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Rapid screening of *Clonorchis sinensis* infection: Performance of a method based on raw-freshwater fish-eating practice

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ABSTRACT

Clonorchis sinensis infection is caused by ingestion of raw freshwater fish containing the infective larvae of *Clonorchis sinensis*. It is highly endemic in East Asia, especially in China. Selective chemotherapy of people who report habitual eating of raw freshwater fish is a control measure. As the performance of this screening technique has not yet been fully evaluated in China, a cross-sectional study was conducted, covering 17 counties in four major clonorchiasis-endemic provinces. About 1 000 participants were enrolled from each county. Fecal samples were collected and examined for helminth eggs and each person enrolled was asked about their practice with respect to eating raw freshwater fish. In total, 16 230 participants from 16 counties were finally included. The overall prevalence of *C. sinensis* infection was 10.8%, ranging from 0 to 53.7% in the 16 counties, while the percentage of inhabitants eating raw freshwater fish was 26.5%, ranging from 0 to 79.1%. The overall sensitivity and specificity of screening for *C. sinensis* infection in this approach was 82.3% and 80.3%, respectively, yielding a Youden's index of 0.6. The overall positive and negative likelihood ratios were 4.2 and 0.2, respectively, while the overall positive and negative predictive values were 33.5% and 97.4%, respectively. Furthermore, the sensitivity was higher with regard to high-intensity infections compared to light infections.

1. Introduction

Clonorchis sinensis, Opisthorchis viverrini and *O. felineus* are common in freshwater fish in East Asia and thus infections with these human liver flukes are high endemic in certain areas due to the habit of consuming such raw fish (Harrington et al., 2017; Qian et al., 2016; Sithithaworn et al., 2012). Globally, an estimation of about 25 million people are afflicted (Qian et al., 2013). Among them, 15 million with *C. sinensis* infection live in China, the Republic of Korea, northern Vietnam and part of Russia; 8.6 million with *O. viverrini* infection live in Southeast Asia, including Thailand, Laos, Cambodia and southern Vietnam; while about 1.5 million people in Russia and some other European countries were estimated to be infected with *O. felineus* (Qian et al., 2012, Qian et al., 2013).

These human liver flukes exert significant loss of healthy lives. Although the symptoms are mild and non-specific at early stage, chronic and long-term infection lead to diverse complications in the liver and biliary system, including cholecystitis, cholangitis, gallstone, etc. (Qiao et al., 2012; Rim, 1986; Upatham et al., 1984). It is argued that the disability weight of clonorchiasis from those sequences, excluding cholangiocarcinoma, is even comparable to that of hepatitis B (Qian et al., 2011, Qian et al., 2013). Especially, both *C. sinensis* and *O. viverrini* are definite human carcinogens, causing fatal cholangiocarcinoma (Bouvard et al., 2009; Brindley et al., 2015). Globally, a conservative estimation of about 7 000 cholangiocarcinoma cases attributable to *C. sinensis* and *O. viverrini* infections occur annually (Fürst et al., 2012; Qian and Zhou, 2017). In humans, the damage caused by these liver flukes is significantly correlated to the worm burden (Qian et al., 2011; Rim, 1986; Upatham et al., 1984). Furthermore, the adult worms survive in human bodies for decades (Attwood and Chou, 1978). Thus,

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treatment with anthelmintic drugs is necessary to control the morbidity, especially for those with heavy infection (Choi et al., 2010; World Health Organization, 2019).

Three treatment strategies could be adopted for human liver fluke infections. Individual treatment is administrated to those with a definite diagnosis. As to the public health control, preventive chemotherapy for whole community and selective chemotherapy for people at risk are advocated after carrying out community diagnosis (Choi et al., 2010; World Health Organization, 2019). In high-risk areas (e.g. prevalence over 20%), mass chemotherapy for whole community is implemented, while in low-risk areas (e.g. prevalence less than 20%), both mass chemotherapy for the whole community and selective chemotherapy for individuals reporting practice of eating raw freshwater fish are advocated (World Health Organization, 2019). A proper treatment strategy determines the effectiveness and cost of control efforts. Usually, selective chemotherapy is simple and rapid, while individual diagnosis is expensive and the cost for drugs and delivery is also high in mass chemotherapy. In China, selective chemotherapy for those at risk of *C. sinensis* infection, namely people eating raw freshwater fish, is recommended (National Health and Family Planning Commission, 2016). A study in Vietnam showed a moderate performance in screening for *C. sinensis* infection by the practice consuming raw freshwater fish: 100% sensitivity and 78% specificity in low endemic areas (a prevalence of 1%) and 98% sensitivity and 58% specificity in highly endemic areas (a prevalence of 25%) (Yajima et al., 2009). In this study, we aimed to evaluate the performance of this rapid screening technique as this has not yet been done in China.

