From awareness to action: NIPD's engagement in the control of food-borne clonorchiasis

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Abstract

Clonorchiasis is caused by ingestion of raw freshwater fish containing infective larvae of *Clonorchis sinensis*. China harbours the largest number of people with *C. sinensis* infection. During the past three decades, the National Institute of Parasitic Diseases, affiliated to the Chinese Center for Disease Control and Prevention (NIPD) conducted many studies to facilitate the control on clonorchiasis. Three national surveys have shown the updated epidemiology of clonorchiasis in China. Recently, a national surveillance system has also been established, which will enable the production of high-resolution map. The evaluation of the disease burden has enhanced the awareness on clonorchiasis. Diverse diagnosis techniques including rapid screening by questionnaire,

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serological tests, faecal examination and a molecular method have been developed or evaluated. The NIPD also participated in the early evaluation of praziquantel against clonorchiasis, which enhanced its application in China. Also, the NIPD has verified the efficacy of tribendimidine against clonorchiasis. A new sustainable strategy is also being explored. However, more research is expected to further facilitate control of clonorchiasis in China, as well as international cooperation in fighting human liver fluke infections in Asia.

1. Introduction

The discovery of *Clonorchis sinensis* could data back to over 140 years ago (Qian et al., 2014b, 2016b). On 21 August 1875, the Lancet first reported the finding of a new fluke, in which Dr. McConnell described a case of a Chinese male who was admitted to the Medical College Hospital in Calcutta, India, on 8 September 1874 (McConnell, 1875). The patient died within a few hours because of severe liver disease. During the post-mortem examination, many narrow, flattened, and lanceolate flukes were detected in the bile ducts. Four weeks later, Dr. Cobbold suggested publicly to name the parasite *Distoma sinense* (Cobbold, 1875). Then, in 1907, Dr. Looss renamed the parasite *Clonorchis sinensis* because of its characteristically branched testes (Looss, 1907). However, the history of the endemicity of clonorchiasis is thought to be much longer. An ancient corpse of the Warring States Period was unearthed in Hubei province, China in 1994 (Wei et al., 1980). Many *C. sinensis* eggs were found in the corpse which was buried about 2400 years ago.

Nowadays, clonorchiasis is predominantly endemic in East Asia, including China (Chen et al., 2012), the Republic of Korea (Jeong et al., 2016), northern Vietnam (Doanh and Nawa, 2016) and part of Russia (Solodovnik et al., 2018) due to the popularity of ingesting raw freshwater fish. Globally, about 15 million people were infected with *C. sinensis*, with 13 million in China, 1 million in the Republic of Korea, another million in northern Vietnam and several thousand in Russia (Qian et al., 2012, 2013b). Adult worms of *C. sinensis* live in the hepatobiliary system and cause major damage there. Early infections usually present no symptoms, but some cases might present some symptoms in nervous and digestive systems, such as asthenia, nausea, indigestion, headache, diarrhoea, or abdominal pain (especially in the right upper quadrant) (Chen et al., 1994; Kim et al., 1982).

Chronic and long-term infections can lead to severe hepatobiliary conditions, particularly cholelithiasis, cholangitis, and cholecystitis (Choi et al., 2008; Qiao et al., 2012). Furthermore, *C. sinensis* has been ascertained as a carcinogen to human beings, leading to fatal bile cancer (cholangiocarcinoma) (Bouvard et al., 2009).

As China harbours most cases with *C. sinensis* infection, research on and control of clonorchiasis in China will determine global progress in the control of clonorchiasis (Qian et al., 2019a). Additionally, the experiences in China benefit other endemic countries. The National Institute of Parasitic Diseases, affiliated to the Chinese Center for Disease Control and Prevention (NIPD) is the only national technical organization in charge of research and control on parasitic diseases (Chen et al., 2020; Zhou et al., 2005). As such, the NIPD has also done much work in combatting clonorchiasis. This paper is summarizing the progress of research and control on clonorchiasis in China promoted by the NIPD in different stages over the previous decades.

2. Implementation of national epidemiological surveys

Since the early 1900s, many surveys were conducted, gradually showing the high endemicity of clonorchiasis in Guangdong province, southern China (Faust, 1925; Whyte, 1908). National surveys were implemented until the late 1980s, which has enabled to map the epidemiology nationally (Qian et al., 2019a). All these national surveys were organized by NIPD, including the design, implementation, supervision, data analysis and report writing.

The first national survey on important parasitic diseases was implemented between 1988 and 1992 (Xu et al., 2000). A total of 1,477,742 participants were included from 726 counties in 30 provinces, and one Kato-Katz thick smear was prepared from each faecal sample. 4606 persons were detected with *C. sinensis* infection, with a prevalence of 0.31% (Chen et al., 2012; Xu et al., 2000). The prevalence was 0.38% (2758/733,143) in males and 0.25% (1848/744,599) in females. In males, the prevalence was low in young people, increased with age peaking in the 30s and then declining in the 50s (Fig. 1). In females, the prevalence peaked in those over 15, and then persisted. Out of 30 provinces, 22 were detected with *C. sinensis* (Fig. 2). The highest prevalence was found in eastern China,

