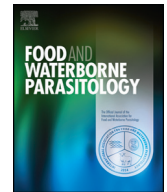




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An investigation of *Human Clonorchiasis* prevalence in an Endemic County in Guangxi Zhuang Autonomous Region, China, 2016



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ABSTRACT

To effectively promote the implementation of interventions, the identification of high-risk groups and the characteristics of *Clonorchis sinensis* infection in endemic regions are needed. In a clonorchiasis-endemic area, local residents were randomly enrolled for helminth egg examination in June 2016. The prevalence in subpopulations as well as the knowledge, attitudes, and behaviours and the factors influencing clonorchiasis in egg-positive populations were analysed. A total of 2282 local residents participated in the survey; the *C. sinensis* prevalence was 48.6% (1109 persons). A higher prevalence was found in males (62.6%) than in females (29.7%). People older than 30 years had the highest prevalence (52.7%–57.6%). Among the 888 persons who were infected with *C. sinensis* and participated the questionnaire investigation, 19.0% (169/888) knew that it could cause cancer. In addition, 60.6% of people reported that they intended to keep eating raw fish despite knowing the risk of infection. The two primary reasons for continuing to eat raw fish were the disease being regarded as not serious (38.3%) and the belief that anti-parasite medications are effective (39.6%). A total of 94.4% (797/844) of responders reported eating raw fish more frequently in the home than outside of the home. Our study revealed a notably high *C. sinensis* prevalence in the study area. Awareness of clonorchiasis disease severity should be increased among high-risk individuals and families in highly endemic areas.

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Abbreviations: *C. sinensis*, *Clonorchis sinensis*.

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1. Introduction

Clonorchiasis is one of the most neglected forms of food-borne trematodiasis; it is caused by infection with *Clonorchis sinensis* after eating raw or insufficiently cooked infected fish. The symptoms of clonorchiasis are related to the worm burden (Kim et al. 2011; Lun et al. 2005; Rim 1986). Hence, patients with a low worm burden are often asymptomatic or experience only mild symptoms, whereas patients with a high worm burden often experience nonspecific symptoms, such as asthenia, nausea, indigestion, or headache (Qian et al. 2016). Typical clinical symptoms of clonorchiasis are jaundice, hepatomegaly and liver tenderness. Furthermore, various complications involving the liver and biliary systems can develop as a result of chronic *C. sinensis* infection; cholelithiasis is the most common complication (Choi et al. 2008; Hou 1955; Qiao et al. 2012, 2014). Currently, *C. sinensis* infection is widely acknowledged to be associated with cholangiocarcinoma—bile duct cancer—and in 2009, *C. sinensis* was classified as a definitive carcinogen by the International Agency for Research on Cancer (IARC) (Bouvard et al., 2009; IARC 2011).

Approximately 15 million people are infected with *C. sinensis* worldwide, and 80% of infected individuals live in China (Qian et al. 2016). Two major endemic regions were identified in China: Guangdong Province and Guangxi Province located in southeast China, and Heilongjiang Province and Jilin Province located in northeast China (Fang et al. 2008; Qian et al. 2012). Three nationwide parasitic disease surveys were conducted in China in 1989–1992, 2001–2004 and 2014–2016 (Chen et al. 2020; Coordinating Office of the National Survey on the Important Human Parasitic Diseases 2005; Fang et al. 2008; Yu et al. 1994). The prevalence of clonorchiasis increased from 0.33% in the first survey to 0.58% in the second survey and then to 0.47% in the third survey, indicating that the number of infected people had increased from 5 million to 12.49 million and then decreased to 5.98 million (Coordinating Office of the National Survey on the Important Human Parasitic Diseases 2005; Fang et al. 2008; Tang et al. 2016a; Yu et al. 1994). Guangxi is one of the most severely impacted regions in China; the *C. sinensis* overall prevalence in Guangxi increased from 3.71% in the second survey (2001–2004) to 6.68% in the third nationwide survey (2014–2015) (Wan et al., 2019). Moreover, the incidence of cholangiocarcinoma, which is the most serious complication caused by *C. sinensis*, was high in South-East China, where Guangxi is located (de Martel et al. 2010). However, *C. sinensis* infection disproportionately occurs in endemic regions, and high-risk populations and risk factors for infection may vary according to the local settings in endemic areas.

To effectively and sustainably implement intervention measures in the context of local endemic areas, in this study, we aim to identify high-risk populations and the risk factors for *C. sinensis* infection in a clonorchiasis-endemic county in Guangxi Zhuang Autonomous Region.