2. Material and methods

2.1. Study design

China harbors over 85% of global clonorchiasis cases (about 13 million) (Qian et al., 2012, Qian et al., 2013). These cases are predominantly found in four highly endemic provinces in eastern China, namely Guangdong, Guangxi, Heilongjiang and Jilin, which harbor over 90% of clonorchiasis cases in China (Coordinating Office of the National Survey on the Important Human Parasitic Diseases, 2005; Fang et al., 2008). In these endemic areas raw freshwater fish, which is called "*yusheng*" in Chinese, is commonly enjoyed by the habitants (Zheng, 2009). The fish is cleaned and sliced into small flesh, which is then enjoyed directly with alcohol and condiments (e.g. ginger, onion, garlic and other vegetables).

In this study we only evaluated one factor, namely the practice of eating raw freshwater fish ("*yusheng*"). The study area was confined to the four highly endemic provinces mentioned.

2.2. Study site and population

In 2017, 17 counties were selected from four provinces, namely Guangxi (6 counties), Heilongjiang (5 counties), Guangdong (3 counties) and Jilin (3 counties). Each county was first classified into five zones based on economic and topography (Qian et al., 2018), and one town was then randomly selected from each zone followed by selection at random of one community (or village) from each town. In all, about 200 habitants were selected as participants from each community making a total study group of about 1 000 persons from each county.

2.3. Investigation procedures

Each participant was asked to report his or her practice of eating raw freshwater fish (yes or no). A stool container was distributed to each participant for collecting one fresh stool sample. The samples were taken to local technical organizations (Centers for Disease Control and Prevention (CDC)).

The Kato-Katz thick smear method was applied (Hong et al., 2003; Qian et al., 2019). Two thick smears were prepared from each stool sample and examined microscopically by experienced technicians for helminth eggs.

2.4. Ethics

The study was approved by the Ethics Committee in the National Institute of Parasitic Diseases, China CDC. The objectives, procedures and potential risks of this study were orally explained and informed to all participants. A written consent form was also obtained with signature of the participant or his/her guardian for a child.

2.5. Statistical analysis

The infection was defined as at least one egg detected in one of the two smears. The infection intensity in terms of eggs per gram (EPG) of feces was calculated by multiplying the average number of eggs in two smears by a factor of 24. Because the EPG was lower than 1 000 in most cases, the intensity of infection was classified into three groups based on the EPG, namely light infection ($0 < EPG < 72$), moderate infection ($72 \leq EPG < 168$) and heavy infection ($EPG \geq 168$).

The prevalence of *C. sinensis* infection was defined as the proportion of persons with *C. sinensis* infection out of all participants. Prevalence was presented by county. Fecal examination was the 'gold standard' and the performance of the screening technique was evaluated based on such indicators as sensitivity, false negative rate (FNR), specificity, false positive rate (FPR), Youden's index, positive likelihood ratio (+LR), negative likelihood ratio (-LR), positive predictive value (PPV) and negative predictive value (NPV). The sensitivity in different infection intensities was also provided, and Pearson χ^2 test was employed to detect the difference. A *p*-value less than 0.05 was set as statistical significance.

3. Results

In one county, people reported they consumed raw marine fish and not freshwater fish, and thus this county was excluded. Finally, 16 230 participants were enrolled from 16 counties.