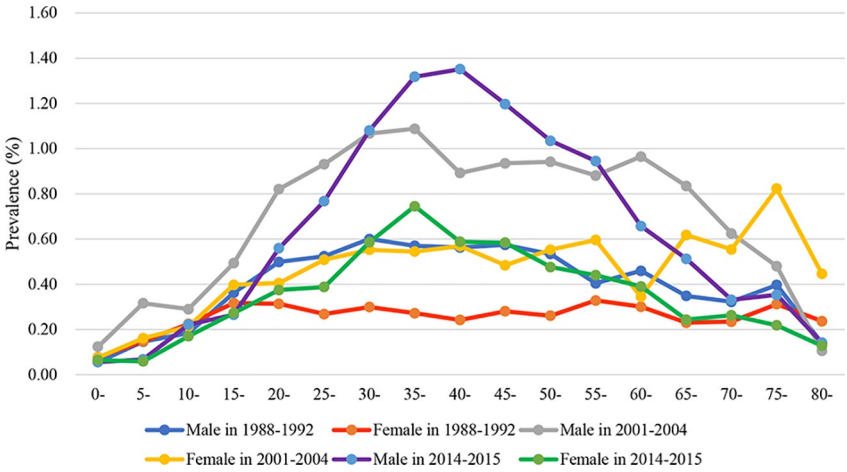

Fig. 1 Epidemiology of clonorchiasis by sexes and ages in China in three national surveys.

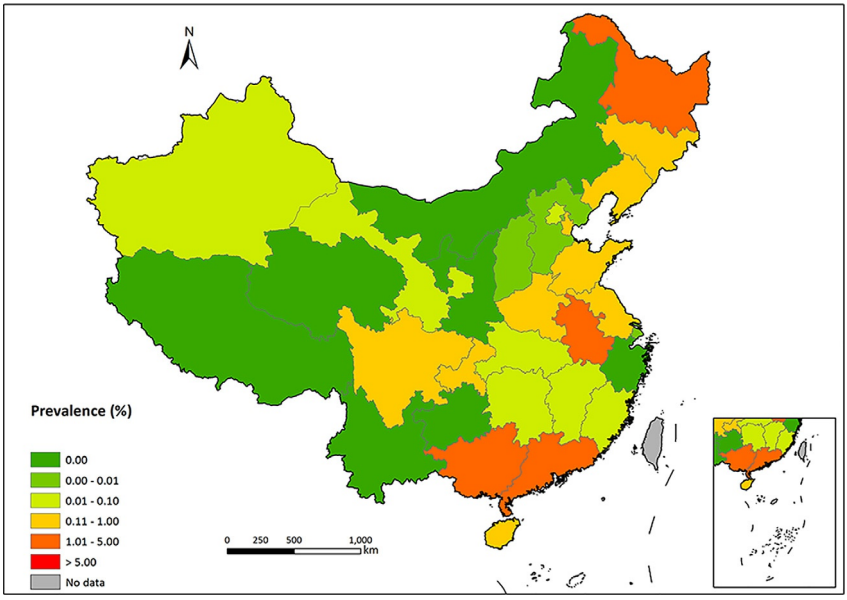

Fig. 2 Epidemiology of clonorchiasis by provincial level in China in 1988–1992.

including Guangdong (1.82%), Guangxi (1.20%) and Heilongjiang (1.18%) provinces. Anhui province in central China showed also a high prevalence (1.44%). Based on this survey, about 4.7 million people were estimated to have *C. sinensis* infection (World Health Organization, 1995).

From 2001 to 2004, another national survey and one special survey were implemented for clonorchiasis (Technical Steering Panel for National Survey of Current Status of Major Human Parasitic Diseases, 2008). *C. sinensis* was embedded in this national survey on important parasitic diseases, which covered 229 counties in 31 provinces. A total of 356,629 persons were included, out of which 2065 persons were detected with *C. sinensis*, with a prevalence of 0.58% (Chen et al., 2012; Technical Steering Panel for National Survey of Current Status of Major Human Parasitic Diseases, 2008). The prevalence was again higher in males (1290/179,341, 0.72%) than in females (775/177,288, 0.44%). In males, the prevalence was high in those aged between 20 and 70, while in females the prevalence increased with age, peaked in the 30s and then fluctuated (Fig. 1). Out of 31 provinces, *C. sinensis* infection was detected in 17 provinces (Fig. 3). High prevalence was found in Guangdong (5.35%), Guangxi (3.71%) and Jilin (4.77%) province. In addition to this, a special survey on clonorchiasis was conducted in the endemic areas in 27 endemic provinces identified on the basis of literature reports (Chen et al., 2012; Fang et al., 2008; Technical Steering Panel for National Survey of Current Status of Major Human Parasitic Diseases, 2008). Out of a total of 217,829

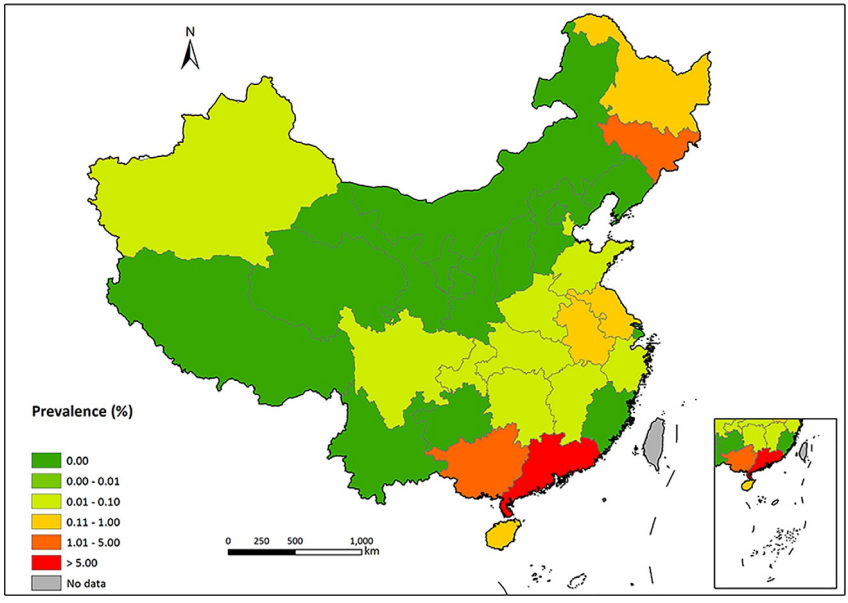

Fig. 3 Epidemiology of clonorchiasis by provincial level in China in 2001–2004.

