Am J Transl Res 2022;14(3):1826-1837
www.ajtr.org /ISSN:1943-8141/AJTR0141435

Original Article

Prevalence of sparganum infection in wild frogs in Hainan province of China involves a risk for sparganosis

Ruijia Fu[1,2], Pei Liang[1], Gang Lu[2], Jinbao Gu[4], Dayong Wang[1,3]

[1]Key Laboratory of Tropical Bioresources of The Ministry of Education of China, Hainan University, Haikou 570228, Hainan, P. R. China; [2]Key Laboratory of Tropical Translational Medicine of Ministry of Education, Hainan Medicine University, Haikou 571199, Hainan, P. R. China; [3]Laboratory of Biopharmaceuticals and Molecular Pharmacology, School of Pharmaceutical Sciences, Hainan University, Haikou 570228, Hainan, P. R. China; [4]Department of Pathogen Biology, Guangdong Provincial Key Laboratory of Tropical Disease Research, School of Public Health, Southern Medical University, Guangzhou 510515, Guangdong, P. R. China

Received December 31, 2021; Accepted March 5, 2022; Epub March 15, 2022; Published March 30, 2022

Abstract: Background: Sparganosis is a serious parasitic zoonosis triggered by intake of and wound contact with frog flesh or the water infected or contaminated by sparganum. Methods: The prevalence of Sparganum infection in wild frogs was assessed. A questionnaire survey was also conducted among the residents in Hainan Province to evaluate people's awareness of the medical and epidemiological relevance of sparganosis. Results: A total of 1556 wild frogs were collected to examine Sparganum infection in different organs. A total of 201 (12.92%) were found to be infected with spargana of the genus *Spirometra*. There were 612 spargana found in those frogs, and the average infection rate was 3.04 per frog. The infection rate in the central region of Hainan Island is higher than that in other regions. Most spargana were found in the hind legs of frog, and the infection rate was not related to the weight of frogs. About 37.05% of the residents on Hainan Island have the knowledge of sparganosis and sparganum infection, and the internet was a major way to learn about sparganosis. The results of logistic regression analysis showed that occupation was identified as a determinant of sparganosis knowledge, and gender, age and residence were the most important risk factors for sparganosis. These results indicated that sparganum infection in wild frogs is common and is a potential threat to the residents on Hainan Island, and unhealthy lifestyle and eating habits may trigger sparganum infections. Conclusion: Wild frogs are commonly infected by Sparganum, which is a potential threat to people. To prevent sparganosis in humans, it is necessary to raise public awareness of sparganum infection in frogs and its association with sparganosis, as well as advocate healthy diet concepts and habits, and abandon the capture, sale and purchase of wild frogs to prevent and decrease the incidence of sparganosis.

Keywords: Sparganum, sparganosis, wild frogs, infection, questionnaire, Hainan

Introduction

Sparganosis is a parasitic disease triggered by sparganum. Sparganum is the larvae of the genus *Spirometra*, a type of tapeworm, which is also the main life stage of infection and causes disease in the human body. Spargana can invade the brain, eyes, abdominal cavity, spinal cord, and subcutaneous tissues of humans. Spargana that cause infection are mostly in vertebrates, but they are mainly parasitic in the bodies of frogs and snakes. Frogs are the second most important intermediate host of *Spirometra* and a very important source of sparganum infection in humans. Human infection results mainly from ingesting raw or incompletely cooked flesh of frogs infected by spargana or from placing frog flesh as a poultice on open wounds to treat skin ulcers or eye inflammation [1-3].

Sparganosis has a global distribution [4-7], but it mostly occurs in East and Southeast Asian countries [8-10]. To date, over 1,300 cases of sparganosis have been reported from 1949 to 2014, mostly in southern and eastern China [11, 12]. On Hainan Island, which constitutes most of Hainan Province, human cases of sparganosis have also been reported [13]. In southern China, eating raw frog meat is a traditional diet for many local people. Sparganum infection in frogs has been reported in a number of prov-

Table 1. Infection rate of sparganum in wild frogs in 18 cities and counties on Hainan Island

Place	Number examined	Number positive	Infection rate (%)	Number of spargana	Intensity of infection	Range
Haikou	124	14	11.29	31	2.21	1-4
Wenchang	97	14	14.43	48	3.43	1-7
Qionghai	83	0	0	0	0	0
Wanning	63	7	11.11	29	4.14	1-6
Lingshui	75	13	17.33	30	2.31	1-3
Sanya	94	9	9.57	28	3.11	2-6
Ding'an	131	36	27.48	62	1.72	1-8
Tunchang	60	4	6.67	13	3.25	2-4
Qiongzhong	103	16	15.53	69	4.31	1-12
Wuzhishan	118	20	16.95	97	4.85	1-22
Baoting	82	27	32.93	73	2.70	1-16
Baisha	64	6	9.38	27	4.50	1-9
Chengmai	102	13	12.75	28	2.15	1-5
Lin'gao	71	4	5.63	11	2.75	1-4
Danzhou	149	10	6.71	39	3.90	2-4
Ledong	51	6	11.76	19	3.17	1-4
Dongfang	43	0	0	0	0	0
Changjiang	46	2	4.35	8	4.00	3-5
Total	1556	201	12.92	612	3.04	1-22

inces in China, such as Anhui, Guangxi, Henan, and Zhejiang (**Table 2**). The natural infection rate of sparganum in frogs varies from region to region and is related to factors such as the living environment, living habits, and the number of hosts investigated. The warm and humid weather on Hainan Island is highly suitable for the survival of frogs, and the local people use frogs as food, which creates conditions for the spread of sparganosis. Therefore, collecting frogs and identifying Sparganosis in their bodies is of great significance for epidemiological investigations related to sparganosis.