3.1. Epidemiology of C. sinensis infection and raw-freshwater fish-eating practice

Out of the 16 230 participants enrolled, 1 747 persons (10.8%) were infected with *C. sinensis* (Table 1 and Fig. 1). Two counties were free from infection, while the prevalence was lower than 1% in four counties, 1%−10% in four counties, 10%−20% in four counties, 20%−30% in one county and over 50% in one county. Among the participants, 4 295 reported a history of eating raw freshwater fish, a proportion of 26.5%. No person reported this practice in three counties and the highest proportion reached 79.1%.

3.2. Screening performance

The overall sensitivity and FNR in 16 counties were 82.3% (1 438/1 747) and 17.7% (309/1 747), respectively (Table 1 and Fig. 2). The overall specificity and FPR were 80.3% (11 626/14 483) and 19.7% (2 857/14 483), respectively. The PPV and NPV values were 33.5% (1 438/4 295) and 97.4% (11 626/11 935), respectively. Youden's index was 0.6, +LR was 4.2 and -LR was 0.2.

The sensitivity could not be calculated in two counties because the infection was zero. The sensitivity was 0 in one county, whose prevalence of *C. sinensis* infection was 0.7% (8/1 110). In other 13 counties, three had a sensitivity less than 50% (25.0%, 37.8% and 45.6%, respectively) and five a sensitivity over 90.0% with the highest 99.4% (Table 1 and Fig. 2).

The sensitivity was 71.1% in light intensity, 86.6% in moderate

Table 1
Epidemiology of *C. sinensis* infection and the screening performance of raw-freshwater fish-eating practice in 16 counties in China.

County	Raw freshwater fish consumption	No infection	Infection shown	No. of participants	Prevalence (%)	Sensitivity (%)	FNR (%)	Specificity (%)	FPR (%)	Youden's Index	+LR	-LR	PPV (%)	NPV (%)
1	No	1000	0	1000	0.0	NA	NA	100.0	0.0	NA	NA	NA	NA	100.0
	Yes	0	0	0	NA									
	Subtotal	1000	0	1000	0.0									
2	No	1020	0	1020	0.0	NA	NA	100.0	0.0	NA	NA	NA	NA	100.0
	Yes	0	0	0	NA									
	Subtotal	1020	0	1020	0.0									
3	0No	880	1	881	0.1	50.0	50.0	88.2	11.8	0.4	4.2	0.6	0.8	99.9
	Yes	118	1	119	0.8									
	Subtotal	998	2	1000	0.2									
4	No	949	1	950	0.1	66.7	33.3	99.7	0.3	0.7	211.6	0.3	40.0	99.9
	Yes	3	2	5	40.0									
	Subtotal	952	3	955	0.3									
5	No	1102	8	1110	0.7	0.0	100.0	100.0	0.0	0.0	NA	1.0	NA	99.3
	Yes	0	0	0	NA									
	Subtotal	1102	8	1110	0.7									
6	No	1018	6	1024	0.6	25.0	75.0	98.9	1.1	0.2	23.4	0.8	15.4	99.4
	Yes	11	2	13	15.4									
	Subtotal	1029	8	1037	0.8									
7	No	969	4	973	0.4	92.2	7.8	99.4	0.6	0.9	149.8	0.1	88.7	99.6
	Yes	6	47	53	88.7									
	Subtotal	975	51	1026	5.0									
8	No	575	17	592	2.9	77.0	23.0	62.1	37.9	0.4	2.0	0.4	14.0	97.1
	Yes	351	57	408	14.0									
	Subtotal	926	74	1000	7.4									
9	No	454	4	458	0.9	94.9	5.1	49.1	50.9	0.4	1.9	0.1	13.8	99.1
	Yes	470	75	545	13.8									
	Subtotal	924	79	1003	7.9									
10	No	650	14	664	2.1	86.4	13.6	68.8	31.2	0.6	2.8	0.2	23.2	97.9
	Yes	295	89	384	23.2									
	Subtotal	945	103	1048	9.8									
11	No	472	44	516	8.5	66.4	33.6	54.3	45.7	0.2	1.5	0.6	18.0	91.5
	Yes	397	87	484	18.0									
	Subtotal	869	131	1000	13.1									
12	No	767	86	853	10.1	45.6	54.4	88.4	11.6	0.3	3.9	0.6	41.6	89.9
	Yes	101	72	173	41.6									
	Subtotal	868	158	1026	15.4									
13	No	728	97	825	11.8	37.8	62.2	85.7	14.3	0.2	2.7	0.7	32.8	88.2
	Yes	121	59	180	32.8									
	Subtotal	849	156	1005	15.5									
14	No	385	1	386	0.3	99.4	0.6	46.1	53.9	0.5	1.8	0.0	26.7	99.7
	Yes	450	164	614	26.7									
	Subtotal	835	165	1000	16.5									
15	No	203	6	209	2.9	97.8	2.2	27.9	72.1	0.3	1.4	0.1	33.6	97.1
	Yes	525	266	791	33.6									
	Subtotal	728	272	1000	27.2									
16	No	454	20	474	4.2	96.3	3.7	98.1	1.9	0.9	49.5	0.0	98.3	95.8
	Yes	9	517	526	98.3									
	Subtotal	463	537	1000	53.7									
Total	No	11,626	309	11,935	2.6	82.3	17.7	80.3	19.7	0.6	4.2	0.2	33.5	97.4
	Yes	2857	1438	4295	33.5									
	Total	14,483	1747	16,230	10.8									

