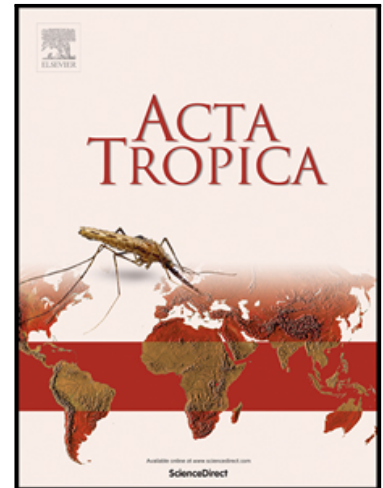


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## **Epidemiology and determinants of clonorchiasis in school children in southeastern China**

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## Abstract

Clonorchiasis is an important food-borne parasitic disease in China because of the popularity in ingesting raw freshwater fish. To explore the epidemiology and determinants of clonorchiasis in children, a cross-sectional survey was implemented in two middle schools in Qiyang county, Hunan province, in southeastern China. Questionnaire survey and fecal examination were implemented. Questionnaires were filled back by 627 students, while stool samples were collected from 557 students, out of which 545 ones also provided questionnaire information. The percentage of students ingesting raw freshwater fish was 40.5% (254/627), while the prevalence with *Clonorchis sinensis* infection was 18.9% (105/557). Such factors contributed significantly to the students' practice in eating raw freshwater fish including boys, fathers' eating raw freshwater fish, mothers' eating raw freshwater fish, and preparation of raw freshwater fish at home, with an adjusted odds ratio of 1.9 (95% confidence intervals (95% CI): 1.3-2.8), 3.9 (95% CI: 2.3-6.5), 3.0 (95% CI: 1.8-4.8) and 2.8 (95% CI: 1.8-4.5), correspondingly. Ingestion of raw freshwater fish, fathers' eating raw freshwater fish and preparation of raw freshwater fish at home were risk factors of *C. sinensis* infection in students, and the adjusted odds ratio was 3.2 (95% CI: 1.9-5.5), 2.1 (95% CI: 1.1-3.9) and 1.8 (95% CI: 1.0-3.2), respectively. Thus, clonorchiasis was endemic in the surveyed schools due to the ingestion of raw freshwater fish, which is influenced by family environment. Education should be implemented in schools to promote behavioral change of eating raw freshwater fish.

**Keywords:** Clonorchiasis; *Clonorchis sinensis*; Epidemiology; Determinants; Students

## 1. Introduction

Infections with the human liver flukes, *Clonorchis sinensis*, *Opisthorchis viverrini* and *O. felineus*, cause high burden in East Asia and part of Europe due to the habit of ingesting raw freshwater fish (Qian et al., 2012; Qian et al., 2016; Suwannatrai et al., 2018). These liver flukes cause significant disability, especially the damage on liver and biliary system. Early symptoms are usually mild, e.g. diarrhea and abdominal pain (Chen et al., 1994; Kim et al., 1982). However, long and chronic infection leads to severe complications including gallstone and cholecystitis (Fedorova et al., 2020; Oh et al., 2014; Qiao et al., 2012). Particularly, both *C. sinensis* and *O. viverrini* are definite carcinogens to humans, causing fatal cholangiocarcinoma (Bouvard et al., 2009; Qian and Zhou, 2017).

Globally, about 25 million people are infected with one of these liver flukes (Qian and Zhou, 2019). Clonorchiasis is endemic in China, the Republic of Korea, northern Vietnam and part of Russia (Chen et al., 2012; Doanh and Nawa, 2016; Jeong et al., 2016), opisthorchiasis *viverrini* in several countries in Southeast Asia including Thailand, Laos, Cambodia, southern Vietnam and Myanmar (Aung et al., 2017; Doanh and Nawa, 2016; Miyamoto et al., 2014; Suwannatrai et al., 2018; Vonghachack et al., 2017), while opisthorchiasis *felineus* in Russia and neighboring countries (Fedorova et al., 2017; Pakharukova and Mordvinov, 2016). Especially, 13 million people are afflicted by clonorchiasis in China (Chen et al., 2012; Qian et al., 2012). In comparison to the significant control and even elimination of many other helminthiases, clonorchiasis is still highly endemic in northeastern and southeastern China, which is attributable to the rooted dietary habit-ingestion of raw freshwater fish and rapid development in aquaculture (Jiang et al., 2015; Qian et al., 2019a; Yang et al., 2014).