participants, 5230 (2.40%) were found with *C. sinensis* infection. The prevalence was 2.94% in males and 1.84% in females. The epidemiological age profile was similar to the national survey. The highest prevalence was also demonstrated in eastern China including Guangdong (16.42%), Guangxi (9.76%), Heilongjiang (4.73%), Jilin (2.90%) and Hunan (1.33%) provinces. Because the distribution of clonorchiasis varied significantly by areas, and three Kato-Katz thick smears were prepared from a single faecal sample in the special survey compared to only one smear in the national survey, the findings in the special survey were believed to be more accurate (Chen et al., 2012; Fang et al., 2008; Technical Steering Panel for National Survey of Current Status of Major Human Parasitic Diseases, 2008). Based on the special survey, about 12.49 million persons were estimated to be infected with *C. sinensis*, most of which distributed in southeastern and northeastern China (Chen et al., 2012; Fang et al., 2008; Technical Steering Panel for National Survey of Current Status of Major Human Parasitic Diseases, 2008). Clonorchiasis is also endemic in Taiwan province, Hongkong Special Administrative Region and Macao Special Administrative Region (Qian et al., 2012; World Health Organization, 1995). It is estimated that about 13 million people were infected in China in the early 2000s (Qian et al., 2012).

Between 2014 and 2015, another national survey was carried out for important parasitic diseases in China (Zhou, 2018). Two parts were performed for clonorchiasis. First, the investigation of clonorchiasis in rural China was combined with that of soil-transmitted helminthiases. Second, in the urban areas, a special survey was implemented for clonorchiasis. Out of 617,441 participants examined by two Kato-Katz thick smears from a single faecal sample, 3466 were infected with *C. sinensis*, which represents a crude prevalence of 0.56% (Zhou, 2018). Unlike the surveys before, the population sampled was not proportional to the total population in different areas. Thus, weighted prevalence should be applied. The weighted prevalence was 0.47%, based on which about 5.98 million people were estimated to be infected with *C. sinensis* (Zhou, 2018). The weighted prevalence was 0.60% in males and 0.34% in females. The prevalence was high in middle aged people in both sexes (Fig. 1). Out of 31 provinces, 18 were detected with *C. sinensis* infection (Fig. 4). Highest prevalences were also demonstrated in Guangxi (6.68%), Guangdong (1.91%), Heilongjiang (1.62%) and Jilin (1.02%) provinces.

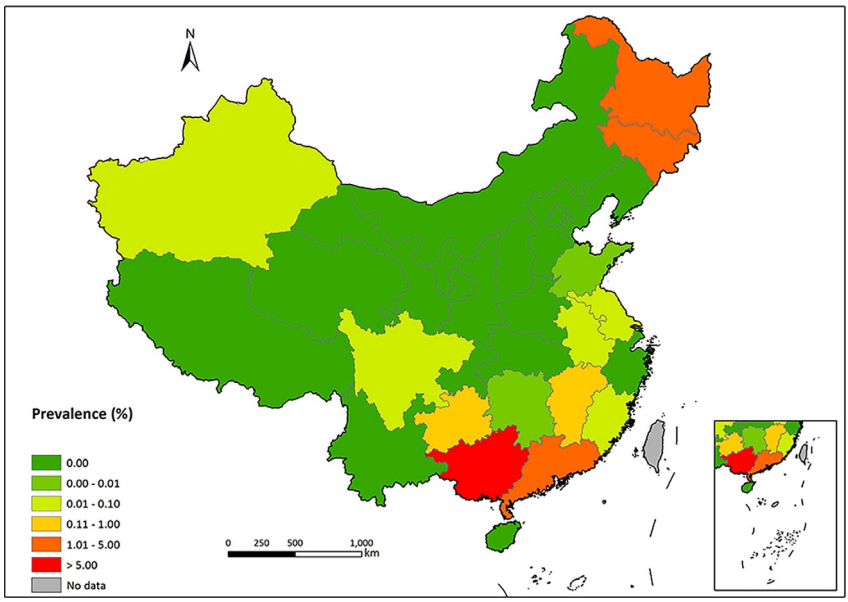

Fig. 4 Epidemiology of clonorchiasis by provincial level in China in 2014–2015.

3. Establishment of a national surveillance system

Surveillance is important in the control of parasitic diseases. Although surveillance systems had been available for many parasitic diseases in China before 2015, clonorchiasis was not included (Qian et al., 2016a, 2019a). Owing to the awareness of the importance of clonorchiasis in China, a national surveillance system for clonorchiasis has been gradually established in China since 2016 after the most recent national survey (Qian et al., 2018).

