Clonorchiasis

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On Aug 21, 1875, James McConnell published in *The Lancet* his findings from a post-mortem examination of a 20-year-old Chinese man—undertaken at the Medical College Hospital in Calcutta, India—in whom he found *Clonorchis sinensis* in the bile ducts. Now, exactly 140 years later, we have a sound understanding of the lifecycle of this liver fluke, including key clinical, diagnostic, and epidemiological features. Developments in the so-called -omics sciences have not only advanced our knowledge of the biology and pathology of the parasite, but also led to the discovery of new diagnostic, drug, and vaccine targets. *C sinensis* infection is primarily related to liver and biliary disorders, especially cholangiocarcinoma. Clonorchiasis mainly occurs in east Asia, as a result of the region's social-ecological systems and deeply rooted cultural habit of consuming raw freshwater fish. The Kato-Katz technique, applied on fresh stool samples, is the most widely used diagnostic approach. Praziquantel is the treatment of choice and has been considered for preventive chemotherapy. Tribendimidine showed good safety and therapeutic profiles in phase 2 trials and warrants further investigation. Still today, the precise distribution, the exact number of infected people, subtle morbidities and pathogenesis, and the global burden of clonorchiasis are unknown. Integrated control strategies, consisting of preventive chemotherapy; information, education, and communication; environmental management; and capacity building through intersectoral collaboration should be advocated.

Published **Online**
August 21, 2015
http://dx.doi.org/10.1016/S0140-6736(15)60313-0

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See **Online** for a short video marking the 140th anniversary of the discovery and subsequent publication of *Clonorchis sinensis*

Introduction

This year marks the 140th anniversary of the discovery and subsequent publication of *Clonorchis sinensis* (video). In brief, on Sept 8, 1874, a 20-year-old Chinese male carpenter was admitted to the Medical College Hospital in Calcutta, India, and died within a few hours from severe liver disease.[1] James F P McConnell, a professor of pathology who did the post-mortem examination the following day, found many narrow, flattened, and lanceolate flukes in the bile ducts, which had not been described before. This finding—the first report on *C sinensis* in the medical literature—was published in *The Lancet* on Aug 21, 1875. 4 weeks later, T Spencer Cobbold publicly suggested naming the parasite *Distoma sinense*.[2] In 1907, Arthur Looss renamed the parasite *Clonorchis sinensis* because of its characteristic branched testes.[3] However, the disease has been in existence for at least two millennia, as shown by the discovery in 1975 of *C sinensis* eggs in the intestine of an excavated corpse of the Western Han Dynasty in Jiangling county, Hubei province, China.[4]

The discovery of *C sinensis* prompted studies on the lifecycle of the parasite (figure 1). In 1910, the Japanese parasitologist Harujiro Kobayashi first proved that freshwater fish act as the second intermediate host, and another Japanese researcher, Masatomo Muto, discovered in 1918 that freshwater snails serve as the first intermediate host.[5] The lifecycle of *C sinensis* is characterised by an alternation of sexual and asexual reproduction in different hosts.[6,7] Briefly, eggs laid by hermaphroditic adult worms, which can survive in man for up to 26 years, reach the intestine with bile fluids and are eliminated with the faeces.[8] After freshwater snails ingest the eggs, miracidia hatch in the intestine and penetrate into the intestinal wall.[9] Subsequently, through asexual reproduction, sporocysts, rediae, and then cercariae are produced. Cercariae escape from the snails about 95 days after infection and adhere to freshwater fish.[10] After encysting in the subcutaneous tissues or muscles of the fish, cercariae develop into mature metacercariae within about 45 days. When people eat raw or insufficiently cooked infected fish, metacercariae will separate from the flesh through gastric juice digestion. Then, through a combined action of extrinsic trypsin and endogenous cysteine proteases, metacercariae excyst in the duodenum.[11] Excysted juvenile flukes migrate quickly, with the help of bile chemotaxis, via the ampulla of Vater to intrahepatic bile ducts, where they develop into adult flukes.[12] Eggs can be detected in faeces around 4 weeks after infection.[13] The egg-laying capacity of an adult *C sinensis* fluke varies, dependent on the final host; in man, this capacity is estimated at around 4000 eggs per worm per day.[14]