The epidemiological profiles of these human liver fluke infections are characterized by the differential prevalence in sex and ages (Fedorova et al., 2020; Qian et al., 2012; Suwannatrai et al., 2018). Males show a higher prevalence compared to females, and prevalence increases by ages. These characteristics are dominated by the difference in ingesting raw freshwater

fish (Qian et al., 2020b). Thus, adults especially men are usually targeted in control. Similarly, few researches have been implemented to explore risk factors of human liver fluke infections in children (Qian and Zhou, 2019). Thus, it is necessary to understand the epidemiological profiles in children. This will not only benefit the adoption of intervention in children, but also promote the overall understanding on transmission of human liver fluke infections in communities. Thus, in this study, a cross-sectional survey was carried out in Qiyang county, Hunan province in southeastern China to capture the epidemiology and determinants of clonorchiasis in school children.

## **2. Materials and methods**

### ***2.1 Study design and study areas***

This study was a cross-sectional study implemented in 2013, aiming to explore the epidemiology and determinants of clonorchiasis in middle school children in Qiyang county, Hunan province. Qiyang county is located in southeast of Hunan province, while Hunan province is located in southern China. A town called Dazhongqiao was selected, where clonorchiasis was endemic (Duan et al., 2009). The town had two middle schools, with 473 and 172 students in 2013, respectively. All students in the two schools were eligible.

### ***2.2 Investigation procedures***

This study contained two parts. A questionnaire was distributed to each student to collect the information on knowledge and practice. The knowledge involved in transmission route and damage of clonorchiasis. The damage referred to the early symptoms (diarrhea and abdominal pain), chronic complications (gallstone and cholecystitis), and severe fatal sequelae (carcinogenicity). Such practices were collected as ingestion of raw freshwater fish in students and their parents, preparation of raw freshwater fish at home, presenting raw freshwater fish to neighbors and accepting raw freshwater fish from neighbors.

Stool containers were then distributed and two stool samples were asked to submit by each student. Stool samples were transferred to local Center for Disease Control and Prevention. Then, the Kato-Katz method using 41.7 mg templates was used to examine helminth eggs

quantitatively and three thick smears were prepared for each sample, which was described in other report (Qian et al., 2019b).

### **2.3 Ethics**

This study was approved by the ethics committee in the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (reference no. 2012–03). A written informed consent form was obtained from the guardian of each participant.

### **2.4 Statistical analysis**

Due to the limited number of students aged below 12 and over 15, ages were classified into  $\leq 12$ , 13, 14 and  $\geq 15$  years old. Eggs per gram of feces (EPG) was calculated for individual by multiplication of the arithmetic average of eggs in available smears by 24. Correspondingly, geometric mean of EPG (GMEPG) and corresponding standard deviation (SD) were calculated for the positive cases. The light, moderate and heavy infections were classified by two cut-off values, namely 1000 and 10000 respectively. Pearson's Chi-square test was applied to compare the prevalence by sex and ages, while Student's t-test and analysis of variance were respectively used to compare EPG after logarithmic transformation. Pearson's Chi-square test was also employed to compare the knowledge and practice by sex and ages. Univariable and multivariable logistic regression analyses were sequentially employed to explore risk factors, in which crude odds ratio (cOR), adjusted odds ratio (aOR) and corresponding 95% confidence intervals (95% CI) were provided. Two multivariable regression models were applied. The first one was used to capture risk factors of students' ingestion of raw freshwater fish, while the second one to explore those of *C. sinensis* infection in students. Wald forward method was applied in selection of variables in multivariable logistic regression. A P value less than 0.05 was set as significance.