NA: not available.

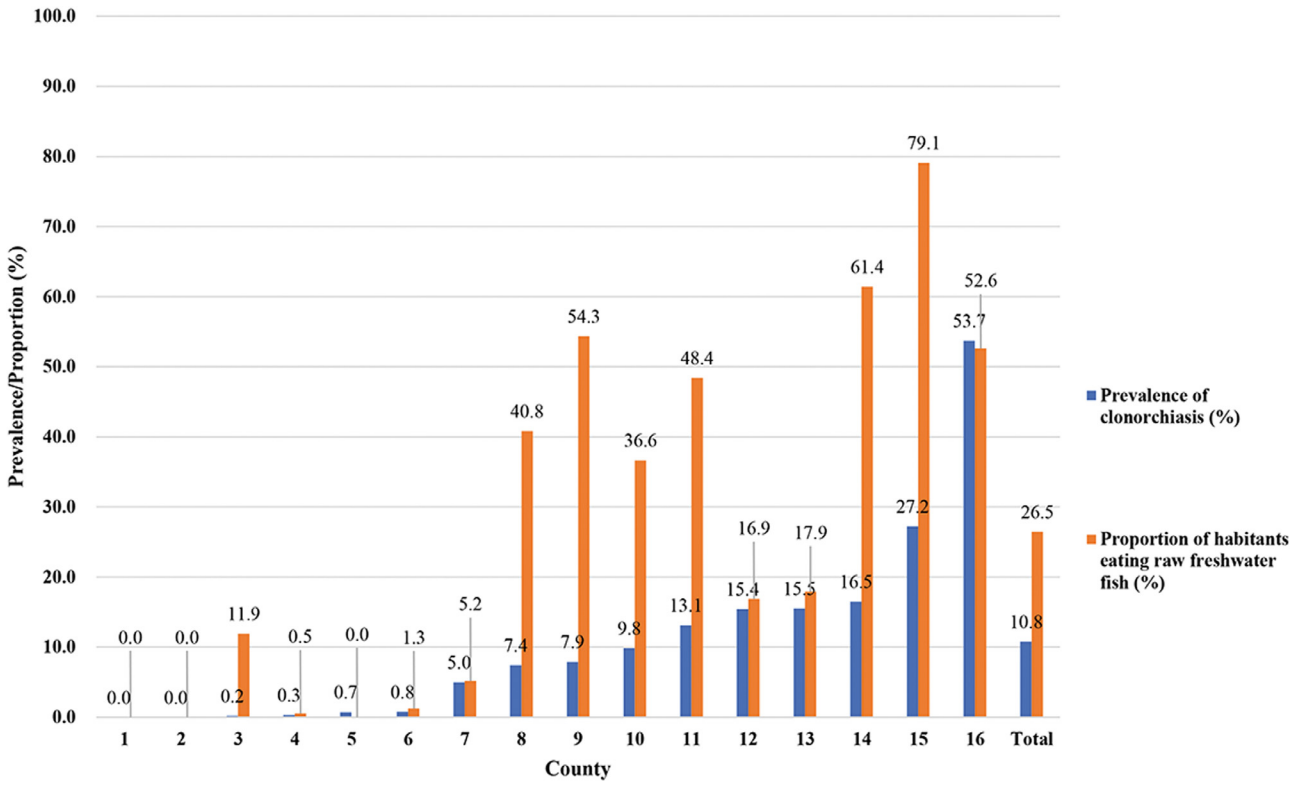

Fig. 1. Epidemiology of *C. sinensis* infection and raw-freshwater fish-eating practice in 16 counties in China.