New insights gained from the -omics sciences

Complete mitochondrial genomes have been decoded for different *C sinensis* isolates from China, South Korea, and Russia, consisting of 13 879 bp, 13 877 bp, and 13 875 bp, respectively.[15,16] The mitochondrial genome has 36 genes, 12 of which code for proteins, two for rRNA, and the remaining 22 for tRNA. The genome of *C sinensis* has a size of 580 Mb, containing 13 634 protein-coding genes.[17] The fatty-acid metabolism of the parasite is completely functional, but some key enzymes in fatty-acid biosynthesis

Search strategy and selection criteria

We searched PubMed and two Chinese databases (China National Knowledge Infrastructure and Wanfang Data) for studies published between inception of these databases (eg, PubMed, 1945) and Dec 30, 2014. We did not apply any language restrictions to our search. The terms used were "clonorchi*" and "liver fluke*" (PubMed) and "huazhigaoxichong" and "ganxichong" (Chinese databases). We hand-searched bibliographies of identified articles for additional relevant publications.

For the **China National Knowledge Infrastructure database** see http://www.cnki.net

For **Wanfang Data** see http://www.wanfangdata.com.cn

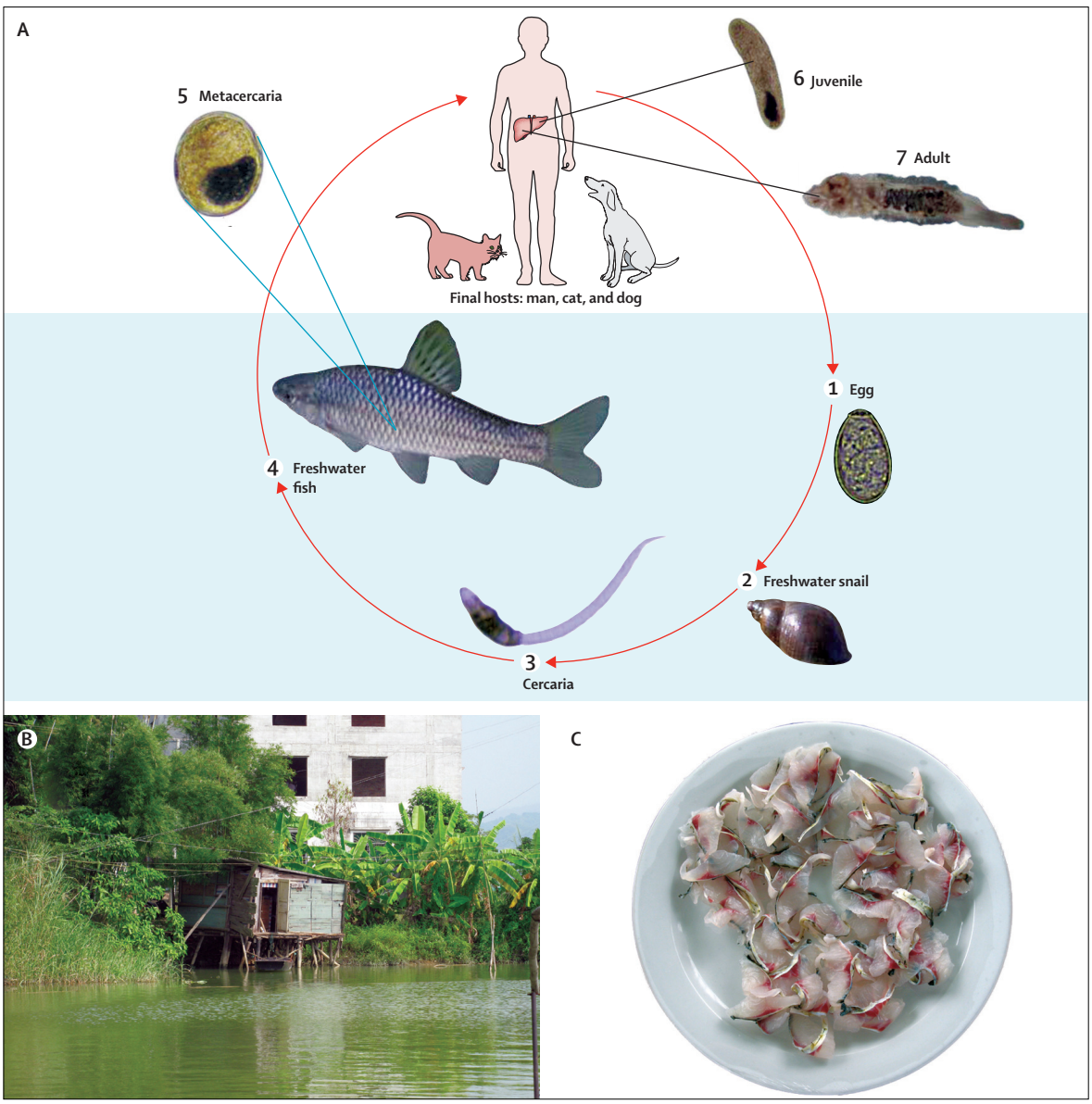

Figure 1: **Lifecycle of *Clonorchis sinensis***
(A) Lifecycle of *C sinensis* (blue colour represents water environment). (1) Eggs enter the water environment from faeces; (2) eggs are ingested by specific freshwater snails, the first intermediate host; (3) cercariae released from the snail; (4) cercariae infect specific freshwater fish, the second intermediate host; (5) cercariae encyst in the subcutaneous tissues or muscles of the fish to form metacercariae; (6) metacercariae are ingested by man (and other hosts) through consumption of raw or undercooked fish, and excyst as juvenile flukes in the duodenum; (7) hermaphroditic adult *C sinensis* predominantly found in intrahepatic bile ducts. (B) A hanging toilet above a river. (C) Raw fish dish.

are absent.[18] The presence of many gene copies encoding fatty-acid-binding proteins suggests the uptake of lipids by the parasite from host bile. Many genes encoding tegumental proteins and excretory-secretory products (ESPs) have been identified, which might lead to the development of new diagnostic assays or serve as drug and vaccine targets. Important molecules contributing to *C sinensis* infection-related pathology, especially cholangiocarcinoma, have also been discovered.[18]

