects of alcohol on mouse infection

Infectivity of third-stage larvae was expressed as the ratio of young adult worms recovered from the brain of the mouse to the number of third-stage larvae consumed by the mouse. Table 5 shows that the infectivity of motile larvae from untreated snail meat was significantly higher than that of larvae obtained from snail meat seasoned for 60 minutes or from snail roasted for 5 minutes ($p < 0.001$). In snail meat treated with seasoning or roasting, coiled larvae can still cause infection, but dead larvae cannot. In mice that ingested larvae orally, the infectivity of third-stage larvae from each snail meat preparation was unaffected by concurrent alcohol consumption.

Discussion

Since Nomura and Lin reported the first human case of eosinophilic meningitis caused by *A cantonensis* in 1945 [23], many epidemiological surveys of this parasitic disease have been reported. Terrestrial and aquatic mollusks, including several species of snails and slugs, are known to be natural intermediate hosts of *A cantonensis* in Taiwan [5,24—26]. Other potential paratenic or transport hosts include planarian, crabs, shrimp, fish, frogs, and toads, although their roles in human infection have not been elucidated [27]. Obviously, food-borne *A cantonensis* infection is still a major public health concern in this country.

A cantonensis infection is endemic to south Taiwan, where the giant African snail is the major intermediate host [4,14]. The incidence of human cases is highest during the

Table 4 Viability of *Angiostrongylus cantonensis* third-stage larvae in meat of *Ampullarium canaliculatus* after three different durations of roasting

Third-stage larvae activity	Numbers of larvae in snail meat for varying durations of roasting			
	Untreated	5 min	10 min	20 min
Motile	323	97	9	0
Coiled	6	177	69	35
Dead	1	73	228	246
Total	330	347	306	281

Chi-square test of motile and coiled larvae from snail meat after three different durations of roasting, $\chi^2 = 111.4$, $p < 0.0001$ and $\chi^2 = 18.0$, $p < 0.01$, r espectively.

Table 5 Infectivity of *Angiostrongylus cantonensis* third-stage larvae from *Ampullarium canaliculatus* snails after seasoning or roasting in *BALB/c* mice and effect of alcoholic drink given to mice immediately after infection with larvae

Snail meat	Alcoholic drink	Infectivity of third-stage larvae with different activity		
		Motile	Coiled	Dead
Untreated	−	68.7 ± 7.1	ND	ND
Control	+	65.6 ± 5.7	ND	ND
Treated with seasoning for	−	53.2 ± 6.1	26.1 ± 4.6	ND
60 min	+	52.5 ± 5.0	25.3 ± 3.5	ND
Treated with roasting for 5 min	−	33.2 ± 4.6	7.0 ± 2.0	0
	+	31.5 ± 4.3	6.2 ± 1.9	0

Infectivity of motile larvae from each group of snails, one-way analysis of variance, $F = 26.2$, $p < 0.001$.
Infectivity of larvae from each group of snails in mice with or without alcoholic drink, Student t test, $p > 0.05$.
ND = insufficient larvae to infect mice.

May—September rainy season [13], when giant African snails are abundant and active in the field [28]. The rate and intensity of *A cantonensis* infection is reportedly associated with the size of the snail [28]. People gather giant African snails for cooking. The viscera and mucus of snails, which reportedly contain 95% of *A cantonensis* larvae [25] are often thrown on the ground during preparation and may be inadvertently ingested by children playing nearby. Thus, about 66% of reported cases are children younger than 9 years who live in rural and mountainous areas of south Taiwan [13]. In adults, infection usually results either from eating partially cooked snails or from eating foods or using dishes that have been contaminated during the cooking process. Snails are rarely eaten raw in Taiwan [4,13].

In contrast to the indigenous patients infected with *A cantonensis* in Taiwan, the three outbreaks in foreign laborers working in southern Taiwan involved adults who had ingested raw golden apple snails rather than giant African snails. In northeastern areas of Thailand, the high incidence of human infection with *A cantonensis* affects the life, health, and productivity of the indigenous people [10]. Koi-hoi, a traditional uncooked snail dish, is eaten with relish by the indigenous population in these areas. Koi-hoi is prepared from Pila snails, which are reportedly a major cause of cerebral angiostrongyliasis. A survey indicated that 0.9% of 423 *Pila polita* snails in northeast Thailand were infected with *A cantonensis* [29]. Because *A canaliculatus* and Pila sp have a similar in size and appearance, we hypothesize that foreign laborers in Taiwan may consume *A canaliculatus* snails because of mistaking them for *P polita* snails.

A canaliculatus was introduced in Taiwan from Argentina in 1979 as a food source. Since then, the species has propagated in paddy field and drainage ditches throughout the island. In 1985, Yen et al. [26] reported a successful experimental infection of the snail with *A cantonensis*. Field surveys in the present study showed that 12—29% of *A canaliculatus* were infected with third-stage *A cantonensis* larvae, which is similar to the results reported 20 years earlier [22]. The number of larvae per infected snail ranged from 36 to 65. Clearly, the rate and intensity of *A cantonensis* infection are significantly higher in the *A canaliculatus* snail in Taiwan than that in the *P polita* snail in Thailand. Thus, consuming raw *A canaliculatus* snail meat in Taiwan is extremely risky.