The surveillance system consists of two major parts, namely the active surveillance and passive surveillance (Qian et al., 2018). The passive surveillance involves collection of *C. sinensis* cases through the national reporting system of infectious diseases. The active surveillance system contains two components, namely fixed surveillance and mobile surveillance. In fixed surveillance, the surveillance spots are fixed for 5 years, while those in mobile surveillance vary each year. Although a national map for clonorchiasis is available owing to the three national and one special surveys, the resolution is low with a precision only down to subprovincial level, which hinders the

implementation of control activities (Technical Steering Panel for National Survey of Current Status of Major Human Parasitic Diseases, 2008; Xu et al., 2000; Zhou, 2018). The fixed surveillance shows the changing trend, while the mobile surveillance aims to demonstrate the prevalence by county level which is expected to be completed within 5–10 years.

In the fixed surveillance part, seven provinces were included, namely Guangdong, Guangxi, Heilongjiang, Jilin, Hunan, Jiangxi and Guizhou, where clonorchiasis is highly endemic based on data from the national survey in 2014 and 2015 (Zhou, 2018). Several counties were selected from each province. Then, five villages are selected from each county. The surveillance system includes a survey on infection in human beings, knowledge, attitudes and practice in human beings, infection in fish, infection in animal reservoirs and other social and economic data. As to the survey on infection in human beings, about 200 villagers are included from each of five villages. The Kato-Katz method is used, and two Kato-Katz smears are prepared for a single faecal sample (Hong et al., 2003; Ting-Jun et al., 2018). In each village, 30 participants are included for the questionnaire survey, with a total of 150 villagers in each county. In each county, 100 fish are investigated by the digestive method (Li et al., 2012) and 25 animals (dogs, cats and pigs) (Chen et al., 1997; Lin et al., 2011) are included using the Kato-Katz method for examination. In mobile surveillance, only the survey on infection in human beings is conducted, in which the methodology is same to that in fixed surveillance.

In 2016, surveillance was completed in 301 counties from 30 provinces (Zhu et al., 2019). Out of 305,081 participants submitting faecal samples, 6226 were infected with *C. sinensis*, yielding a crude prevalence of 2.04%. *C. sinensis* infection was detected in 70 counties, distributed in 15 provinces. Nearly 90% persons with infection were distributed in four highly endemic provinces in southeastern and northeastern China, namely Guangdong, Guangxi, Heilongjiang and Jilin provinces. The epidemiological profiles in sex and age were similar to those in national surveys. Especially, high prevalence was demonstrated in those counties nearing to rivers.

4. Evaluation of disease burden

Compared to other helminthiases, clonorchiasis has long been neglected globally (Qian et al., 2013c; World Health Organization, 2010). It has not been listed among the global burden of disease before 2010. Thus, to fully demonstrate the burden of clonorchiasis, scholars from NIPD have implemented several studies.

First, the global epidemiology of clonorchiasis is captured in an article by Qian et al. from NIPD, who comprehensively reviewed the literature and summarized the data (Qian et al., 2012). It was found that globally the number of people with *C. sinensis* infection has increased from 7 million in the early 1990s to 15 million in the early 2000s, mainly occurring in China, the Republic of Korea, northern Vietnam and part of Russia. Regular national surveys have only been conducted in China and the Republic of Korea, every 5–10 years. Due to a lack of data, the number of people infected in the 2000s in Vietnam and Russia was assumed to be the same to those in the 1990s, as estimated by the World Health Organization. The people with *C. sinensis* infection didn't change significantly from the 1990s to the 2000s in the Republic of Korea. The increase in China contributed to the significant increase globally, with China harbouring about 85% of the global clonorchiasis cases. Even taking into consideration that opisthorchiasis is caused by *Opisthorchis viverrini* in southeast Asia and by *O. felineus* in Russia and neighbouring countries, China also shares over 50% of the global cases of human liver fluke infections (Qian et al., 2013b). The control of clonorchiasis in China is therefore likely to determine the global progress against human liver fluke infections. Furthermore, more surveys are expected in Vietnam and Russia, which will enable to capture accurate epidemiology there. Additionally, even in China and the Republic of Korea, where national surveys have been conducted every several years, high-resolution maps are expected with more surveys and the use of new mapping techniques (Qian et al., 2016a).

Second, the disability weight of *C. sinensis* infection has been estimated (Qian et al., 2011). Disability-adjusted life years (DALYs) are used to assess the health burden due to diseases, and consist of years of life lost (YLLs) and years lived with disability (YLDs) (King and Bertino, 2008). Thus, disability weight is a crucial parameter in calculation of YLDs, which reflects the average degree of loss of quality of life due to certain conditions. It ranges between 0 (complete health) and 1 (death). However, this parameter is not available for clonorchiasis. Additionally, although *C. sinensis* infection rarely causes death directly, it can lead to fatal cholangiocarcinoma (Bouvard et al., 2009). In a strategic diagram, Qian et al. argued that the burden of clonorchiasis could be calculated through the division of the sequelae of *C. sinensis* infection into two parts, namely fatal sequelae and non-fatal sequelae (Qian et al., 2011). The former includes early death and the period lived with cancer before death. Thus, the disability weight of early death is equal to the figure of one while that of living with cancer before death could be similar to those of other cancers. As to the non-fatal sequelae, the

disability weight was captured through the multiplication of the probability and disability weight of single sequela caused by *C. sinensis* infection, then added together. The probability was captured by a field survey in a clonorchiasis endemic village in Guangdong province, while the disability weight of single sequela was found through literature searches. Then Monte Carlo simulation was applied. It was found that the overall disability weight is 0.075, and 0.101 in males and 0.050 in females. The disability weight is proportional to the geometric mean of eggs per gram of faeces (EPG), which is consistent to the fact that the morbidity of *C. sinensis* infection is positively correlated to the worm burden (Chen et al., 1994; Kim et al., 1982). The disability weight of *C. sinensis* infection is similar to that of hepatitis B, which also attacks the liver (Qian et al., 2013b).