Developmental transcriptomes show that most genes differentially expressed in the developmental phases of the parasite are consistent with the biological and physical features in each lifecycle stage.[19] Genes coding for components of energy metabolism, motility, and reproduction are highly expressed in adults. Proteases and antioxidant enzymes are also highly expressed in adults to defend against host immune attacks. Many aminoacid sequences encoded in the transcriptome of adults have homology to those associated with human cancer development.[19,20] Furthermore, transcriptomes of different tissues in adult *C sinensis*, such as oral sucker, muscle, ovary, and testis, also correspond to their

 Published online August 21, 2015 http://dx.doi.org/10.1016/S0140-6736(15)60313-0

respective biological functions.[17] Genes in pathways relevant to lipid binding, stimulus response, and muscle differentiation are highly expressed in the oral sucker region, whereas those related to metabolic function are highly expressed in muscle tissue. Motility, growth, and development are important for newly excysted juvenile flukes in the bile environment; the upregulation of many genes involved in energy production and regulatory process after stimulation with bile corresponds to these activities.[21]

ESPs are a key focus in proteomic research into *C sinensis*. Diverse biological and pathological functions of these products, such as glycometabolism, detoxification, and carcinogenesis, have been identified.[22,23] Among the ESPs, diverse glutathione transferases are activated differentially in response to host bile and oxidative stressors.[24] Additionally, immunoproteomic analysis of them might help in the discovery of potential serodiagnostic antigens.[25]

Clinical manifestation and complications

Symptoms caused by an infection with *C sinensis* are related to worm burden.[14,26,27] Hence, patients with low infection intensity are often asymptomatic or show only mild symptoms, whereas patients with high infection intensity often show unspecific symptoms, such as asthenia, nausea, indigestion, headache, dizziness, vertigo, abdominal discomfort, diarrhoea, or abdominal pain, especially in the right upper quadrant.[28,29] Typical physical signs of *C sinensis* infection are jaundice, hepatomegaly, and liver tenderness.