## **3. Results**

### **3.1 Profiles of students and infection status**

Overall, 627 students submitted questionnaire and 557 students provided at least one stool sample. Among them, 545 students submitted both stool and questionnaire.

Among 557 students, there were 282 boys (50.6%) and 275 girls (49.4%). Age ranged from 10 to 17, with an average of 13.5. Most (523, 93.9%) ranged between 12 and 15. Among them, the number detected with *C. sinensis*, *Trichuris trichiura*, and *Ascaris lumbricoides* was 105 (18.9%), 29 (5.2%) and 4 (0.7%). No hookworm was detected in this study.

The EPG of *C. sinensis* infection ranged between 4 and 4904, with a GMEPG of 75. Most students (97, 92.4%) belonged to light infection and another 8 belonged to moderate infection (7.6%). The prevalence of *C. sinensis* infection was 18.4% (52/282) in boys and 19.3% (53/275) in girls ( $\chi^2=0.063$ ,  $P=0.802$ ). The GMEPG ( $\pm$ SD) was 81 ( $\pm$ 6) in boys and 70 ( $\pm$ 6) in girls ( $t=0.457$ ,  $P=0.648$ ). The prevalence was 13.7% (18/131) in aged below 12 years, 19.6% (31/158) in aged 13 years, 20.3% (31/153) in aged 14 years and 21.7% (25/115) in aged over 15 years, but the increasing trend was nonsignificant ( $\chi^2=3.124$ ,  $P=0.373$ ). The corresponding GMEPG ( $\pm$ SD) was 76 ( $\pm$ 4), 65 ( $\pm$ 7), 68 ( $\pm$ 5) and 101 ( $\pm$ 6), which was also nonsignificant ( $F=0.338$   $P=0.798$ ).

### 3.2 Knowledge in students

Among 627 students with questionnaire information, 143 (22.8%) knew transmission route of clonorchiasis (**Table 1**). This piece of knowledge was higher in boys (26.9%) than girls (18.4%) ( $\chi^2=6.448$ ,  $P=0.011$ ), and higher in older students ( $\chi^2=10.468$ ,  $P=0.015$ ). The proportion with knowledge on early symptoms, chronic complications and carcinogenicity was 18.8%, 6.2% and 2.6%, respectively. The knowledge on chronic complications was higher in boys (8.7%) than girls (3.6%) ( $\chi^2=6.848$ ,  $P=0.009$ ), but no difference was demonstrated in different ages ( $\chi^2=0.527$ ,  $P=0.913$ ). No difference was demonstrated in sex or ages as to knowledge on early symptoms and carcinogenicity.

<Table 1 near here>

### 3.3 Practice in students and their families

In total, 254 students (40.5%) reported ingestion of raw freshwater fish, which was higher in boys (47.1%) than girls (33.6%) ( $\chi^2=11.854$ ,  $P<0.001$ ). Although an increasing trend by ages in ingesting raw freshwater fish was shown, it was not significant ( $\chi^2=0.857$ ,  $P=0.836$ ). The proportion of families with fathers' eating raw freshwater fish was 48.6% and that with mothers' practice was 31.3% ( $\chi^2=228.290$ ,  $P<0.001$ ) (**Figure 1**). In total, 39.7% students reported the preparation of raw freshwater fish by their households, 7.3% reported the presentation of raw freshwater fish to neighbors and 14.2% reported the acceptance of raw freshwater fish from neighbors.

<Figure 1 near here>

### 3.4 Epidemiological determinants

In univariable logistic regression, sex, fathers' eating raw freshwater fish, mothers' eating raw freshwater fish, preparation of raw freshwater fish at home, presentation of raw freshwater fish to neighbors and acceptance of raw freshwater fish from neighbors were significantly correlated to the ingestion of raw freshwater fish in students (**Table 2**). However, in multivariable model, only sex (boys), fathers' eating raw freshwater fish, mothers' eating raw freshwater fish, and preparation of raw freshwater fish at home were significant, and the aOR was 1.9 (95% CI: 1.3-2.8), 3.9 (95% CI: 2.3-6.5), 3.0 (95% CI: 1.8-4.8) and 2.8 (95% CI: 1.8-4.5), correspondingly.