Although many inhabitants in rural areas of Taiwan collect *A canaliculatus* for food, the snail is rarely consumed raw. In Thailand, the traditional koi-hoi dish is prepared from chopped raw snail meat flavored with seasoning and vegetables. Third-stage *A cantonensis* larvae in chopped snail meat are easily exposed to the seasoning used in koi-hoi. After snail meat is seasoned for 10 minutes or longer, the number of motile third-stage larvae in coiled state increases [30]. However, a mouse infection model in a recent study revealed infectivity in coiled third-stage larvae in snail meat even after 1 hour seasoning [31]. Foreign laborers in Taiwan may have difficulty finding the ingredients they traditionally use to flavor raw snail meat and may therefore modify the ingredients. They usually slice the snail meat to a thickness of about 0.5 cm before seasoning with the modified ingredients. They typically consume the snail meat immediately after seasoning with the modified ingredients, before the seasoning can permeate. Within 5 minutes after exposure, almost all *A cantonensis* larvae observed in snail meat in this study were motile, and infectivity was 68.7%. Longer exposure to seasoning decreased the numbers of motile larvae in the snail meat and increased the numbers of coiled larvae. However, either motile or coiled larvae can still cause infection in mice, and even coiled larvae in snail meat seasoned for 60 minutes had 26.1% infectivity. Clearly, the seasoning ingredients used by foreign laborers in Taiwan only partially inhibit the viability of third-stage *A cantonensis* larvae.

A literature review reveals no studies of the viability of third-stage *A cantonensis* larvae in *A canaliculatus* snails roasted over open flame. Foreign laborers in Taiwan often gather after work for dining, drinking, and chatting. On such occasions, they typically use a simple roasting net to roast snails over an open fire started from tree branches. This study showed that, in *A canaliculatus* snails larger than 4 cm, snails roasted over an open flame for 20 minutes are safer for consumption compared with snails eaten raw. According to interviews with the three hospitalized patients analyzed in this study, most foreign laborers believe that raw snail meat is safe if eaten with alcoholic drinks. However, analysis of mice infected with third-stage *A cantonensis* larvae in this study indicated that consuming alcohol did not confer significant protection from infection.

In conclusion, the high rate of *A cantonensis* infection in *A canaliculatus*, the consumption of the snail, and the method of preparation are major risk factors for *A cantonensis* infection in foreign laborers in Taiwan. Some authors recommend that foreign laborers be legally prohibited from consuming raw snail meat to reduce the risk of *A cantonensis* infection. However, changing dietary habits that have existed for generations in foreign laborers would be difficult. To reduce infection risk, the authors therefore suggest an informational program to advise foreign laborers in safely preparing snail meat for human consumption (i.e. seasoning for longer than 2 hours and roasting for longer than 20 minutes).

References

[1] Alicata JE. Biology and distribution of the rat lungworm, Angiostrongylus cantonensis, and its relationship to eosinophilic meningoencephalitis and other neurological disorders of man and animals. Adv Parasitol 1965;3:223—48.

[2] Rosen L, Loison G, Laigret J, Wallace GD. Studies on eosinophilic meningitis. 3 Epidemiologic and clinical observarions on Pacific islands and the possible etiologic role of Angiostrongylus cantonensis. Am J Epidemiol 1967;85:17—44.

[3] Punyagupta S, Bunnag T, Juttiyudata P, Rosen L. Eosinophilic meningitis in Thailand, Epidemiologic studies of 484 typical cases and the etiologic role of Angiostrongylus cantonensis. Am J Trop Med Hyg 1970;19:950—8.

[4] Chen ER. Angiostrongyliasis and eosinophilic meningitis on Taiwan: a review. Special Publication No. 44. In: Cross JH, editor. Study on angiostrongyliasis in Eastern Asia and Australia. Taipei, Taiwan: NAMRU-2; 1979. p. 57—73.

[5] Otsuru M, Sato Y, Asato R, Sekikawa H. Epidemiological observations of angiostrongyliasis in the southwest islands of Japan and in Taiwan. J Formos Med Assoc 1982;81:819—32.

[6] Pien FD, Pien BC. *Angiostrongylus cantonensis* eosinophilic meningitis. Int J Infect Dis 1993;3:161—3.

[7] Luessi F, Sollors J, Torzewski M, Muller HD, Siegel E, Blum J, et al. Eosinophilic meningitis due to *Angiostrongylus cantonensis* in Germany. J Travel Med 2009;16:292—4.

[8] Wang QP, Lai DH, Zhu XQ, Chen XG, Lun ZR. Human angiostrongyliasis. Lancet Infect Dis 2008;8:621—30.

[9] Lima AR, Mesquita SD, Santos SS, Aquino ER, Rosa Lda R, Duarte FS, et al. Alicata disease: neuroinfection by *Angiostrongylus cantonensis* in Recife, Pernambuco, Brazil. Arq Nouropsiquiatr 2009;67:1093—6.