Chronic *C sinensis* infection results in various complications in the liver and biliary systems, mainly cholelithiasis, cholangitis, and cholecystitis.[30–34] Cholelithiasis is one of the most frequent complications of such infection. Additionally, liver abscess and pancreatitis are suspected to be linked to *C sinensis* infection.[30,31,35] Developmental retardation has been reported in children with heavy infection. These children often present with inappetence, diarrhoea, malnutrition, anaemia, and hepatomegaly.[36] *C sinensis* infection is now widely acknowledged to be associated with cholangiocarcinoma, the bile duct cancer (figure 2).[37,38] Despite demonstration of an association between *C sinensis* infection and cholangiocarcinoma in case studies, cross-sectional surveys, and case-control studies, *C sinensis* was—by contrast with *Opisthorchis viverrini* (another major liver fluke)—only in 1994 classified as a probable carcinogen (group 2A) by the International Agency for Research on Cancer.[39] As additional evidence accrued, *C sinensis* was reclassified in 2009 as a definite carcinogen (group 1).[37,38] Systematic reviews and meta-analyses show pooled odds ratios for *C sinensis* infection and cholangiocarcinoma ranging between 4·5 and 6·1, as assessed by cross-sectional and case-control studies.[40–42] The estimated yearly incidence of cholangiocarcinoma attributable to clonorchiasis is 25 per 100 000 in female and 35 per 100 000 in male individuals.[42]

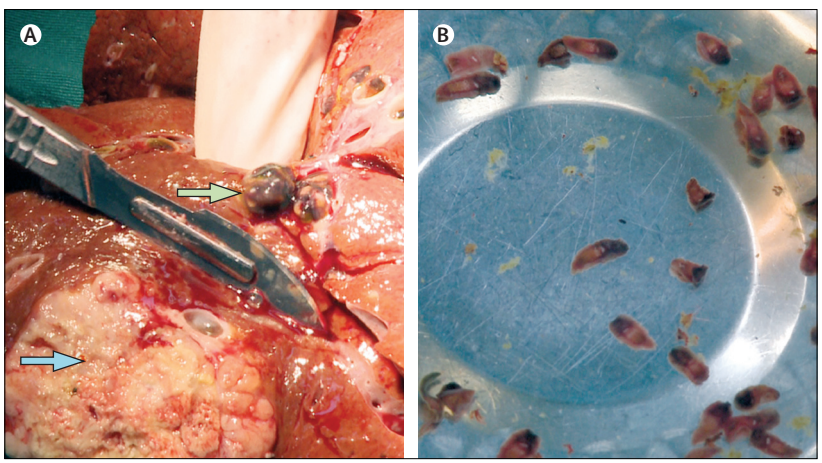

Figure 2: **Liver with cholangiocarcinoma and *Clonorchis sinensis***
(A) A human liver with cholangiocarcinoma (blue arrow) and adult *C sinensis* flukes (green arrow). (B) Adult *C sinensis* flukes (length: 15–20 mm; width: 3–5 mm) isolated from a human liver with cholangiocarcinoma.

Pathogenesis and carcinogenesis

The pathogenesis of *C sinensis* infection encompasses several factors, such as mechanical obstruction of the bile ducts by the worms, mechanical injury by feeding activities of the worms through the mucosa of the bile ducts, immunopathology caused by infection-related inflammation, including secondary bacterial infection, and toxic effects of the worms' ESPs.[7,43,44] The accumulation of worms and the narrowing of the bile ducts by adenomatous hyperplasia cause an obstruction, sequential bile stagnation, and bile pigment deposition, which may give rise to the formation of stones in bile ducts with eggs or dead worms as nuclei.[30,32,43,44] After cholestasis, a favourable environment for secondary bacterial infection is established, especially for *Escherichia coli*, which might result in cholangitis. Cholecystitis associated with *C sinensis* infection consists of fibrosis, infiltration of mast cells and eosinophils, and mucosal hyperplasia of the gallbladder wall.[7] Consequently, a poor function of the gallbladder causes precipitation of bilirubinate, calcium carbonate crystals, and mucin on the eggs, which causes stone formation in the gallbladder.[33,34] Hepatomegaly is usually more pronounced in the left lobe than in the right lobe, which has been explained by the presence of a higher number of worms in the left lobe because of small anatomical differences between the hepatic ducts; the left hepatic duct is wider than its right counterpart and there is a smaller tilt angle between the left hepatic duct and the common hepatic duct.[45]