The present study aimed to improve the awareness of the food safety issue of sparganosis through investigation and revealing of the prevalence of Sparganum infection in wild frogs in Hainan and other provinces in China, the possible routes of Sparganum transmission to humans, and the awareness of sparganosis and potential risks.

Materials and methods

Survey region

The present study was carried out on Hainan Island located in the north western South China Sea. This area is geographically separated from the Leizhou Peninsula on the continent of Asia by the Qiongzhou Strait between the eastern longitudes of 108°37'-117°50' and northern latitudes of 3°58'-20°20'. This area has a tropical monsoon climate. As a province and the largest island of mainland China, Hainan Province consists of 19 cities and counties with a total population of approximately 9.45 million. There are eighteen cities on Hainan Island with an area of approximately 35,400 square kilometres. According to different geographic locations and the distribution of water systems, the 18 cities and counties located on Hainan Island were selected as the survey sites. We divided the 18 cities and counties of Hainan Province into eastern coastal regions (Haikou, Wenchang, Qionghai, Wanning, Lingshui, and Sanya), central regions (Ding'an, Tunchang, Qiongzhong, Wuzhishan, Baoting, and Baisha) and western coastal regions (Chengmai, Lin'gao, Danzhou, Changjiang, Dongfang, and Ledong). The 19th city, located on coral reefs with a small population of approximately 500 people, was not included in the survey. In addition, we collected 362 cases of sparganum infection from 2015 to 2020, and analyzed the related factors affecting sparganum infection.

Table 2. Comparison of infection prevalence among provinces of China in the past 20 years

Province	Year	Number examined	Number positive	Infection rate (%)	Reference
Hainan	2021	1556	201	12.92	This study
Guangdong	2016	1949	229	11.8	[18]
	2016	1304	148	11.35	[22]
	2014	107	44	41.12	[32]
Jiangsu	2017	300	31	10.33	[23]
Zhejiang	2018	521	61	11.71	[17]
	2010	254	13	5.12	[26]
	2015	348	40	11.49	[26]
Guangxi	2013	632	69	10.92	[15]
	2003	76	59	77.6	[31]
Henan	2012	1149	306	26.63	[16]
Hunan	2012	428	126	29.44	[20]
	2010	292	59	20.2	[21]
Guizhou	2007	4472	836	18.69	[19]
Anhui	2006	4986	404	8.1	[14]
Sichuan	2015	127	40	31.5	[24]
Chongqing	2015	37	2	5.41	[24]
Fujian	2001	61	25	40.98	[25]

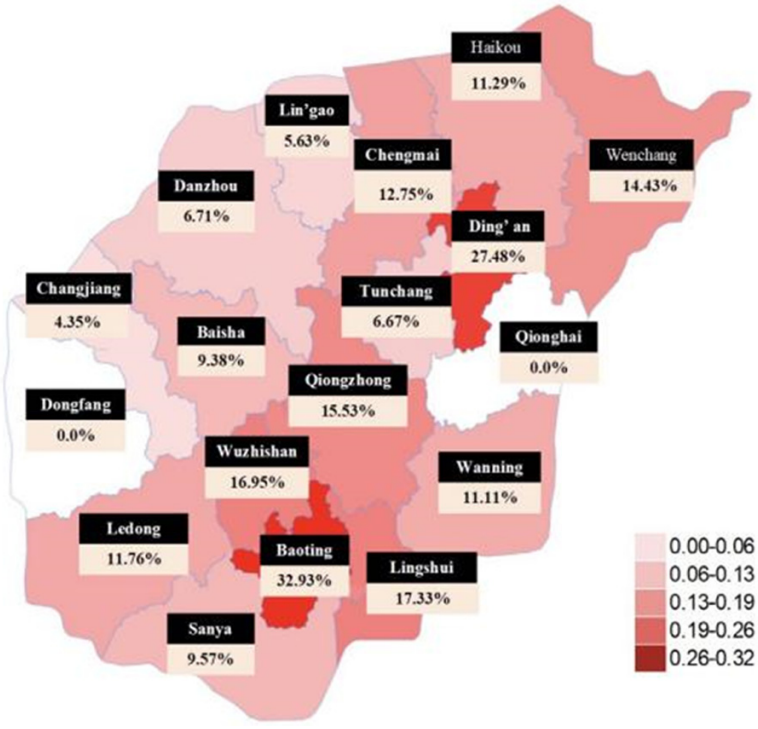

Figure 1. Prevalence and distribution of sparganum infection in wild frogs on Hainan Island. Geographic map of the areas surveyed in Hainan Province, China. Variation in the prevalence of Sparganum derived from frogs examined in 18 cities and counties. Differences in prevalence values are indicated with the colour gradient (from white to red).