<Table 2 near here>

In univariable logistic regression, students' ingestion of raw freshwater fish, fathers' eating raw freshwater fish, mothers' eating raw freshwater fish, and preparation of raw freshwater fish at home were significantly related to *C. sinensis* infection in students (**Table 3**). In multivariable regression, only students' ingestion of raw freshwater fish, fathers' eating raw freshwater fish, and preparation of raw freshwater fish at home were significant, and the aOR was 3.2 (95% CI: 1.9-5.5), 2.1 (95% CI: 1.1-3.9) and 1.8 (95% CI: 1.0-3.2), respectively.



<Table 3 near here>

#### 4. Discussion

This study demonstrated the high prevalence of clonorchiasis in school children in one county in southeastern China. On comparison, the prevalence of soil-transmitted helminthiasis was quite low. Thus, children should not be neglected in clonorchiasis endemic areas. The high prevalence of clonorchiasis is strongly related to the high popularity in ingesting raw freshwater fish there. Additionally, most students submitted two samples in this study, which promoted the diagnostic sensitivity (Qian et al., 2019b). However, most infection belonged to light intensity and no heavy intensity was detected, which should be attributable to the low frequency in eating raw fish.

The knowledge on the harm could increase the awareness to prevent clonorchiasis, while that on the transmission route guides the adoption of proper prevention measures. Thus, these two aspects were both inquired through questionnaire survey. Unsurprisingly, the knowledge in students was quite low, which is consistent to other reports (Qian et al., 2013; Qian et al., 2020b). Particularly, knowledge on severe complications and carcinogenicity was lower compared to that on transmission route and early symptoms. Few students had gone through the experiences of these severe conditions (complications and cancer), and thus it is difficult for children to capture such knowledge unless they had received special education. Especially, carcinogenicity of *C. sinensis* had not been ascertained until 2009, which could explain the extreme low awareness on it (Bouvard et al., 2009).

The proportion in ingestion of raw freshwater fish was higher in boys than girls, which indicates early divergence of raw-eating practice in different sexes. This is consistent to the higher percentage of ingesting raw freshwater fish in fathers than mothers in this study as well as the higher popularity of raw-eating practice in adult males compared to adult females in other reports (Qian et al., 2013). However, the difference by ages was nonsignificant. That is probably attributed to the narrow range of ages in this study. The percentage of ingesting raw freshwater fish was higher in fathers than that in mothers, which is consistent to the higher prevalence of clonorchiasis in men than women (Chen et al., 2012; Qian et al., 2014). Preparing raw freshwater fish at home was also frequent in this study. What is interesting is

the presentation and acceptance of raw freshwater fish as gifts between neighbors. This is firstly documented in clonorchiasis endemic areas in China, although such food-share had been reported in clonorchiasis endemic areas in Vietnam and opisthorchiasis endemic areas in Thailand (Phimpraphai et al., 2018; Saenna et al., 2017; Vinh et al., 2017). Only 7.3% students reported the presentation of raw freshwater fish to neighbors at their homes, while 14.2% ones reported the acceptance of such dish from neighbors. When children are at home, their neighbors are more likely to presenting dish, which shows high kindness to children. However, the children of the neighbors presenting raw fish are probably not at home and thus don't witness this process. Obviously, this phenomenon demonstrates the low awareness on harm of ingesting raw freshwater fish in adults.