intensity and 86.0% in heavy intensity, which was significantly different ($\chi^2 = 53.4$, $p < 0.001$) (Table 2). The sensitivity in moderate intensity was significant higher than that in light one ($\chi^2 = 40.0$, $p < 0.001$) and so was that in heavy intensity compared to that in light one ($\chi^2 = 37.0$, $p < 0.001$). However, there existed no significant difference between moderate and heavy ones ($\chi^2 = 0.1$, $p = 0.769$).

The specificity was all 100% in the three counties without eating raw freshwater fish in habitants. In other 13 counties, three had a specificity less than 50% (27.9%, 46.1% and 49.1%, respectively) (Table 1 and Fig. 2). The specificity was over 90% in four counties, in which the highest was 99.7%.

The PPV was calculated in 13 counties, ranging from 0.8% to 98.3%, with four counties 10%−20%, two counties 20%−30%, two counties 30%−40%, two counties 40%−50% and two counties over 80% (including the highest one) (Table 1 and Fig. 2).

The NPV was 100% in the two counties without raw-freshwater fish-eating practice and *C. sinensis* infection, and 99.3% in the county with *C. sinensis* infection but without the practice of eating raw freshwater fish (Table 1 and Fig. 2). In other 13 counties, the NPV was over 90% in 11 counties and another two was 88.2% and 89.9%, respectively.

4. Discussion

Clonorchiasis ranks today among the most important food-borne parasitic diseases in China, especially compared to other parasitic diseases that have been controlled or eliminated, e.g. lymphatic filariasis, leishmaniasis, malaria and schistosomiasis (Hu et al., 2016; Qian et al., 2019; Tang et al., 2016; Yang et al., 2014). Similarly, the techniques used for clonorchiasis control lag behind those used for other parasitic diseases (Qian and Zhou, 2019). Thus, it is compulsory to develop and evaluate applied techniques, out of which the chemotherapy strategy is important.

In this study, we demonstrated the value in screening for *C. sinensis* infection through the practice of eating raw freshwater fish. The sensitivity and specificity overall reached 82.3% and 80.3%, respectively. The results here have high universality. Firstly, 16 counties were included from four provinces, which harbor over 90% of clonorchiasis cases in China. Secondly, the prevalence of *C. sinensis* infection covered a high range from 0 to over 50% in this study. Thus, this screening technique is useful for the selection of target populations for subsequent treatment. It is suitable for use in the field owing to simplicity and low cost. On the comparison, the low compliance with respect to fecal collection weakens the effectiveness of individual diagnosis and subsequent treatment (Mao et al., 2008). Side effects of treatment (praziquantel) also contribute to reducing the compliance leading to lower coverage of mass chemotherapy (Qian et al., 2013; Xu et al., 2014). Thus, the rapid screening by oral enquiry or written questionnaires regarding this food practice is of high importance. In particular, higher sensitivity is also demonstrated in high-intensity infections, which is of crucial importance in treatment. The level of EPG value provides a measure of the worm burden, which is positively correlated with the degree of harm to the human body (Kim et al., 2011). Not only the early symptoms, but also the chronic harm as well as carcinogenicity are all related to the worm burden (Choi et al., 2005; Qian et al., 2011, 2012; Rim, 1986; Upatham et al., 1984). Thus those with high-intensity infections urgently need treatment.