Collection and examination of samples

All frogs were collected from different sites in the 18 cities and counties on Hainan Island, including local rural ponds, rivers and farmlands. The collected frogs were wild frogs, which were weighed, numbered, registered and then anaesthetized and sacrificed in the laboratory. The Sparganum in the frog muscles were examined under a stereomicroscope with the naked eye after stripping off the skin. Once being identified, the spargana were removed from the muscle and placed in a culture dish containing physiological saline. After measurements and morphological observations were obtained, most of the white spargana appeared as flat ribbons of 1 cm to 8 cm in length, and the maximum length of some individuals reached 12 cm. They were highly active and able to change shape in a Petri dish containing physiological saline. The number of spargana collected from each infected frog was counted to estimate the intensity of sparganum infection (Under the microscope, a complete, active sparganum can be observed and recorded as 1. The number of sparganum in all muscle tissues throughout the body of each frog was counted).

Questionnaire survey

We sent the questionnaires to local residents through WeChat, microblogs and other network platforms, which were completed online. The Eight epidemiological ques-

Table 3. Infection incidence in three geological areas on Hainan Island

Region	Number examined	Number positive	Infection rate (%)	Number of spargana	Intensity of infection	Range
Eastern coastal	536	57	10.63	166	2.91	1-7
Midland	558	109	19.53	341	3.13	1-22
Western coastal	462	35	7.58	105	3.00	1-5
Total	1556	201	12.92	612	3.04	1-22

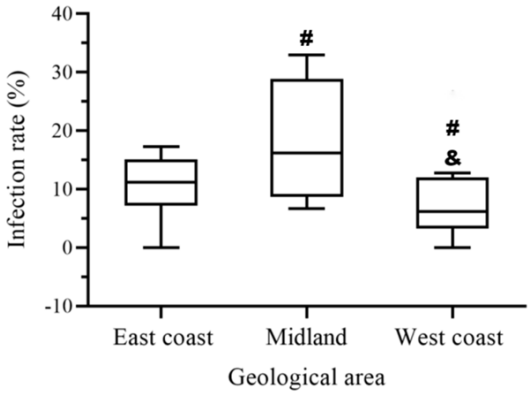

Figure 2. Summary of sparganum infection in the three geographical areas of Hainan Island. #indicates a difference compared with East coast; &indicates a difference compared with Midland.

tions in the questionnaire were as follows [18]: (1) Do you know sparganum? (2) Do you know the parasitic tissues in sparganum? (3) Do you know the harmfulness of sparganosis? (4) Do you know the transmission route of sparganosis? (5) Have you ever eaten frog meat? (6) Do you have a history of covering inflamed eyes, wounds, or skin abscesses with frog muscle slices as a treatment? (7) Do you have the habit of eating raw or undercooked meats? (8) Do you often or occasionally drink unboiled water? The number of respondents answered "yes" to each of the questions was recorded.

Statistical analysis

Original data were recorded and sorted using Microsoft® Excel, and all statistical analyses were conducted using SPSS 13.0. Counting data were expressed in the form of n (%), and compared via the chi-square test between groups. Measurement data were presented in ($\bar{x} \pm s$), and compared via the independent sample t test between groups and via the one-way ANOVA and post hoc LSD test among groups. $P < 0.05$ was notated as a significant difference.

Results

Prevalence of sparganum in wild frogs

A total of 1556 individual wild frogs collected from all 18 survey sites were examined, and 201 wild frogs were found to be parasitized by Sparganum, showing a total natural infection rate of 12.92% (201/1556). A total of 612 spargana were found in those frogs, showing an average infection rate of 3.04 per frog. The maximum number of spargana identified in a single frog was 22. There was a statistically significant difference in the infection rate of spargana in the wild frogs collected from different areas, and the highest prevalence of infection in wild frogs, up to 32.93% (27/82), was in Baoting County (**Table 1**; **Figure 1**).

A statistically significant difference was found in the prevalence rate of Sparganum in the wild frogs in these three geological regions, with the highest infection rate found in central Hainan Island, up to 19.53% (109/558) (**Table 3**; **Figure 2**).

The frogs were divided into 4 groups according to their body weights, namely, the < 30 g group, 30-60 g group, 60-90 g group, and > 90 g group. The infection rates of Sparganum in these groups were 14.11% (46/326), 14.12% (62/439), 11.42% (70/613) and 12.92% (23/178), respectively. There was no significant difference in the infection rate among groups. In comparison to the other two groups, the 60-90 g group and > 90 g group had higher average intensities of infection at 3.77 per frog and 4.22 per frog, respectively (**Table 4**; **Figure 3**).

Sparganum can be widely parasitic in various parts of a frog's body, and it is more common in muscle tissue. By analysing the data of the 201 frogs infected by spargana, we found that most spargana were located in the hindleg muscle,

Table 4. Incidence of sparganum infection in frogs of different weight on Hainan Island

Region	Number examined	Number positive	Infection rate (%)	Number of spargana	Intensity of infection	Range
< 30 g	326	46	14.11	112	2.43	1-12
30~60 g	439	62	14.12	139	2.24	1-8
60~90 g	613	70	11.42	264	3.77	1-22
> 90 g	178	23	12.92	97	4.22	1-9
Total	1556	201	12.92	612	3.04	1-22