Third, cholangiocarcinoma burden attributed to *C. sinensis* infection has also been calculated (Qian et al., 2012). Although the relationship between *C. sinensis* infection and cholangiocarcinoma has been observed long ago by scholars and verified in several cross-sectional and case-control studies, it is not until 2009 that *C. sinensis* was classified as a definite carcinogen to human beings (Bouvard et al., 2009). As to the cholangiocarcinoma burden attributed to *C. sinensis* infection, different approaches have been used (Furst et al., 2012; Plummer et al., 2016). In a meta-analysis by scholars from NIPD, it was found that the odds ratio between *C. sinensis* infection and cholangiocarcinoma reaches 4.47 (Qian et al., 2012). Then, based on the cholangiocarcinoma data in the Republic of Korea, it was estimated that the incidence of cholangiocarcinoma attributed to *C. sinensis* infection is 35/100000 in males and 25/100000 in females infected with *C. sinensis* (Qian et al., 2012). On the basis of this, it was estimated that in the early 2000s, nearly 5000 cholangiocarcinoma cases each year could be attributed to *C. sinensis* infection (Qian and Zhou, 2017).

5. Capturing social determinants

To allow a scientific design of interventions, the determinants of clonorchiasis should be first captured (Qian et al., 2016a). The national surveys demonstrate the spatial and temporal profiles, as well the distribution of clonorchiasis in populations (Technical Steering Panel for National Survey of Current Status of Major Human Parasitic Diseases, 2008; Xu et al., 2000; Zhou, 2018). While the transmission of clonorchiasis is caused by ingestion of *C. sinensis* metacercariae present in freshwater fish, infection may be acquired in various ways, such as the ingestion of raw or undercooked fish or through contamination of other foods (Fan et al., 2014). Usually, the

direct ingestion of raw freshwater fish is believed to be the most important transmission route in China (Zheng, 2009). Thus, several field surveys have been carried out by scholars from the NIPD to further explore the epidemiology and practice of eating raw freshwater fish.

Hengxian county is located in the southeast part of China, and it is believed to be one of the most afflicted counties by clonorchiasis in China. Two earlier surveys embedded in the national surveys demonstrated the increasing prevalence of clonorchiasis in Hengxian county. To capture the update epidemiology, in 2011 another survey was organized by the NIPD (Qian et al., 2014a). 3437 villagers were enrolled in this survey from 24 villages. After comparison with two earlier surveys, it was demonstrated that the prevalence of clonorchiasis had increased from 19.7% in 1989 to 30.5% in 2002 and then 46.5% in 2011. What is a distinct comparison was the significant decrease of soil-transmitted helminthiases from 86.3% in 1989 to 25.1% in 2002 and 7.0 in 2011. Social and economic development promotes the great achievement in the control of soil-transmitted helminthiases in China, while the prevalence of clonorchiasis increases due to the development of pisciculture, which makes more people affordable to ingest raw freshwater fish.

In a cross-sectional survey in 16 counties in four major clonorchiasis endemic provinces, namely Guangdong, Guangxi, Heilongjiang and Jilin (Qian et al., 2019c), 16,230 participants were enrolled to explore the relationship between ingestion of raw freshwater fish and *C. sinensis* infection. There existed a linear relationship between the practice of eating of raw freshwater fish and *C. sinensis* infection by county level (coefficient of determination (R^2) = 0.44). The prevalence of clonorchiasis was 33.5% in those with raw-eating practice, while it was 2.6% in those without the practice. Thus, the relative risk of *C. sinensis* infection was 12.9. The attributable risk, attributable risk per cent, population attributable risk and population attributable risk per cent was 30.9%, 92.3%, 8.2% and 75.9%, respectively. This study clearly demonstrates the crucial role of ingestion of raw freshwater fish in the transmission of clonorchiasis in China. However, it also indicates the potential role of other transmission routes.

In another study, the knowledge and raw-eating practice in those with *C. sinensis* infection was analysed in different populations within one community in Guangdong province. First, it was demonstrated that a high proportion of persons ingested raw freshwater fish at restaurants (Qian et al., 2013a). It was also demonstrated that the proportion was higher in adult males (80.3%) than in adult females (54.9%) and children (25.0%). Thus, restaurants could not be neglected in the control of clonorchiasis in some endemic areas. Second, it was

demonstrated that compared to children, adults had more knowledge on transmission and harm of clonorchiasis, but they also had more practice in ingesting raw freshwater fish. This disparity indicates that education in adults is likely to be less effective and that education should target children. Of course, adults sometimes have some misunderstanding on clonorchiasis, such as the killing effect of *C. sinensis* larvae in fish by alcohol, which still needs education to be rectified (Qian, 2014).

6. Applied research on diagnostics and drugs

6.1 Diagnostics

Both diagnosis and treatment of clonorchiasis caught the attention of the NIPD. Scholars from the NIPD have evaluated the effectiveness of different diagnostic methods. Li et al. conducted a study to compare the performance of serological diagnostics for clonorchiasis in China (Li et al., 2018). Four commercial tests were included. The performance was low for the one detecting IgM. The sensitivity was high for the other three detecting IgG and ranged from 81.51% (194/238) to 99.16% (236/238). It was also demonstrated that the sensitivity was higher in heavy infection compared to that in light infection. The specificity ranged from 73.42% (174/237) to 87.34% (207/237), and was lower in participants from clonorchiasis endemic areas as compared to those from non-endemic areas. Additionally, high cross reaction was demonstrated with paragonimosis. Overall, more research is expected to improve the sensitivity in light infection and the specificity for immunological tests.