However, the performance of this technique varied significantly between the areas investigated. In two counties, both sensitivity and specificity were over 90%, while in others one indicator could be good and another less so. Firstly, the treatment history could impact the performance. Among the 16 counties in this study, 15 counties had already been included in national surveillance for clonorchiasis since 2016 (Qian et al., 2018). In surveillance, fecal samples were collected and examined, and the results were provided to the participants. Thus, some persons who showed false positive result in this study could have been examined and accepted treatment before. Secondly, other studies have also demonstrated differences regarding fish infection by *C. sinensis* in areas included in this study, which is due to the differences in fecal contamination of water from human beings and animals (Chen et al., 2010; Sohn et al., 2009; Zhang et al., 2014). Obviously, the

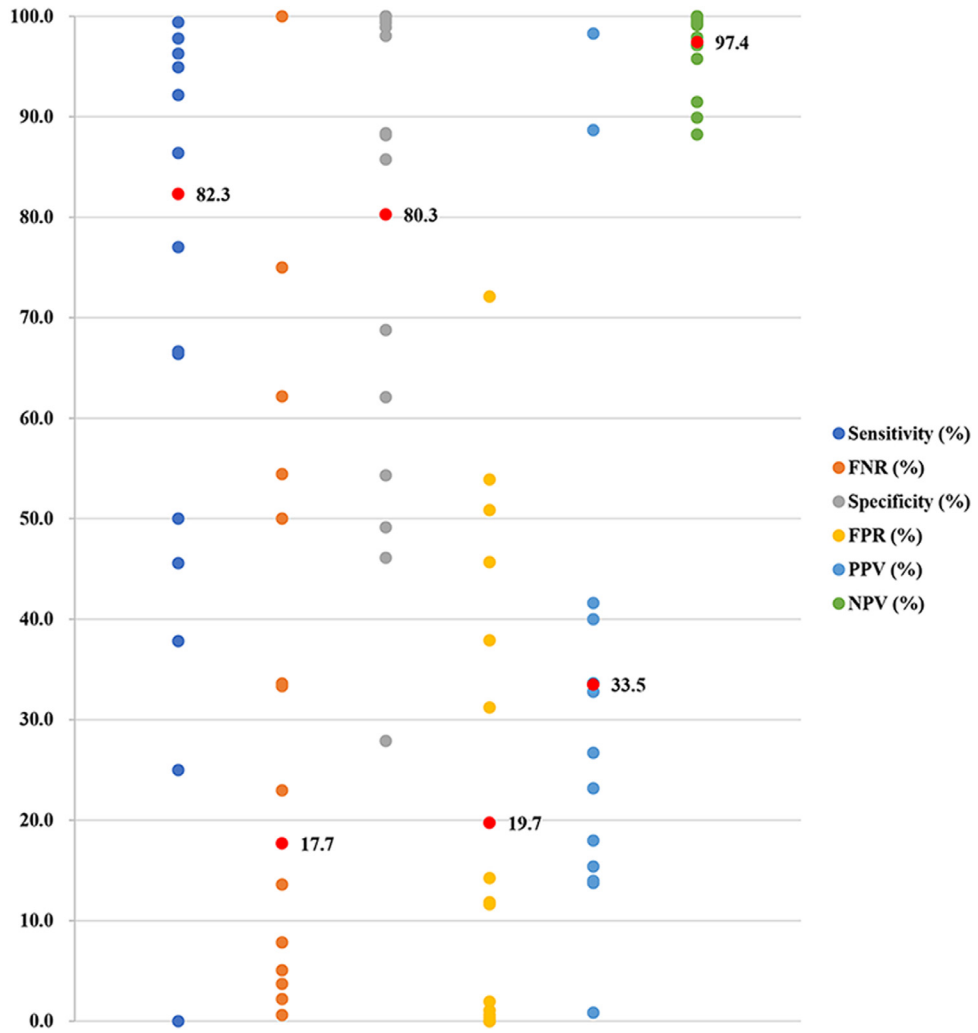

Fig. 2. The performance of raw-freshwater fish-eating practice in screening of *C. sinensis* infection in 16 counties in China. Red cycles represent the overall values in 16 counties.

infection status of fish also impacts the performance of the screening technique.