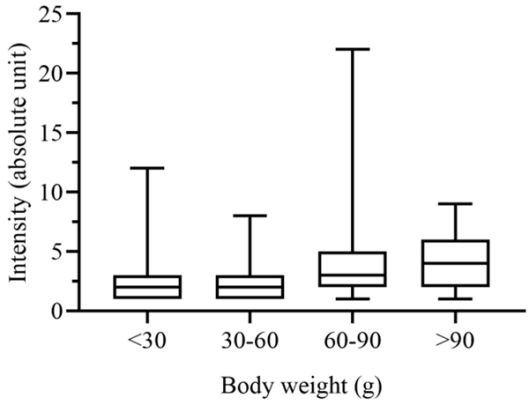

Figure 3. Illustration of the incidence of sparganum infection in frogs of different weights on Hainan Island.

with up to 447 parasites and an average infection intensity of 3.08 per frog. The infection rate was also the highest in the hindleg muscles, reaching 72.14% (n = 201), followed by 20.89% on the back, 13.93% on the forelegs, 10.45% on the abdomen, and 3.48% on the body cavity (**Table 5**; **Figure 4**).

Analysis of the questionnaire survey

A total of 876 residents from different cities and counties in Hainan Province participated in the questionnaire survey. Questions 1 to 4 represented the level of awareness of sparganosis among the local residents. Among the residents investigated, 392 (44.8%) were aware of sparganum, 294 (33.6%) were aware of the frog tissues that can be infected, 325 (37.1%) were aware of its harmfulness, and 286 (32.7%) were aware of its route of infection (**Table 6**). The main ways by which participants learned about sparganosis are shown in **Table 7**.

Questions 5 to 8 were related to the possible routes of getting sparganosis. A higher rate indicates a greater possibility that behaviour was typical of acquiring sparganosis. Among the residents investigated, 546 (62.3%) ate frog meat, 20 (2.3%) used frog meat for external application, 278 (31.7%) had the habit of eating raw or undercooked meat, and 439 (50.1%) drank untreated water (**Table 6**).

The factors influencing acknowledgement of sparganosis were separated into two kinds of variables: categorical variables including gender, residence, and occupation, and continuous variables including age and educational status. The factors were analysed by the logistic regression model. Among the variables, only the occupation (OR = 3.937; $CI_{95\%}$ = 1.878-8.254; P < 0.01) was identified as determinant of awareness of sparganosis. Among them, the awareness rate in public servants was 3.937 times higher than that in businessmen (**Table 8**).

Analysis of related factors for sparganum infection

First of all, we analyzed the related factors of sparganosis infection due to eating frog meat, and found that women were 0.673 times more likely to be infected with sparganosis than men (P < 0.05), and people face a higher risk of sparganosis infection due to eating frog meat as they grow older (P < 0.05). In addition, occupation may also be one of the reasons for the increased risk of sparganosis infection due to eating frog meat (P < 0.05, **Table 9**). Then, we analyzed the related factors of sparganosis infection due to drinking unboiled water, and found that the risk of sparganosis infection due to drinking unboiled water in women was 0.488 times higher than that in men, and the risk of sparganosis infection due to drinking unboiled water in rural population was 2.119 times higher than that in urban population (P < 0.05, **Table 10**). Finally, analysis of the related factors of sparganosis infection due to eating raw

Table 5. Incidence of sparganum infection at different location of frogs on Hainan Island

Region	Number positive	Infection rate (%)	Number of spargana	Intensity of infection	Range
Foreleg	28	13.93	42	1.50	1-3
Hindleg	145	72.14*	447	3.08	1-11
Backside	42	20.89#	79	1.88	1-4
Abdomen	21	10.45#	36	1.71	1-3
Coelomic	7	3.48#	8	1.14	1-2
x^2		150.600			
P		< 0.001			

Note: *indicates that there is a statistical difference in the infection rate with Foreleg, #indicates that there is a statistical difference in the infection rate with Hindleg.

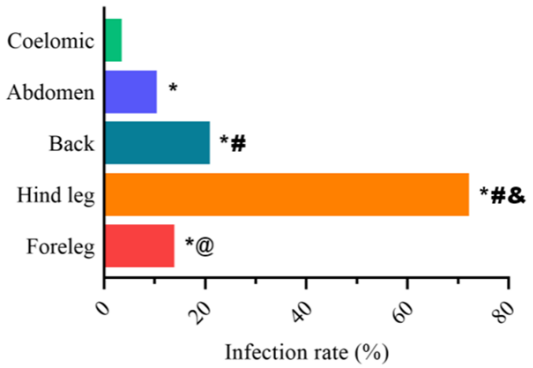

Figure 4. Illustration of the difference in the parasitic locations on the frogs collected from the 18 cities and counties on Hainan Island. *indicates a difference compared with Coelomic; #indicates a difference compared with Abdomen; &indicates a difference compared with Back; @indicates a difference compared with hind leg.

Table 6. Participation decomposition of the questionnaire survey (n = 876)

Question	Number (%)
1	392 (44.8)
2	294 (33.6)
3	325 (37.1)
4	286 (32.7)
5	546 (62.3)
6	20 (2.3)
7	278 (31.7)
8	439 (50.1)

or undercooked meats showed that women face a lower risk of sparganosis infection due to eating raw or undercooked meats than men (P < 0.05) and the possibility of infection in rural population was significantly higher than that in urban population (P < 0.05). Moreover, the increase of age was also a risk factor for sparganosis infection due to eating raw or undercooked meats (P < 0.05, **Table 11**).