[10] Eamsobhana P, Tungtrongchitr A. Angiostrongyliasis in Thailand. In: Arizono N, Chai JY, Nawa Y, editors. Food-borne helminthiasis in Asia. Chiba, Japan: The Federation of Asian Parasitologists; 2005. p. 183—97.

[11] Cross JH, Chen ER. Angiostrongyliasis. In: Black SJ, Seed JR, editors. Food-borne parasitic zoonoses. New York, NY: Springer Science; 2007. p. 263—92.

[12] Hsieh HC. Outline of parasitic zoonoses in Taiwan. Formosan Sci 1959;13:99—109.

[13] Yii CY, Chen CY, Chen ER, Hsieh HC, Shih CC, Cross JH, et al. Epidemiologic studies of eosinophilic meningitis in southern Taiwan. Am J Trop Med Hyg 1975;24:447—54.

[14] Cheng CW, Sato Y, Chen ER. Epidemiological and clinical observations on eosinophilic meningitis and meningoencephalitis of children caused by infection with *Angiostrongylus cantonensis* in southern Taiwan. Ryukyu Med J 1984;7:1—9.

[15] Hwang KP, Chen ER, Chen TS. Eosinophilic meningitis and meningoencephalitis in children. Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi 1994;35:124—35.

[16] Tsai HC, Lee SS, Huang CK, Yen CM, Chen ER, Liu YC. Outbreak of eosinophilic meningitis associated with drinking raw vegetable juice in southern Taiwan. Am J Trop Med Hyg 2004;71: 222—6.

[17] Punyagupta S, Juttijudata P, Bunnag T. Eosinophilic meningitis in Thailand. Clinical studies of 484 typical cases probably caused by *Angiostrongylus cantonensis*. Am J Trop Med Hyg 1975;24:921—31.

[18] Tsai HC, Liu YC, Kunin CM, Lee SS, Chen YS, Lin HH, et al. Eosinophilic meningitis caused by *Angiostrongylus cantonensis*: report of 17 cases. Am J Med 2001;111:109—14.

[19] Tsai TH, Liu YC, Wann SR, Lin WR, Lee SJ, Lin HH, et al. An Outbreak of meningitis caused by *Angiostrongylus cantonensis* in Kaohsiung. J Microbiol Immunol Infect 2001;34:50—6.

[20] Lin CW, Liu YL, Lin HC, Wand SD, Wei ST, Wu EC, et al. An outbreak of Angiostrongylus cantonensis infection in Tainan County, Taiwan, 2009. Taiwan Epidemiol Bull 2010;26:50—6.

[21] Chye SM, Chang JH, Yen CM. Immunodiagnosis of human eosinophilic meningitis using an antigen of *Angiostrongylus cantonensis* L5 with molecular weight 204 kDa. Acta Trop 2000;75:9—17.

[22] Yen CM, Chen ER, Cheng CW. A survey of *Ampullarium canaliculatus* for natural infection of *Angiostrongylus cantonensis* in south Taiwan. J Trop Med Hyg 1990;93:347—50.

[23] Beaver PC, Rosen L. Memorandum on the first report of angiostrongylus in man, by Nomura and Lin, 1945. Am J Trop Med Hyg 1964;13:589—90.

[24] Chiu JK. Snail hosts of *Angiostrongylus cantonensis* in Taipei, Taiwan. Bull Inst Zoology Acad Sinica (Taipei) 1964;3:55—62.

[25] Wen YF. Distribution patterns of Angiostrongylus cantonensis in Achatina fulica. Chinese J Microbiol 1973;6:116—21.

[26] Yen CM, Chen ER, Hsieh HC. Experimental infection of Angiostrongylus cantonensis larvae to Ampullarium canaliculatus. J Formos Med Assoc 1985;84:35—40.

[27] Prociv P, Spratt DM, Carlisle MS. Neuro-angiostrongyliasis: unresolved issues. Int J Parasitol 2000;30:1295—303.

[28] Chen ER, Hsieh HC, Shih CC. First report on biological study of *Achatina fulica* (an intermediate host of *Angiostrongylus cantonensis*) in south Taiwan. J formos Med Assoc 1971;70: 364.

[29] Pipitgool V, Sithithaworn P, Pongmuttasaya P, Hinz E. *Angiostrongylus* infections in rats and snails in northeast Thailand. Southeast Asian J Trop Med Public Health 1997;28 (Suppl 1):190—3.

[30] Eamsobhana P, Yoolek A, Punthuprapasa P, Yong HS. Thai "koi-hoi" snail dish and angiostrongyliasis cantonensis: effects of food flavoring and alcoholic drink on the third-stage larvae in infected snail meat. Foodborne Pathog Dis 2009;6:401—5.

[31] Eamsobhana P, Yoolek A, Yong HS. Effect of Thai "koi-hoi" food flavoring on the viability and infectivity of the third-stage larvae of *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae). Acta Trop 2010;113:245—7.