2. Material and methods

2.1. Study sites

The study was carried out in Hengxian County, Nanning District, Guangxi Zhuang Autonomous Region, P.R. China (Fig. 1). According to previous studies, the main clonorchiasis-endemic region in Guangxi is located in the Zhujiang River basin, where Hengxian County is located (Jiang et al. 2015). Hengxian County has 17 towns and a population of approximately 1.3 million people. The annual disposable income per capita was 4469 dollars for urban residents and 1273 for rural residents in 2017. The area of Hengxian County comprises 3464 km² with an agriculture area of 1105 km². The main economic industry is agriculture products, including jasmine tea, corn, etc.

2.2. Study design and data collection

Among the 17 towns in Hengxian County, 7 were selected as survey areas according to a random sampling method, and two villages from each town were then further selected. In each surveyed village, 200 residents were randomly enrolled. A uniform questionnaire was used to collect the demographic information of all the enrolled study subjects. Furthermore, one stool sample was collected from each study participant. Triple Kato-Katz thick smears were prepared for each sample and examined for eggs under a light microscope by experienced and trained public health staff (Katz et al. 1972; Qian et al. 2013).

After the faecal examination, participants confirmed to be infected with *C. sinensis* without any other helminth infection were asked to complete a structured questionnaire that was administered by trained investigators. The questionnaire assessed the participants' awareness of clonorchiasis infection, its health outcomes and behaviours as risk factors of infection. The questionnaire contained four parts: demographic characteristics, previous illness history, knowledge of and attitude towards clonorchiasis, and behaviour related to *C. sinensis* infection.

2.3. Data analysis

The data were double-entered and cross-checked in EpiData 3.1 (<http://www.epidata.dk/>). Analyses were run in R statistical software (version 3.1.4, R Foundation for Statistical Computing, Vienna, Austria). The overall prevalence was computed by dividing the infected people with the overall subjects that were tested. Moreover, the prevalence for different population groups (i.e., according to sex, age, occupation and education level) were also calculated and compared. Age was classified into five categories (<15, 15–29, 29–44, 45–59, and > 59 years of age).

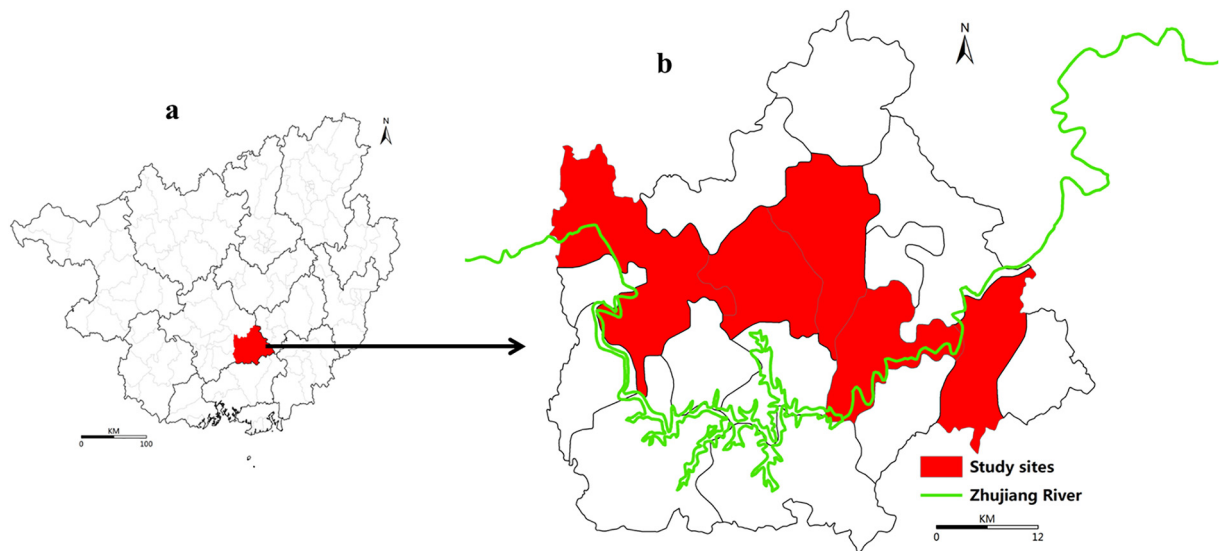


Fig. 1. The geographic location of the study area in Hengxian County, Guangxi Zhuang Autonomous Region (a. Location of Hengxian County in Guangxi Zhuang Autonomous Region. b. Location of the study area in Hengxian County).