Discussion

As a tropical region located in the very south of China, Hainan Island has warm climate throughout the year, with plentiful rainfall, suitable humidity, and abundant crops from farmlands, which are very suitable for the growth and reproduction of a large number of frogs. This area also provides optimal natural conditions for the reproduction and spread of spargana.

The investigation showed that Sparganum was found in 1,556 wild frogs, and a total of 612 parasites were detected. Among them, up to 22 spargana were detected in a single frog. The natural infection rate of Sparganum in wild frogs on Hainan Island was 12.92%, and the intensity of infection was 3.04 per frog, indicating that spargana were widely distributed in various regions of the island, and sparganum infection in wild frogs was common (**Table 1**). As shown in **Table 2**, the prevalence of sparganum in different regions ranged from 5.41 to 40.98 percent, probably due to different temperatures and humidity, ecological and geographical environment, the time of sample collection as well as the number of samples which can influence the detection, so, the actual reasons for the various prevalence of sparganum in different studies are hard to explain. Although the infection rate in wild frogs on Hainan Island is not very high, which is even lower than that in other provinces and cities in China as indicated by the surveys in Guizhou (18.69%), Henan (26.6%), Hunan (29.44%, 20.2%) and so on. There are still many people unaware of sparganum infection in wild frogs and without sufficient protection against sparganosis. Especially in recent years, with the changes in eating habits, an increasing number of people have begun to eat frog meat. Some local people on Hainan Island have the habit of eating wild frogs. Although there is no definite evidence showing that locals eat raw frog meat, insufficient heat-

Table 7. The main way for people to acknowledge information of sparganosis (n = 362)

Source of the knowledge	Number of people (%)
Media	93 (25.7)
Network	136 (37.6)
School	14 (3.9)
Newspapers and magazines	51 (14.1)
Other	68 (18.7)

ing or incomplete cooking processes may still cause sparganum infection.

In the study, a total of 341 spargana were collected from the central region, with a higher rate of sparganum infection in wild frogs compared with that in other regions (19.53%) (**Table 3**). The infection rates in Baoting and Ding'an Counties located in the central region were higher (32.93% and 27.48%, respectively) than those in other places, suggesting that people in these two regions face a higher risk of sparganosis than those in other regions (**Figure 1**). The central area of Hainan Island is the source of the main rivers on the island, with an average annual rainfall of more than 2,200 mm, rich freshwater resources and good water quality. There are many ponds, reservoirs and rice fields suitable as habitats for frogs and their reproduction. In Baoting county and Wuzhishan city, the original ecological environment is relatively well preserved, with more free-range cats and dogs. Free-range cats and dogs have more chances to eat wild frogs as food and thus catch infection of spargana. The eggs in their faeces can easily enter the water after being washed away by rain, thereby forming a natural circulation and reiterative transmission process, which provides a better chance for completion of the life cycle of *Spirometra* [26]. The infection rate of sparganum in wild frogs in Qionghai city and Dongfang city was 0, and the infection rate in Changjiang County and Lin'gao County was not high (**Figure 1**). This result may be related to the fact that the collection point was close to the sea. The sea is relatively far from highly crowded residential areas, so there are fewer cats and dogs, which are also important sources of sparganum infection; actions should be taken to prevent the faeces of cats and dogs from entering into water bodies closely related to human daily life [27]. It has been reported in the literature that no sparganum infection occurs in manually bred frogs, which may be due to the lack of intermediate hosts, such as cats, dogs, and *Cyclops*, required for a complete life cycle of *Spirometra* on farms during the manual breeding process [28, 29]. Manually bred frogs are fed artificial foods, and frogs have less chance to contact the outside world and prey on *Cyclops*. In addition, manual breeding environments are generally sterilized and disinfected, which reduces the sparganum infection rate [30].

Reportedly, sparganum can parasitize in frogs of different weights. Although a trend in the difference in the infection rate was found among frogs of different weights, it was not statistically significant (**Figure 3**). Therefore, the infection rate was not related to weights but to the growth environments of the frogs. However, the sparganum infection intensity in large frogs was relatively high (**Table 4**), indicating that the flesh of the hypertrophied frogs rich in protein, calcium and phosphorus, especially the muscles of the hindlegs, provides abundant nutrients and plentiful space for the growth and development of spargana. In addition, large frogs have lived for a longer time, and the prolonged exposure to the natural environment increases the probability of recurrent infection, resulting in large-scale infection. Sparganum can widely parasitize various locations of a frog's body, and it is more common in muscle tissue. There will be oedema in the area infected by spargana, and some frogs may have congestion or bleeding. The current results showed that frog hindleg muscles had the most parasites of spargana, with 447 parasites, and had a higher infection rate (72.14%) than other tissues, with an average infection intensity of 3.08 per frog (**Table 5**). The observation that spargana mainly parasitized the leg muscles of frogs is consistent with the findings of other domestic scholars [14, 16, 22, 31, 32]. This result further indicates that spargana have strong contractility and mobility, and they mostly extend under the skin or curl to live in the cavities in leg muscles since in comparison to other tissues, frog leg muscles have more nutrients and provide better conditions for the survival of spargana.