The exact mechanism by which carcinogenesis occurs remains to be elucidated; many pathways could be implicated.[43,46] ESPs are immunogenic, they stimulate inflammation and promote proliferation, and they suppress apoptosis.[46–49] Differential transcriptional and proteomic profiles in human cholangiocarcinoma

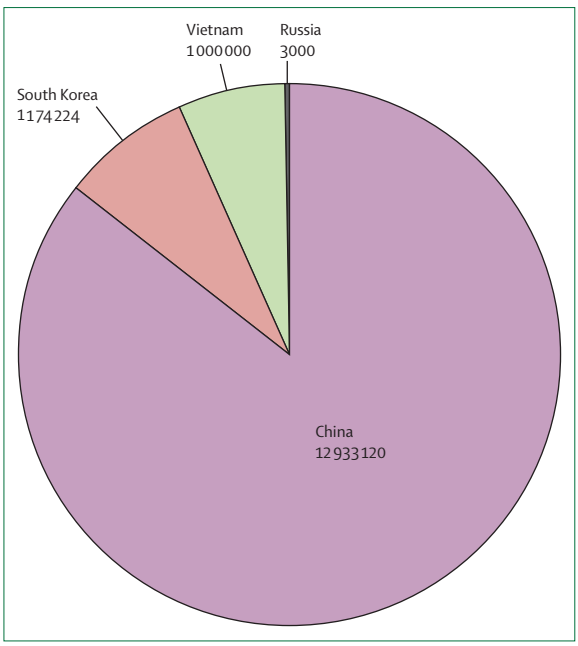

Figure 3: **Global distribution of clonorchiasis**
Adapted with permission from Qian and colleagues.[58]

cells treated with ESPs showed upregulation or downregulation of host molecules.[50–52] The upregulated genes are related to oncogenesis, or cell proliferation or differentiation, whereas the downregulated genes have a role in apoptosis.[50,52] For example, the gene coding minichromosome maintenance protein 7 (MCM7), which is associated with various cancer types, is upregulated.[50] Further investigations showed that ESPs from *C sinensis* induce histone acetyltransferase recruitment and MCM7 transactivation, subsequently giving rise to cholangiocarcinoma.[53] ESPs from *C sinensis* also trigger an increase in free radicals through several enzymes, which, in turn, induce the increase in mRNA and protein expression of the pro-inflammatory cytokines interleukin 1β and interleukin 6, in a nuclear factor-κB-dependent manner.[54] In opisthorchiasis, parasite-specific interleukin 6 is implicated in the pathogenesis of advanced periductal fibrosis, with a possible link to cholangiocarcinoma.[55] *O viverrini* granulin, the major growth factor present in ESPs, was proved to induce proliferation of host cells and to contribute to the development of a tumorigenic environment.[56] Unsurprisingly, the gene encoding *O viverrini* granulin has a homology in the genome of *C sinensis*, and it is highly transcribed in adult worms, especially in the sucker region.[17–19] Comparison between cholangiocarcinoma caused by *O viverrini* infection and cholangiocarcinoma resulting from other non-infectious causes shows differences in mutation patterns.[57] Thus, it is reasonable to propose that differences also exist in the induction of cholangiocarcinoma by *C sinensis* infection compared with other causes.

Epidemiology

An estimated 15 million people are infected with *C sinensis*, predominantly in east Asia (ie, China, South Korea, northern Vietnam, and parts of Russia; figure 3).[41,42,58] China has the biggest share with an estimated 13 million people infected with the parasite. There are two major endemic regions in China—namely, provinces in the southeast, including Guangdong and Guangxi, and provinces in the northeast, such as Heilongjiang and Jilin.[42,59] Clonorchiasis is a major public health problem in South Korea, with an estimated 1·2 million people infected with *C sinensis*.[60] Especially high prevalence rates are reported along the four major rivers in the southern part of South Korea.[61] About 1 million people are infected with *C sinensis* in Vietnam, mainly in the northern part.[62,63] Finally, about 3000 people in the far east of Russia are reported to be infected with *C sinensis*.[62] Clonorchiasis coexists in opisthorchiasis-endemic areas of Thailand, as identified by PCR-based examination.[64] Historically, *C sinensis* infection was highly endemic in Japan, although only a few cases are reported nowadays.[65] In other countries, cases are reported in either immigrants from endemic countries or travellers visiting endemic areas who consume raw freshwater fish.[42]