Qian et al. have conducted two studies evaluating the performance of faecal examination techniques (Qian et al., 2013e, 2019e), of which one was nested in a drug evaluation trial (Qian et al., 2013e). In total 74 persons infected with *C. sinensis* were examined at baseline by faecal examination (the Kato-Katz method and the formalin-ether concentration technique (FECT)). Three weeks after treatment, the infection status was re-examined. 38 (51.4%) people were still infected as determined by a combination of the two methods. The number of positive cases was 23 (31.1%) with a single Kato-Katz smear, 29 (39.2%) with three Kato-Katz smears, 35 (47.3%) with six Kato-Katz smears from two samples, 13 (17.6%) with a single FECT examination and 17 (23.0%) with a duplicate FECT examination. In another natural setting, the diagnostic performance of Kato-Katz method in school children was evaluated, while the practice of ingestion of raw freshwater fish was also assessed (Qian et al., 2019e). With the "gold" standard of six smears from two faecal samples, 77 out of 397 students were detected with *C. sinensis* eggs. However, only 45 students were detected

when a single smear was examined, which showed an underestimation of 41.6%, while the infection intensity in term of eggs per gram of faeces was overestimated. The practice of ingesting raw freshwater fish in false negative cases was comparable to those in the detected cases, while the raw-eating practice in both groups was significantly higher than that in the negative group, which indicates that the higher performance by multiple smears was due to improved diagnostic capacity rather than low specificity or contamination. Based on these two studies, it is argued that the underestimation of *C. sinensis* infection is difficult to be avoided in low endemicity situations when limited Kato-Katz smears are examined. Multiple smears from repeated samples should be used in such settings for the examination of those individuals ingesting raw freshwater fish, for the evaluation of drug efficacy or for the verification of elimination.

Xu et al. have compared the performance of the Kato-Katz method with a PCR technique in detecting *C. sinensis* eggs in human faeces (Xu et al., 2019). Out of 133 faecal samples, 103 (77.4%) were positive with the combination of Kato-Katz method and PCR technique. The positive rate was higher in PCR technique (70.7%) than Kato-Katz method (57.1%). Among 76 positive samples by Kato-Katz method, 67 (88.2%) were positive in PCR technique, while it was 47.4% (27/57) in the negative sample by Kato-Katz method. Although the difference was insignificant, the detection rate by PCR technique was a little higher in those with high infection intensity. The Kappa value of the consistency reached 0.73 between two methods. Thus, it is argued that in low-endemic areas, PCR technique is expected to improve the sensitivity.

The probability to introduce a rapid screening technique to identify people for treatment of clonorchiasis is important. By further analysing the data on *C. sinensis* infection and raw-freshwater fish-eating practice of villagers in 16 counties from four endemic provinces mentioned above (Qian et al., 2019c), Qian et al. evaluated the screening performance by practice of ingesting raw freshwater fish to potentially select those persons with *C. sinensis* infection (Qian et al., 2020). Overall, the sensitivity and false negative rate were 82.3% (1438/1747) and 17.7% (309/1747), respectively, while the specificity and false positive rate were 80.3% (11,626/14,483) and 19.7% (2857/14,483), respectively. Youden's index reached 0.6. The positive and negative predictive values were 33.5% (1438/4295) and 97.4% (11,626/11,935), respectively. Additionally, the sensitivity was related to the infection intensity: 71.1% in light intensity, 86.6% in moderate intensity and 86.0% in heavy intensity. Thus, the rapid screening for raw-freshwater fish-eating practice could be applied. However, it has also

been noticed that other parameters should be explored to optimize the screening performance, e.g., treatment history and frequency and duration of the practice of eating raw freshwater fish.

6.2 Drugs

In the late 1980s, scholars from the NIPD initiated an efficacy trial of praziquantel against *C. sinensis* infection (Chen et al., 1982). In a lightly infected group of patients (EPG <1000) to whom praziquantel was administrated at a dose of 20 mg/kg trice in 1 day, the cure rate was 81.8%, the eggs reduction rate was 96.47% and the proportion of side effects was 50.0% (Table 1). In another group that received praziquantel 25 mg/kg trice in 1 day, the cure rate, egg reduction rate and proportion of side effects was 82.8%, 99.77%, 67.9%, respectively. The cure rate was 91.4% in light infection (EPG < 1000) and 65.4% in heavy infection (EPG ≥ 1000). In the heavily infected group (EPG ≥ 1000) treated with praziquantel 25 mg/kg trice in 1 day plus 15 mg/kg trice in another day, the cure rate, egg reduction rate and proportion of side effects was 94.4%, 99.995% and 80.6%, respectively. In the control group treated with hexachloropxylol (17.5 g divided into 10 times within 5 days), the cure rate, egg reduction rate and proportion of side effects was 79.5%, 99.81%, 87.5%, respectively. The cure rate was 80.8% in light infection (EPG < 1000) and 76.9% in heavy infection (EPG ≥ 1000). This trial demonstrated that praziquantel is efficacious and

Table 1 Efficacy and safety of praziquantel against *C. sinensis* infection in a clinical trial (Chen et al., 1982).