The overall proportion of infected people who knew the infection route of *C. sinensis* and whether it caused cancer was calculated. Additionally, the attitudes towards *C. sinensis*, including whether one would continue to eat raw fish despite knowing it could lead to clonorchiasis and the main reasons for continuing to eat it, were analysed. The knowledge of and attitudes towards *C. sinensis* in the different sex and age groups were further calculated and compared. Pearson's χ^2 tests or Fisher's exact tests, when appropriate, were applied to assess the associations between categorical variables. A p -value lower than 0.05 was considered statistically significant.

3. Results

3.1. Prevalence of *C. sinensis* infection

A total of 2282 residents participated in our study; 1109 were infected with *C. sinensis*. The overall unadjusted prevalence was 48.6%. Among the 1109 people infected with *C. sinensis*, 7 (0.6%), 5 (0.5%) and 2 (0.2%) were also infected with hookworms, trichurids and pinworms, respectively. The prevalence among males (62.6%) was higher than that among females (29.7%) ($\chi^2 = 241.435$, $p < 0.01$). Adults in the 30–44 (55.3%), 45–59 (57.6%) and > 59 (53.0%) age groups had the highest prevalence, while children (4.8%) had a lower prevalence than those in the other age groups. Farmers (55.4%) had a higher prevalence than those in other occupations, and no significant difference was identified for people with different levels of education ($\chi^2 = 1.215$, $p > 0.05$) (Table 1).

3.2. Perception of *C. sinensis* and associated risk behaviours

Among the 1109 individuals with confirmed *C. sinensis* infection, 888 (80.1%) participated in the questionnaire-based investigation (Table 2). The overall percentage of individuals who knew the infection route of *C. sinensis* was 59.1%; this knowledge was more prevalent among males (61.6%) than among females (51.4%) ($\chi^2 = 7.172$, $p < 0.01$). No significant difference was found regarding knowledge of the infection route between the different age groups ($\chi^2 = 5.553$, $p > 0.05$). Nineteen percent of infected individuals knew that *C. sinensis* could cause cancer, with no significant difference between males (20.4%) and females (14.7%) ($\chi^2 = 3.552$, $p > 0.05$); however, a difference was identified among the different age groups ($\chi^2 = 8.135$, $p = 0.043$).

Among those who responded to the questionnaire, 92 (10.4%) had been previously diagnosed with clonorchiasis, and 68 (73.9%) of them had been treated with medicine. Thirty-eight (41.3%, 38/92) had not taken medicine at the last time of diagnosis, and the main reason for this was the belief that clonorchiasis is not serious (65.8%).

Eight hundred and forty-four infected people reported eating raw fish; most of them (57.1%) reported eating 1–11 times in the past year, and 50.9% reported eating at least 20 slices every time they ate raw fish. Most responders (94.4%) consumed raw fish at home (Table 2).

Regarding attitudes towards eating raw fish, 60.6% of people reported that they intended to keep eating raw fish despite knowing it could lead to clonorchiasis. The two main reasons for this risk behaviour were the belief that clonorchiasis is not

Table 1
Demographic and infection characteristics of the screened study subjects.

Characteristic	Total	No. infected (%)
Overall	2282	1109(48.6)
Sex		
Male	1312	821(62.6)
Female	970	288(29.7)
Age group		
Median (IQR)	50 (41–56)	
<15	289	14(4.8)
15–29	175	80(45.7)
30–44	499	276(55.3)
45–59	905	521(57.6)
>59	400	212(53.0)
Occupation		
Student or child	293	16(5.5)
Farmer	1958	1085(55.4)
Other	31	8(25.8)
Education		
Illiterate or primary school	483	263(54.5)
Junior high school	1158	663(57.3)
Senior high school or university	167	85(50.9)

Table 2
Characteristics, perceptions and risk behaviours with respect to clonorchiasis of those infected with *C. sinensis*.