Logistic regression analysis further demonstrates the epidemiological determinants of ingesting raw freshwater fish and *C. sinensis* infection in children. Sex, fathers' and mothers' ingestion of raw freshwater fish, preparation of raw freshwater fish at home, presentation and acceptance of raw freshwater fish between neighbors were all significantly related to the ingestion of raw freshwater fish in students in univariable regression. However, presentation and acceptance of raw freshwater fish between neighbors were excluded in multivariable analysis, which is probably attributed to the collinearity of these factors with other ones, e.g. preparation of raw freshwater fish at home. These findings demonstrate the importance of family environment in the development of raw-eating practice in children. This further supports our recent finding of familial assimilation theory which drives the transmission of ingesting raw freshwater fish among family members (Qian et al., 2020b). Further analysis on the risk factors of *C. sinensis* infection demonstrated that raw-eating practice in children is of crucial importance in transmission of *C. sinensis*, indicated by a high aOR. However, after adjusted by the practice in children themselves, fathers' raw-eating practice and households' preparation of raw freshwater fish were still significantly related to the infection in children. This indicates that food contamination during preparation and consumption also contributes to *C. sinensis* infection.

Our studies have important implications. Students should not be neglected and schools need to be integrated in the control of clonorchiasis. Education should be implemented to increase the awareness on harm and prevention of clonorchiasis (Qian et al., 2020b). The recent pilot in primary schools demonstrates the performance of comprehensive education in increasing the knowledge, decreasing the practice and strengthening the belief related to ingestion of raw freshwater fish in pupils, which should also be tested in middle and high

schools (Qian et al., 2020a). Additionally, education at home couldn't be neglected. Parents could be told that their ingestion of raw freshwater fish not only causes *C. sinensis* infection to themselves but also to their children through behavioral transmission and food contamination. It also needs to be emphasized that the presentation of raw freshwater fish to neighbors is not desirable.

This study had limitations, which are expected to be explored in future. The practice in ingesting raw freshwater fish is complex including interaction among family members. Thus, more quantitative information needs to be captured, e.g. the amount, occasion, frequency and duration in ingesting raw fish in different family members. Our study indicates the potential interaction among neighbors in ingesting raw freshwater fish in communities. Thus, further social analysis will be useful. The caption of information above will promote the understanding of the transmission of the practice and benefit the design of a more precise strategy against clonorchiasis.

## 5. Conclusions

Clonorchiasis is high prevalent in children in this county due to the high popularity in ingesting raw freshwater fish. On the comparison, students have a little knowledge on transmission and harm of clonorchiasis, especially the latter. The raw-eating practice in students is highly impacted by their family environment including the raw-eating practice of parents and preparation of raw freshwater fish at home. *C. sinensis* infection in students is majorly determined by raw-eating practice of themselves. However, fathers' raw-eating practice and preparation of raw fish at home are also contributors, which indicates the food contamination during preparation and consumption. Education is urgently needed through school platform to promote the behavioral change in students, and the parents should also be persuaded through community education to avoid ingesting raw freshwater fish for the sake of both themselves and their children.

### Author statement:

**Men-Bao Qian:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration

**Shi-Feng Zhuang:** Investigation, Resources, Supervision, Project administration

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**Zheng-Xiang Li:** Conceptualization, Resources, Supervision, Project administration

**Xiao-Nong Zhou:** Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition

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**Table 1.** Knowledge and practice related to clonorchiasis in students

Knowledge and practice	Groups	Yes	No	Percentage (%)	$\chi^2$	P
Knowledge-transmission route	All	143	484	22.8	6.448	0.011
	Boys	87	236	26.9		
	Girls	56	248	18.4		
	≤12	22	116	15.9	10.468	0.015
	13	43	134	24.3		

	14	36	143	20.1		
	≥15	42	91	31.6		
	All	118	509	18.8		
Knowledge-early symptoms (diarrhea/abdominal pain)	Boys	59	264	18.3	0.134	0.715
	Girls	59	245	19.4		
	≤12	24	114	17.4		
	13	34	143	19.2	0.740	0.864
	14	32	147	17.9		
	≥15	28	105	21.1		
	All	39	588	6.2		
Knowledge-chronic complications (gallstone/cholecystitis)	Boys	28	295	8.7	6.848	0.009
	Girls	11	293	3.6		
	≤12	8	130	5.8		
	13	10	167	5.6	0.527	0.913
	14	11	168	6.1		
	≥15	10	123	7.5		
	All	16	611	2.6		
Knowledge- carcinogenicity	Boys	8	315	2.5	0.015	0.902
	Girls	8	296	2.6		
	≤12	3	135	2.2		
	13	9	168	5.1	6.715	0.082
	14	2	177	1.1		
	≥15	2	131	1.5		
	All	254	373	40.5		
Practice-eating raw freshwater fish	Boys	152	171	47.1	11.854	<0.001
	Girls	102	202	33.6		
	≤12	53	85	38.4	0.857	0.836