Overall, the number of people infected with *C sinensis* has more than doubled from 7 million in the 1990s to 15 million in the 2000s.[41,42,58,62] A total of 275 370 disability-adjusted life-years have been attributed to clonorchiasis.[41] However, this might be a serious underestimation because of the exclusion of light and moderate infections, although disability might be noted in these groups.[66] The prevalence of *C sinensis* infection in male individuals is usually higher than that in female individuals.[42,59–61] In general, prevalence increases with age and is usually highest in the 50–59-year age group. The infection intensity shows similar trends with regard to sex and age compared with infection prevalence. Prevalence and intensity of infection are determined by the deeply rooted culture of eating raw fish, as well as the accumulative effect of long survival of the parasites.[8,67–69]

Environmental determinants and social-ecological systems

The distribution of intermediate host snails is a key factor affecting the endemicity of clonorchiasis (figure 4). *Parafossarulus striatulus*, *Bithynia fuchsiana*, *Alocinma longicornis*, and *Melanoides tuberculata* are the most important intermediate host snail species.[27,62,63] *P striatulus* is predominantly found in ponds, ditches, streams, and swampy areas with water grasses and abundant organic materials in the beds. Water temperature affects the survival and reproduction rate of *P striatulus*.[70] Temperature above 20°C promotes egg-laying of the snail intermediate host. Hibernation in mud prevents snail death in winter. More than 100 species of freshwater fish have been implicated in

 Published online August 21, 2015 http://dx.doi.org/10.1016/S0140-6736(15)60313-0

the transmission of *C sinensis*, because they can harbour metacercariae. Most of these fish belong to the Cyprinidae family.[27,62] Additionally, several species of crayfish can harbour metacercariae of *C sinensis* and are thus contributing to the transmission of the disease in focal areas of China, where consumption of raw crayfish is a deeply rooted sociocultural practice.[71]

Traditionally, high prevalence of *C sinensis* has been reported in villages in close proximity to freshwater bodies used for aquaculture.[72] Piscivorous animals, especially cats and dogs (both wild or reared as pets or guardians), serve as reservoir hosts for *C sinensis*, and these animals are widely distributed.[73,74] Cats and dogs can maintain the lifecycle of the parasite in endemic areas without involvement of people.

Land exploitation affects the distribution of bodies of water, which then affects the endemicity of clonorchiasis.[72,75] Climate change might also have a role; rising temperature will affect the survival and reproduction of intermediate hosts, and changes in rainfall pattern will affect the presence of water bodies, especially ponds.[75]

The epidemiology of clonorchiasis is determined by social-ecological systems, especially consumption of raw freshwater fish,[68,76] a practice that is passed from one generation to the next. In some areas, eating of raw fish is strongly encouraged to protect traditional cultural habits; in some instances special festivals are organised in this regard.[76] In addition to unawareness of the presence, transmission, and effect on health of clonorchiasis, many people believe that raw fish is highly nutritious and that metacercariae can be killed by concurrent consumption of alcohol or hot spices.[77] Offering raw fish to guests is deemed a hospitable gesture.[76] Untreated human and animal faeces are still used to feed fish in traditional aquaculture, which promotes infection of fish.[78] Importantly, economic development is a key factor in disease transmission because more people can afford to eat raw fish, not only at home but also in restaurants.[69] The expansion of aquaculture—nowadays also making use of rivers, lakes, and water reservoirs to enhance inland fish cultivation—increases the difficulties for environmental management as a means of control. Additionally, because of improved trade and transportation channels, which help the distribution of fish, clonorchiasis is no longer restricted to villages located near bodies of water.[72]