The purposes of the questionnaire survey were to understand the residents' knowledge about sparganosis and whether their lifestyle and eating habits are related to human sparganosis in Hainan Province. As shown in **Table 6**, the mean

Table 8. Logistic regression analysis on factors influencing awareness of sparganosis

Variable	β	Standard error	Wald χ^2	*P* value	Odds ratio	95% CI for OR
Gender						
Male					1.000	
Female	-0.176	0.154	1.301	0.254	0.839	0.620-1.135
Age, years						
< 18						
18~40	0.082	0.161	0.256	0.613	1.085	0.791-1.489
41~60						
> 60						
Residence					1.000	
Urban	-0.272	0.210	1.681	0.195	0.762	0.505-1.149
Rural			39.639	0.000*		
Occupation						
Businessman					1.000	
Public servant	1.370	0.378	13.167	0.000*	3.937	1.878-8.254
Farmer	0.426	0.611	0.486	0.486	1.531	0.462-5.073
Other	0.279	0.397	0.495	0.482	1.322	0.608-2.876
Educational status						
Illiteracy						
Primary school						
Secondary school	0.216	0.146	2.181	0.140	1.241	0.932-1.652
Junior college and above						

Note: *represents P < 0.05.

Table 9. Logistic regression analysis of factors influencing eating frog meat induced sparganosis (Part 1)

	Variable	β	Standard error	Wald χ^2	*P* value	Odds ratio	95% CI for OR
Eating frog meat	Gender						
	Male					1.000	
	Female	-0.397	0.149	7.123	0.008*	0.673	0.503-0.900
	Age, years						
	< 18						
	18~40	0.332	0.150	4.925	0.026	0.717	0.535-0.962
	41~60						
	> 60						
	Residence						
	Urban					1.000	
	rural	-0.223	0.181	1.526	0.217	0.800	0.562-1.140
	Occupation			10.430	0.015		
	Businessman					1.000	
	Public servant	0.382	0.285	1.803	0.179	1.466	0.839-2.562
	Farmer	-0.529	0.462	1.314	0.252	0.589	0.238-1.456
	Other	-0.094	0.291	0.104	0.747	0.910	0.515-1.609
	Educational status						
	Illiteracy						
	Primary school						
	Secondary school	-0.161	0.116	1.915	0.166	0.851	0.678-1.069
	Junior college and above						

Note: *represents P < 0.05.

Table 10. Logistic regression analysis of factors influencing drinking unboiled water induced sparganosis (Part 2)

	Variable	*β*	Standard error	Wald χ^2	*P* value	Odds ratio	95% CI for OR
Drinking unboiled water	Gender						
	Male					1.000	
	Female	-0.717	0.145	24.440	0.000*	0.488	0.367-0.649
	Age, years						
	< 18						
	18~40	-0.176	0.149	1.387	0.239	0.839	0.626-1.124
	41~60						
	> 60						
	Residence						
	Urban					1.000	
	Rural	0.751	0.185	16.471	0.000*	2.119	1.474-3.044
	Occupation			7.253	0.064		
	Businessman					1.000	
	Public servant	-0.391	0.289	1.834	0.176	0.677	0.384-1.191
	Farmer	-1.014	0.469	4.669	0.051	0.363	0.145-0.910
	Other	-0.147	0.298	0.244	0.621	0.863	0.481-1.548
	Educational status						
	Illiteracy						
	Primary school						
	Secondary school	0.122	0.112	1.180	0.277	0.885	0.711-1.103
	Junior college and above						

Note: *represents P < 0.05.

value for questions 1 to 4 was approximately 37.05%. Although this value was not very low, there is still opportunity to improve residents' awareness of sparganosis through health education. The results in **Table 7** show that information provided by the internet was the most important way for residents to learn about sparganosis. Most people have not systematically conducted research, and the knowledge obtained is from the internet without high accuracy. Logistic regression analysis revealed that the public servants had a better awareness of sparganosis, which may be related with their education, relatively rich sources of safe food, and more common sense of health (**Table 8**). This study indicated the necessity that food safety knowledge should be propagated to people beyond local residents in Chinese provinces to improve the awareness of sparganosis; many parasitic diseases are caused by ignorance and inappropriate habits. The consumption of frogs is common in Hainan. Improper ways of ingesting frogs and other animal meat are risky factors causing infection of sparganum. In addition, drinking raw water and swallowing the *Cyclops* inadvertently is a way to infect sparganum (**Table 6**). The results in **Tables 9-11** show that the risk of acquiring sparganosis is higher in males than in females, which is consistent with reported cases of sparganosis in China [18, 27, 33]. The possible reason is that females have better living habits than males who have frequent social activities with higher chance of having undercooked meats. The habit of drinking unboiled water and eating raw or undercooked meat were more common in rural residents than in urban residents, due to the lower awareness of sparganosis, limited drinking water resources, and traditional habits in rural areas. The occurrence probability of eating raw or undercooked meats was increased with age. Although no case of human sparganosis has been reported in the province in recent years, as long as the problems highlighted above occur, there is a potential risk of sparganosis in humans.