Item	% (n/N)
Sex (n = 888)	
Male	75.5(670/888)
Female	24.5(218/888)
Age group (n = 888)	
15–29	6.3(56/888)
30–44	26.6(236/888)
45–59	50.0(444/888)
>59	17.1(152/888)
Occupation (n = 888)	
Farmer	96.4(856/888)
Other	3.6(32/888)
Education (n = 888)	
Illiterate or primary school	28.0(249/888)
Junior high school	62.7(557/888)
Senior high school or university	9.2(82/888)
Perception of clonorchiasis (n = 888)	
Knew the infection route of clonorchiasis	59.1(525/888)
Knew it causes cancer	19.0(169/888)
Clonorchiasis risk behaviours	
Frequency of eating raw fish (n = 844)	
<1 time in the past year	7.9(67/844)
1–11 times in the past year	57.1(482/844)
12–50 times in the past year	27.6(233/844)
>50 times in the past year	7.3(62/844)
Quantity of raw fish eaten (n = 844)	
≤1 slice ^a	4.9(41/844)
2–9 slices	18.1(153/844)
10–19 slices	26.1(220/844)
≥20 slices	50.9(430/844)
Most frequent location for raw fish consumption (n = 844)	
Restaurant or other	5.6(47/844)
Home	94.4(797/844)
Intend to keep eating raw fish despite knowing it can cause clonorchiasis (n = 888)	60.6(538/888)
Reasons for continuing to eat raw fish despite knowing it can cause clonorchiasis (n = 538)	
Clonorchiasis is not serious	38.3(206/538)
Anti-parasite medications are effective	39.6(213/538)
Others	22.1(119/538)

^a About 8–10 g per slice.

serious (38.3%) and the belief that anti-parasite medications are effective for treatment (39.6%). A significant difference in the intention to keep eating raw fish was found between males (68.2%) and females (37.2%) ($\chi^2 = 66.420, p < 0.01$). No statistically

significant difference was identified between the different age groups ($\chi^2 = 3.055, p > 0.05$). The leading reasons for the intention to keep consuming raw fish in each age group were similar to those in the overall age group.

4. Discussion

In this field survey, we found that the overall *C. sinensis* prevalence was strikingly high (48.6%). More than half of the infected individuals (59.1%) knew the infection route of *C. sinensis*, but only a small portion (19.0%) knew that *C. sinensis* could cause cancer. In addition, although male adults had greater awareness of the infection route of *C. sinensis* (61.1%) than adult females (51.4%), they had a higher proportion of infection (62.6%) and were less willing to change their behaviour of eating raw fish (68.2%) than females (29.7%, 37.2%, respectively). Most (94.4%) of the infected individuals reported eating raw fish more frequently at home than away from home.

The *C. sinensis* prevalence in Hengxian County identified in this study (48.6%) was much higher than the average rate in Guangxi Province (6.68%) (Wan et al., 2019), highlighting the disproportionate infection risk in endemic regions. The epidemiology of clonorchiasis is determined by local social-ecological systems, especially the consumption of raw freshwater fish (Phan et al. 2011; Zheng 2009), a practice that is passed from one generation to the next. In some areas, eating raw fish is strongly encouraged to protect traditional cultural habits (Zheng 2009). In addition to a lack of awareness regarding the presence, transmission, and effect of clonorchiasis on health, many people believe that raw fish are highly nutritious and that metacercariae can be killed by the concurrent consumption of alcohol or hot spices (Qian et al. 2016). Historically, a high prevalence of *C. sinensis* has been reported in villages in close proximity to bodies of freshwater used for aquaculture (Keiser and Utzinger 2005). Previous studies revealed that under the current expansion of aquaculture, rivers, lakes and water reservoirs are used to enhance inland fish cultivation, resulting in increased challenges for environmental management (Keiser and Utzinger 2005; Qian et al. 2016). Our study site was located in the central-southern part of Guangxi, which is rich in water resources, including 9 large inland rivers and 194 reservoirs that are heavily used for aquaculture. Thus, these resources provide suitable conditions for the growth of host snails and freshwater fish (such as *Squaliobarbus curriculus*, *Ctenopharyngodon idella* and *Oreochromis niloticus* which are the most common fish species making raw fish locally). Currently, in some local endemic village regions of China, toilets are built directly above or beside fish ponds, and allow unprocessed faeces to contaminate the water, which would increase *C. sinensis* infection in snails and freshwater fish (Huang et al. 2017). A previous study demonstrated that snails and freshwater fish in Hengxian County were significantly infected by cercariae and metacercariae (Xu et al. 2016). Furthermore, the economy of our study site has been rapidly developing in recent years, with the annual gross domestic product (GDP) increasing from 1.9 billion dollars in 2010 to 4.4 billion dollars in 2017. Economic development has promoted the development of aquaculture and made it economically feasible for more residents to include freshwater fish in their diet (Keiser and Utzinger 2005; Qian et al. 2014). Additionally, economic development improves trade and transportation channels, aiding the distribution of fish; thus, clonorchiasis is no longer restricted to villages located near bodies of water (Keiser and Utzinger 2005; Qian et al. 2016). All these environmental and socioeconomic factors might contribute to the high prevalence rate of *C. sinensis* in our study area.