	Cure rate (%)[a]				
Regimens	**Light infection (EPG < 1000)**	**Heavy infection (EPG ≥ 1000)**	**Total**	**Eggs reduction rate(%)[a]**	**Side effects (%)**
PZQ (20 mg/kg, t.i.d)	81.8 (27/33)	–	81.8 (27/33)	96.47	50.0
PZQ (25 mg/kg, t.i.d)	91.4 (96/105)	65.4 (34/52)	82.8 (130/157)	99.77	67.9
PZQ (25 mg/kg, t.i.d + 15 mg/kg, t.i.d)	–	94.4 (34/36)	94.4 (34/36)	99.995	80.6
Hexachloropxylol (17.5 g divided into 10 times within 5 days)	80.8 (21/26)	76.9 (10/13)	79.5 (31/39)	99.81	87.5

[a]Evaluated 1 month after drug administration.

safe against *C. sinensis* infection. Both the efficacy and side effects are influenced by the infection intensity. A higher dose increases the efficacy but more side effects also occur. In the light of evidence also from other trials, praziquantel was gradually introduced as the treatment of clonorchiasis in China.

Tribendimidine is a new drug, developed in the 1970s by NIPD (Xiao et al., 2005, 2013). It was approved in 2004 by the Chinese Food and Drug Administration for treating soil-transmitted helminth infections in humans. Several experiments in vivo and in vitro also demonstrated the potential of tribendimidine against *C. sinensis* infection (Keiser et al., 2009; Xiao et al., 2008a,b, 2009a,b, 2011; Xue et al., 2009, 2010). Finally, in 2012, two clinical trials were conducted in China, which further demonstrated the potential of tribendimidine in the treatment of *C. sinensis* infection (Qian et al., 2013d; Xu et al., 2014).

In the first randomized open-label trial in Guangxi, the efficacy and safety of tribendimidine was assessed in two different arms, namely 400 mg once and 400 mg daily for 3 days compared to the standard dose of praziquantel (75 mg/kg, divided into three times in 1 day) (Qian et al., 2013d). In total 74 patients with *C. sinensis* were enrolled. The cure rate was 44% in the tribendimidine "once" group, 58% in the group that received tribendimidine for 3 days and 56% in the praziquantel group. The egg reduction rate was 97.6%, 98.8% and 98.8%, respectively. Especially, the cure rate was 100% in light infection (EPG $<$ 1000) in all three groups. Additionally, the overall percentage of side effects was only 44% in the tribendimidine "once" group, 67% in the group that received tribendimidine for 3 days and 92% in the praziquantel group.

Another randomized clinical trial was implemented in Hunan province (Xu et al., 2014). A total of 156 patients were divided into four arms, 400 mg tribendimidine once, 200 mg tribendimidine twice a day, 75 mg/kg praziquantel divided into twice a day for 2 days, and 400 mg mebendazole single dose. The cure rate against *C. sinensis* was 50%, 33.3%, 56.8% and 0%, respectively, while the egg reduction rate was 98.3%, 97.1%, 98.0% and 71.2%, respectively. In a second part of the study, those uncured in the praziquantel group received the same regime of praziquantel, while those uncured in the tribendimidine groups (400 mg tribendimidine once and 200 mg tribendimidine twice per day) received 400 mg tribendimidine once. The cure rates were 75% and 78.1%, respectively, while the egg reduction rate was 75.8% and 74.2%, respectively. Additionally, it was also demonstrated that less side effect occurred in the tribendimidine groups as compared to the praziquantel group.

7. Exploration of an optimal control strategy and interventional measures

Between 2001 and 2004, an international cooperation between China and the Republic of Korea was implemented to tackle helminthiases. Heilongjiang province in northeastern China was selected for clonorchiasis (Choi et al., 2010). Seven villages were included, in which seven different chemotherapy strategies were tested. Among them, five were classified as heavy endemic areas in Zhaoyuan county with a prevalence of over 40%, while another two were moderately endemic areas with a prevalence between 20% and 30% (from Ningan county and Hailin county, respectively). After 3 years, the prevalence of *C. sinensis* infection decreased significantly in all seven groups, but to different degrees **(**Table 2**)**. In all seven groups, 75 mg/kg praziquantel divided into three doses in 1 day was employed. The prevalence decreased by 72.9% from 69.5% in 2001 to 18.8% in 2004 in one heavily endemic village through mass treatment every 2 years. It decreased by 82.5% from 48.0% in 2001 to 8.4% in 2004 in one heavily endemic village through mass treatment every year. It decreased by 72.7% from 54.9% to 15.0% in one heavily endemic village

Table 2 Effectiveness of different chemotherapy strategies using praziquantel against *C. sinensis* infection in a community-based trial (Choi et al., 2010).

Endemic level	Prevalence in 2001 (%)	Prevalence in 2004 (%)	Declining rate (%)	Intervention measures
Heavily endemic villages	69.5	18.8	72.9	Mass treatment every 2 years
	48.0	8.4	82.5	Mass treatment every year
	54.9	15.0	72.7	Selective treatment every year
	73.2	12.3	83.2	Selective treatment and reservoir animal control every year
	59.5	7.5	87.4	Selective treatment every 6 months
Moderately endemic villages	24.8	1.9	92.3	Selective treatment every 2 years
	29.7	1.3	95.6	Selective treatment every year

through selective treatment every year. It decreased by 83.2% from 73.2% to 12.3% in one heavily endemic village through selective treatment and reservoir animal control every year. It decreased by 87.4% from 59.5% to 7.5% in one heavily endemic village through selective treatment every 6 months. In two moderate villages, the prevalence decreased by 92.3% from 24.8% to 1.9% through selective treatment every 2 years and by 95.6% from 29.7% to 1.3% through selective treatment every year. Thus, it was demonstrated that chemotherapy could effectively decrease the prevalence of *C. sinensis* infection. Particularly, high frequency of treatment (mass chemotherapy every year or selective treatment every 6 months in heavily endemic areas and selective treatment every year in moderately endemic areas) is indicated. Additional treatment of reservoir animals increased programme effectiveness.