Wild frogs are commonly infected by Sparganum, which is a potential threat to people. To prevent sparganosis in human, it is necessary

Table 11. Logistic regression analysis on factors influencing eating raw or undercooked meats induced sparganosis (Part 3)

	Variable	*β*	Standard error	Wald χ^2	*P* value	Odds ratio	95% CI for OR
Eating raw or undercooked meats	Gender						
	Male					1.000	
	Female	0.532	0.151	12.378	0.000*	0.703	1.266-2.290
	Age, years						
	< 18						
	18~40	0.540	0.174	9.624	0.002*	1.716	1.220-2.413
	41~60						
	> 60						
	Residence						
	Urban					1.000	
	Rural	0.702	0.205	11.747	0.001*	2.018	1.351-3.015
	Occupation			1.661	0.646		
	Businessman					1.000	
	Public servant	0.370	0.291	1.610	0.204	1.447	0.818-2.561
	Farmer	0.361	0.537	0.453	0.501	1.435	0.501-4.111
	Other	0.294	0.301	0.956	0.328	1.342	0.744-2.422
	Educational status						
	Illiteracy						
	Primary school						
	Secondary school	-0.067	0.124	0.293	0.588	0.935	0.734-1.192
	Junior college and above						

Note: *represents P < 0.05.

to raise public awareness of sparganum infection in frogs and its association with sparganosis, advocate healthy diet concepts and habits, and abandon the capture, sale and purchase of wild frogs to prevent and decrease the incidence of sparganosis [34].

However, our study still has many limitations. For instance, the accurate morphological identification of tapeworm species must be based on the morphology of adults, but the specimens obtained in the field are usually larvae. Therefore, we need to further distinguish sparganum by multiplex PCR sequencing and conduct a more comprehensive morphological analysis to determine its species classification. In addition, the specific infection mechanism of sparganum is also the focus of further research. Moreover, due to the lack of clinical infection cases, we are currently unable to judge the specific pathogenic effect and mechanism of sparganum in human body. In the follow-up experiments, we will conduct more in-depth and accurate research and analysis as soon as possible to provide more reliable reference opinions for clinical practice.

Acknowledgements

This work was supported by the National Nature Science Foundation of China (Grant No. 31760246 to D.W. and No. 81560332) and by the Program of the Hainan Association for Science and Technology Plan for Youth R&D Innovation (QCXM201918).

Disclosure of conflict of interest

None.

Address correspondence to: Dayong Wang, Laboratory of Biopharmaceuticals and Molecular Pharmacology, School of Pharmaceutical Sciences, Hainan University, Haikou 570228, Hainan, P. R. China. Tel: +86-18789556728; E-mail: wangdy@hainanu.edu.cn

References

[1] Hong D, Xie H, Zhu M, Wan H, Xu R and Wu Y. Cerebral sparganosis in mainland Chinese patients. J Clin Neurosci 2013; 20: 1514-1519.

[2] Prasetyo RH and Safitri E. Sparganum in frog meat: a warning for the occurrence of human sparganosis. Trop Parasitol 2019; 9: 130-131.