Currently, the management of *C. sinensis* in highly endemic areas involves treatment with praziquantel (Qian et al. 2016; World Health Organization 2013) combined with information, education and communication (IEC) measures to enhance sustainability (Oh et al. 2014). Our study found that 59.1% of infected individuals knew the infection route of *C. sinensis*, indicating that approximately 40% of infected individuals did not know the infection route. Furthermore, even among those who knew the infection route, many, particularly adult males, reported that they would keep eating raw fish. One of the primary reasons was that the disease was regarded as not being serious. Therefore, additional health education about *C. sinensis*, particularly the severity of this disease (as a carcinogen), should be provided to people in highly endemic areas, especially adult males. For instance, in 2014 the China Central Television, which is the most influential media in China, broadcasted an episode on the habit of raw-fish eating and its potential to cause clonorchiasis. This program focused on the cholangiocarcinoma that the chronic *C. sinensis* infection might result in, providing wide-scale health education for Chinese, particularly to people experienced habits of raw fish consumption (Qian 2014). In addition, based on the fact that it is difficult to change the habit among adults in high endemic areas in China, health education implemented in school might be an alternative solution option in the long run (Tang et al. 2016b). Besides, remove the contaminated raw freshwater fish from market would also be an effective way to decrease the prevalence of clonorchiasis. Infection condition for freshwater fish and snails should be surveillance periodically, infected ponds should be marked under surveillance. Additionally, metacercaria-tainted fish should be barred from markets. Currently, PCR-based molecular techniques including nested PCR, real-time PCR, multiplex PCR and a LAMP have been reported to test the infection of freshwater fish and snails in recent years (Tang et al. 2016b). These test methods are more rapid, convenient and accurate compared to traditional microscope detection method (Tang et al. 2016b), which in turn could further preventing the contaminated freshwater fish come into markets.

Among those who ate raw fish, over 90% reported eating it most often at home. Although raw fish are not cooked, the preparation process is complex. For instance, the fish must be cut into ultra-thin slices without bleeding (Qian et al. 2013). Thus, it is possible that the preparation process of raw fish, which is usually the responsibility of the housewife at home, might introduce hygiene problems. Other foods, such as fruits and vegetables, could be contaminated by the blood or left-over material from the preparation of the freshwater fish, or cross-contamination could occur if the same cooking tools used to prepare the raw fish are used to prepare other foods. Children or females who do not eat raw fish might become infected by eating contaminated food. Previous studies have suggested that household hygiene is important in the control of parasite infections (Ellis and McManus 2009; Ellis et al. 2007). Therefore, family health education should be enhanced to change the high-risk behaviour of

eating raw fish. Additionally, family members of infected individuals should be actively screened for early detection and treatment.

Our study had the following three major limitations: First, not all the subjects who were infected with *C. sinensis* participated in our questionnaire investigation. However, we compared the basic demographic information between the analysis subjects and the excluded subjects, and no significant differences were identified; thus, this issue should have had a limited influence on the results. Second, the infection status of the first intermediate host, snails; the second intermediate host, freshwater fish; and the reservoir host, cats or dogs, was not investigated in the current study. Therefore, it was not clear whether high prevalences were also observed in these animals, which is an important factor for future control measures. Further studies are needed to explore this issue. Third, a previous study revealed that other minute intestinal flukes were highly endemic in our study area, with the prevalence of *Haplorchis taichui* being 57.08% in people around our study site (Yang et al. 2012). Based on the fact that it is somewhat difficult to differentiate the eggs between *C. sinensis* and minute intestinal flukes by using the Kato-Katz method, our results might be influenced. However, in our study, each stool sample was examined by three different experienced people, and the stool could not be classified as *C. sinensis* positive until all three people reached consensus by obtaining the same results. This quality control measure could decrease the misdiagnosis in our study.

Based on our study, the awareness of *C. sinensis* was inadequate in both males and females, particularly with respect to the severity of this infection and its link with cancer. In addition, the main setting for eating raw fish at home needs to be addressed. More efforts should be taken to enhance awareness of this disease, especially its severity (as a carcinogen), which may contribute to reducing the consumption of raw fish in endemic areas.

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Role of the funding source

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability

Data supporting the conclusions of this article are included within the article. The datasets generated during and/or analysed during the present study are available from the corresponding author upon reasonable request.

Ethical approval

This study was approved by the ethics committees of the Chinese Center for Disease Control and Prevention (Approval Notice no: 201617). The objectives, procedures and potential risks were orally explained to all participants. A written consent form was also obtained from each participant, with their own signature or the signature of a proxy.

Informed consent

Not applicable

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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