Following increased awareness of the importance of clonorchiasis after the second national survey between 2001 and 2004, two control pilots were established for clonorchiasis between 2006 and 2009, and NIPD was in charge of the implementation of the projects (Ji et al., 2012; Xin-Hua et al., 2011). One pilot was established in Yangshan county, Guangdong province in southeastern China, and another one in Zhaoyuan county, Heilongjiang province in northeastern China. In Yangshan county, the prevalence of clonorchiasis was 14.0% (356/2541) in 2006 (Xin-Hua et al., 2011). A comprehensive intervention including education and selective chemotherapy (once every year with 75 mg/kg praziquantel divided into twice per day for 2 days or 64 mg/kg albendazole divided into eight times in 4 days) was used. The prevalence decreased to 6.9% (73/1062) in 2009. In Zhaoyuan county, the prevalence of clonorchiasis was 67.5% (1806/2677) in 2006 (Ji et al., 2012). Similarly, health education and chemotherapy (mass chemotherapy once every year with 75 mg/kg praziquantel divided into four times by 2 days) was employed. The persons who accepted treatment were 147,120, 190,991 and 146,644 in 2007, 2008 and 2009, respectively. The prevalence of clonorchiasis decreased to 7.1% (72/1011) in 2009.

The practice of eating raw freshwater fish is key in the transmission of clonorchiasis, and thus the interruption of this practice will determine the sustainability of control. Qian et al. explored a behavioural change strategy against clonorchiasis. To explore this strategy, behavioural change communication products were to be provided. Thus, a national screening was conducted (Qian et al., 2019d). The evaluation system was set up through experts' consultancy (Hui-Hui et al., 2018). A total of 96 groups of products on helminthiases were collected (Qian et al., 2019d).

However, most products targeted schistosomiasis and echinococcosis, and only five focused on clonorchiasis. Out of 34 products awarded, none targeted clonorchiasis. Based on the awarded products targeting schistosomiasis and echinococcosis, a clonorchiasis-specific product was designed and produced. Because of the difficulty to change adults' behaviour, a strategy targeting children was used. The product was a cartoon, which introduced the harm and prevention of clonorchiasis for children. Finally, a cluster trial was carried out (Qian et al., 2019b). In clonorchiasis endemic areas, one school was selected as intervention, and another one as control. Comprehensive education activities were implemented in interventional school, including the erection of a bulletin board, the presentation of the educational cartoon and distribution of educational brochures, as well as drawing and essay writing competitions. After 6 months, the effectiveness was evaluated. In interventional school (251 students), the percentage of students with knowledge on transmission route, early symptoms, complications and carcinogenicity of clonorchiasis increased from 15.1% to 92.4%, from 6.8% to 43.4%, from 4.8% to 94.8% and from 4.8% to 57.8% after 6 months. In the control group (153 students), the proportion increased from 9.2% to 56.9%, from 0.7% to 33.3%, from 2.6% to 42.5%, and from 2.6% to 20.9%. Although the percentage also increased in control school, the effect was lower compared to that in interventional school. Especially, the percentage of students determined not to eat raw freshwater fish in the future increased from 71.3% at baseline to 97.2% at evaluation survey in interventional school, but it didn't change significantly from 79.7% to 83.0% in control school.

8. Conclusions and perspectives

During the past 70 years, significant progresses have been achieved in the control of neglected tropical diseases in China owing to great social developments and control activities (Qian et al., 2019a). However, clonorchiasis is still highly prevalent in eastern China due to the deep-rooted habit of eating raw freshwater fish and the development of aquaculture. According to predictions of the national control programme, the prevalence of clonorchiasis should have decreased by 30% in major endemic areas in China in 2020, as compared to 2015 (National Health and Family Planning Commission of the People's Republic of China, 2016). In the near future, clonorchiasis is yet to be considered as still the most important food-borne parasitic disease in eastern China. It is therefore essential to take

more actions to control clonorchiasis transmission in highly endemic areas. NIPD, the key organization in designing the strategic workplan to this purpose, will focus on several aspects to control clonorchiasis (Qian and Zhou, 2019). First, national surveillance will be strengthened. It is expected that a high resolution map, with details down to the county level will be available around 2020. Second, more research will be carried out to develop innovative diagnostic and treatment tools. Rapid screening tools both at the community and individual level will be operationalized. More research is also expected to validate the efficacy of tribendimidine against clonorchiasis. Third, detailed studies on the transmission mechanism will be explored to guide control. Especially, the determinants of *C. sinensis* transmission will be explored. Fourthly, control strategies and measurements will be further explored, including information, education and communication techniques, chemotherapeutic strategies, and integrated strategies. Additionally, NIPD will put more efforts on the cooperation with international institutions on other liver fluke infections, such as opisthorchiasis in southeastern Asia—Thailand, Laos, Cambodia, Vietnam and Myanmar (Qian and Zhou, 2019). As a result, NIPD is expected to become one of the leading institutions in the control of liver fluke infections globally.

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