In the recommended guideline, only the qualitative information on raw-freshwater fish-eating practice is included, while other information on the treatment history as well as the frequency and duration of such practice has not been considered (National Health and Family Planning Commission, 2016). Based on the findings in this study, this rapid screening technique should be further optimized. Especially, multiple rounds of chemotherapy are usually needed to control the morbidity of *C. sinensis* infection due to the re-infection. Thus, it is expected that the performance level of the screening technique will decrease in those areas already subjected to strong control efforts, namely high chemotherapy coverage. Thus, it is needed to carry out further studies to explore the best algorithm to increase the universality of the approach, which should include the treatment history and more information regarding the raw-freshwater fish-eating practice (e.g. the frequency and duration).

5. Conclusion

The strategy to screen target populations for potential *C. sinensis* infection by the practice of eating raw freshwater fish is overall valuable at the current control stage. In particular, rapid screening technique shows high sensitivity for high-intensity infections. However, more parameters including treatment history and frequency and duration of the practice of eating raw freshwater fish are needed to optimize the screening technique, especially in areas where chemotherapy has already been implemented.

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CRediT authorship contribution statement

Men-Bao Qian: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Zhi-Hua Jiang:** Investigation, Resources, Supervision. **Tao Ge:** Investigation, Resources, Supervision. **Xin Wang:** Investigation, Resources, Supervision. **Chang-Hai Zhou:** Methodology, Resources, Supervision. **Hui-Hui Zhu:** Methodology, Data curation, Supervision. **Xiao-Nong Zhou:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Table 2
Sensitivity of raw-freshwater fish-eating practice in screening of *C. sinensis* infection by intensity in 16 counties in China.

County	Raw freshwater fish consumption	No. of cases				Sensitivity (%)			
		Light	Moderate	Heavy	Total	Light	Moderate	Heavy	Total
1	No	0	0	0	0	NA	NA	NA	NA
	Yes	0	0	0	0				
	Subtotal	0	0	0	0				
2	No	0	0	0	0	NA	NA	NA	NA
	Yes	0	0	0	0				
	Subtotal	0	0	0	0				
3	No	0	0	1	1	NA	100.0	0.0	50.0
	Yes	0	1	0	1				
	Subtotal	0	1	1	2				
4	No	1	0	0	1	0.0	NA	100.0	66.7
	Yes	0	0	2	2				
	Subtotal	1	0	2	3				
5	No	5	2	1	8	0.0	0.0	0.0	0.0
	Yes	0	0	0	0				
	Subtotal	5	2	1	8				
6	No	2	4	0	6	0.0	33.3	NA	25.0
	Yes	0	2	0	2				
	Subtotal	2	6	0	8				
7	No	3	1	0	4	90.6	83.3	100.0	92.2
	Yes	29	5	13	47				
	Subtotal	32	6	13	51				
8	No	5	2	10	17	81.5	87.5	67.7	77.0
	Yes	22	14	21	57				
	Subtotal	27	16	31	74				
9	No	0	4	0	4	100.0	90.0	100.0	94.9
	Yes	16	36	23	75				
	Subtotal	16	40	23	79				
10	No	7	6	1	14	86.3	79.3	95.7	86.4
	Yes	44	23	22	89				
	Subtotal	51	29	23	103				
11	No	23	7	14	44	55.8	78.1	70.2	66.4
	Yes	29	25	33	87				
	Subtotal	52	32	47	131				
12	No	37	12	37	86	37.3	36.8	53.8	45.6
	Yes	22	7	43	72				
	Subtotal	59	19	80	158				
13	No	42	35	20	97	44.0	32.7	31.0	37.8
	Yes	33	17	9	59				
	Subtotal	75	52	29	156				
14	No	1	0	0	1	97.7	100.0	100.0	99.4
	Yes	42	75	47	164				
	Subtotal	43	75	47	165				
15	No	4	2	0	6	94.4	98.4	100.0	97.8
	Yes	67	123	76	266				
	Subtotal	71	125	76	272				
16	No	2	11	7	20	91.3	95.4	97.5	96.3
	Yes	21	226	270	517				
	Subtotal	23	237	277	537				
Total	No	132	86	91	309	71.1	86.6	86.0	82.3
	Yes	325	554	559	1438				
	Subtotal	457	640	650	1747				

NA: not available.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2020.105380.

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