13	70	107	39.5
14	73	106	40.8
≥15	58	75	43.6

**Table 2.** Univariable and multivariable logistic regression analysis on students' ingestion of raw freshwater fish

Determinants	No. without eating	No. with eating	Univariable regression		Multivariable regression*		
			cOR (95% CI)	P	aOR (95% CI)	P	
Sex							
Girls	202	102					
Boys	171	152	1.8 (1.3-2.4)	<0.001	1.9 (1.3-2.8)		0.002
Age (years)				0.836			
≤12	85	53					
13	107	70	1.0 (0.7-1.7)	0.837			
14	106	73	1.1 (0.7-1.7)	0.668			
≥15	75	58	1.2 (0.8-2.0)	0.384			
Fathers' eating raw freshwater fish							
No	275	47					
Yes	98	207	12.4 (8.4-18.3)	<0.001	3.9 (2.3-6.5)		<0.001
Mothers' eating raw freshwater fish							
No	322	109					
Yes	51	145	8.4 (5.7-12.4)	<0.001	3.0 (1.8-4.8)		<0.001

Preparing raw freshwater fish at home							
No	297	81					
Yes	76	173	8.3 (5.8-12.0)	<0.001	2.8 (1.8-4.5)	<0.001	
Presenting raw freshwater fish to neighbors							
No	357	224					
Yes	16	30	3.0 (1.6-5.6)	<0.001			
Accepting raw freshwater fish from neighbors							
No	339	199					
Yes	34	55	2.8 (1.7-4.4)	<0.001			

\* Sex, fathers' eating raw freshwater fish, mothers' eating raw freshwater fish and preparing raw freshwater fish at home were included.

**Table 3.** Univariable and multivariable logistic regression analysis on students' infection with *C. sinensis*

Determinants	No. without infection	No. with infection	Univariable regression		Multivariable regression <sup>*</sup>	
			cOR (95% CI)	<i>P</i>	aOR (95% CI)	<i>P</i>
Sex						
Girls	222	51				
Boys	221	51	1.0 (0.7-1.5)	0.984		
Age (years)				0.400		
≤12	111	18				
13	123	30	1.5 (0.8-2.8)	0.210		

14	122	29	1.5 (0.8-2.8)	0.243		
≥15	87	25	1.8 (0.9-3.5)	0.093		
<hr/> Students' eating raw freshwater fish						
No	313	30				
Yes	130	72	5.8 (3.6-9.3)	<0.001	3.2 (1.9-5.5)	<0.001
<hr/> Fathers' eating raw freshwater fish						
No	268	23				
Yes	175	79	5.3 (3.2-8.7)	<0.001	2.1 (1.1-3.9)	0.027
<hr/> Mothers' eating raw freshwater fish						
No	338	48				
Yes	105	54	3.6 (2.3-5.7)	<0.001		
<hr/> Preparing raw freshwater fish at home						
No	306	34				
Yes	137	68	4.5 (2.8-7.1)	<0.001	1.8 (1.0-3.2)	0.039
<hr/> Presenting raw freshwater fish to neighbors						
No	416	93				
Yes	27	9	1.5 (0.7-3.3)	0.320		
<hr/> Accepting raw freshwater fish from neighbors						
No	387	88				
Yes	56	14	1.1 (0.6-2.1)	0.768		

\* Students' eating raw freshwater fish, fathers' eating raw freshwater fish and preparing raw freshwater fish at home were included.

## Legends to figures

**Fig. 1** The proportion of practice related to eat raw freshwater fish