[3] Nithiuthai S, Anantaphruti MT, Waikagul J and Gajadhar A. Waterborne zoonotic helminthiases. Vet Parasitol 2004; 126: 167-193.
[4] Berger L, Skerratt LF, Zhu XQ, Young S and Speare R. Severe sparganosis in Australian tree frogs. J Wildl Dis 2009; 45: 921-929.
[5] Bracaglia G, Ranno S, Mancinelli L, Santoro M, Cerroni L, Massone C, Sangueza O, Bravo FG, Diociaiuti A, Nicastri E, Muraca M, El Hachem M, Boldrini R, Callea F and Putignani L. A waterborn zoonotic helminthiase in an Italian diver: a case report of a cutaneous Sparganum infection and a review of European cases. Pathog Glob Health 2015; 109: 383-386.
[6] Griffin MP, Tompkins KJ and Ryan MT. Cutaneous sparganosis. Am J Dermatopathol 1996; 18: 70-72.
[7] Czyzewska J, Namiot A, Koziolkiewicz K, Matowicka-Karna J, Dzieciol J and Kemona H. The first case of human sparganosis in Poland and a review of the cases in Europe. Parasitol Int 2019; 70: 89-91.
[8] Kim JG, Ahn CS, Sohn WM, Nawa Y and Kong Y. Human sparganosis in Korea. J Korean Med Sci 2018; 33: e273.
[9] Yamashita K, Akimura T, Kawano K, Wakuta Y, Aoki H and Gondou T. Cerebral sparganosis mansoni. Report of two cases. Surg Neurol 1990; 33: 28-34.
[10] Anantaphruti MT, Nawa Y and Vanvanitchai Y. Human sparganosis in Thailand: an overview. Acta Trop 2011; 118: 171-176.
[11] Lu G, Shi DZ, Lu YJ, Wu LX, Li LH, Rao LY and Yin FF. Retrospective epidemiological analysis of sparganosis in mainland China from 1959 to 2012. Epidemiol Infect 2014; 142: 2654-2661.
[12] Liu Q, Li MW, Wang ZD, Zhao GH and Zhu XQ. Human sparganosis, a neglected food borne zoonosis. Lancet Infect Dis 2015; 15: 1226-1235.
[13] Li MW, Song HQ, Li C, Lin HY, Xie WT, Lin RQ and Zhu XQ. Sparganosis in mainland China. Int J Infect Dis 2011; 15: e154-156.
[14] Chen SH, Zhang YN, Li H, Cai YC and Chen JX. Analysis on parasitic infection of clinical samples from hospitals in Shanghai during 2011-2013. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 2014; 32: 446-451.
[15] Deng Y, Liu CJ, Chen WQ, Zhou RM, He LJ and Lin XM. Investigation on prevalence of Spirometra mansoni sparganum infection in frogs from Henan province. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi 2012; 24: 82-84.
[16] Lin XM, Liu CJ, Zhang HW, Zheng LY, Yan QY, He LJ and Zhao XD. Epidemiological investigation on sparganosis mansoni and animal experiments. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 2010; 28: 132-134.
[17] Pilsczek FH. Helminthic infections mimicking malignancy: a review of published case reports. J Infect Dev Ctries 2010; 4: 425-429.
[18] Hong Q, Feng J, Liu H, Li X, Gong L, Yang Z, Yang W, Liang X, Zheng R, Cui Z, Wang W and Chen D. Prevalence of spirometra mansoni in dogs, cats, and frogs and its medical relevance in Guangzhou, China. Int J Infect Dis 2016; 53: 41-45.
[19] Li H and Taylor DW. Vessel-bearing stems of ASOVINEA TIANIIgen. et sp. nov. (Gigantopteridales) from theUpper Permian of Guizhou province, China. Am J Bot 1999; 86: 1563-1575.
[20] Yamagishi M, Hori M, Fujikawa D, Ohsugi T, Honma D, Adachi N, Katano H, Hishima T, Kobayashi S, Nakano K, Nakashima M, Iwanaga M, Utsunomiya A, Tanaka Y, Okada S, Tsukasaki K, Tobinai K, Araki K, Watanabe T and Uchimaru K. Targeting excessive EZH1 and EZH2 activities for abnormal histone methylation and transcription network in malignant lymphomas. Cell Rep 2019; 29: 2321-2337, e2327.
[21] Polito U, Andreis ME, Di Giancamillo A, Modina SC, Scurati R, Marmotti A, Michielon G, Domenicucci M, Lombardo MDM, Di Giancamillo M, Herrera V, Mangiavini L, Agnoletto M, Brambilla L and Peretti GM. Clinical anatomy of the meniscus in animal models: pros and cons. J Biol Regul Homeost Agents 2020; 34: 197-202.
[22] Kim HR, Lee SM, Won JW, Lim W, Moon BI, Yang HJ and Seoh JY. Functional changes in regulatory T cells during an experimental infection with sparganum (plerocercofid of Spirometra mansoni). Immunology 2013; 138: 57-67.
[23] Zhang XP, Jiang SF, Hong GB, Fu YH, He YY, Ma XJ, Wang ZY, Zhang YG, Zhu Q and Ma XB. Investigation on food contamination with parasites in Shanghai market. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi 2012; 24: 404-409.
[24] Cui J, Wei T, Liu LN, Zhang X, Qi X, Zhang ZF and Wang ZQ. Molecular characterization of a Spirometra mansoni antigenic polypeptide gene encoding a 28.7 kDa protein. Parasitol Res 2014; 113: 3511-3516.
[25] Xie D, Wang M, Chen X and Tuo HZ. A case report: 1-year follow-up of cerebral sparganosis mansoni with a stroke-like onset. BMC Neurol 2019; 19: 105.
[26] Mao JZ, Zhou BJ, Wang KG and Wen M. Field investigation of sparganosis in frogs in Huaxi area. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 2009; 27: 187-188.
[27] Cho SY, Kang SY and Kong Y. Purification of antigenic protein of sparganum by immunoaffini-

ty chromatography using a monoclonal antibody. Kisaengchunghak Chapchi 1990; 28: 135-142.
[28] Simpson C, Jabbar A, Mansfield CS, Tyrrell D, Croser E, Abraham LA and Gasser RB. Molecular diagnosis of sparganosis associated with pneumothorax in a dog. Mol Cell Probes 2012; 26: 60-62.
[29] Khurana S, Appannanavar S, Bhatti HS and Verma S. Sparganosis of liver: a rare entity and review of literature. BMJ Case Rep 2012; 2012: bcr2012006790.
[30] Woldemeskel M. Subcutaneous sparganosis, a zoonotic cestodiasis, in two cats. J Vet Diagn Invest 2014; 26: 316-319.
[31] Zhang H, Cai C, Fang W, Wang J, Zhang Y, Liu J and Jia X. Oxidative damage and apoptosis induced by microcystin-LR in the liver of Rana nigromaculata in vivo. Aquat Toxicol 2013; 140-141: 11-18.
[32] Jia X, Yan R, Lin H, Liu Z, Shen L, Yang H, Wu H, Shan X and Zhang H. TBBPA and its alternative TCBPA induced ROS-dependent mitochondria-mediated apoptosis in the liver of Rana nigromaculata. Environ Pollut 2022; 297: 118791.
[33] Guangming G, Tao Z, Chao L and Moyan Z. Different morphologic formation patterns of dark patches in the black-spotted frog (Pelophylax nigromaculata) and the Asiatic toad (Bufo gargarizans). Anat Sci Int 2017; 92: 130-141.
[34] Yamada T, Nishio T, Sano Y, Kawago K, Matsuda K and Uchiyama M. Effects of arginine vasotocin and vasopressin receptor antagonists on Na+ and Cl- transport in the isolated skin of two frog species, Hyla japonica and Rana nigromaculata. Gen Comp Endocrinol 2008; 157: 63-69.