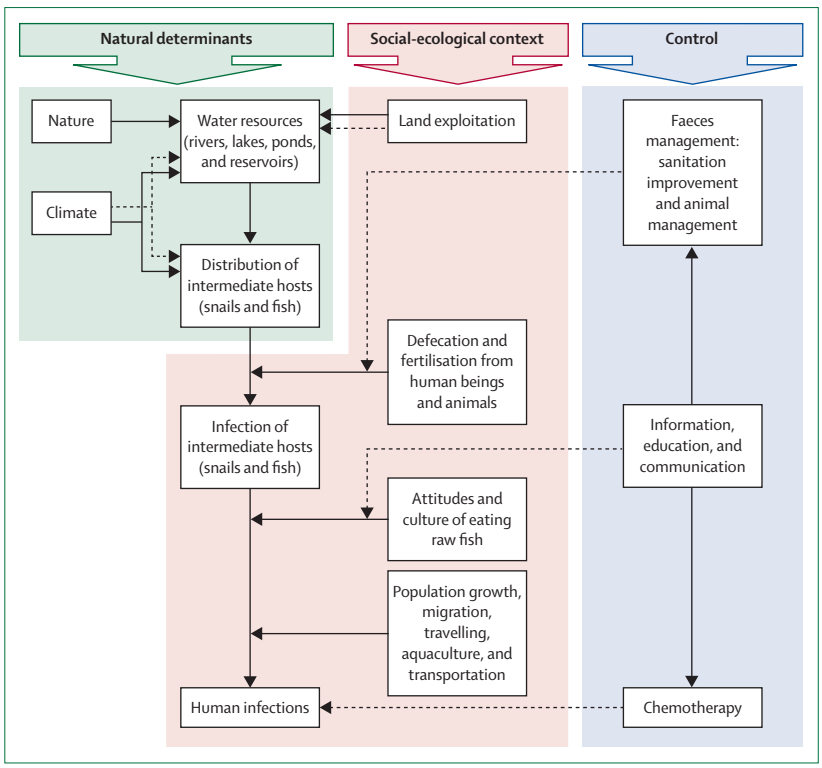

Figure 4: **Epidemiological determinants of clonorchiasis**
Solid arrows represent promoting agents. Dashed arrows represent inhibitory agents.

Diagnosis

The detection of eggs in stool is the gold standard for diagnosis of *C sinensis* infection. However, eggs cannot be detected in faeces during biliary obstruction.[79] The Kato-Katz method is the most widely used technique because of its simplicity, low cost, and the ability to quantify infection intensity.[14,80] A disadvantage of this method is low sensitivity, especially for detection of low-intensity infection. Therefore, several Kato-Katz thick smears, ideally prepared from consecutive stool samples, are needed to enhance sensitivity.[81] Additionally, to distinguish *C sinensis* eggs from other liver flukes (eg, *O viverrini* and *Opisthorchis felineus*) and minute intestinal flukes with microscopy is very difficult.[82] Direct faecal smear is often applied in hospitals.[80] The formalin-ether concentration technique is mainly used in laboratories, but the repeated washing steps may result in a serious underestimation of infection intensity.[81] Diagnosis can also be based on *C sinensis* eggs detected in the gallstone and bile.[33,34,79] Collection of worms after purgation with anthelmintic drugs is useful for differentiating between helminth species.[83]

Immunodiagnosis for clonorchiasis is usually used as a supplementary method. Intradermal testing for clonorchiasis has been applied since the 1950s and was widely used before the introduction of ELISA in the late 1970s.[26,84,85] Serodiagnosis by ELISA using crude antigens is not ideal because of cross-reactivity, but use of ESPs can lead to improved performance.[47] Application of recombinant antigens, such as proteins in tegument or ESPs, raises specificity but results in decreased sensitivity.[86] Furthermore, ELISA tests that detect antibodies cannot differentiate between past and active infections, especially in patients with high infection intensity.[87] Assays that detect antigens avoid this challenge and could be used for post-chemotherapy assessment.[88] Other immunodiagnostic techniques have also been attempted in clonorchiasis, such as complement fixation,

	Protein	Worm reduction rate	Egg reduction rate
DNA vaccine (pcDNA3·1)[114]	Cysteine proteinase	31·5%	15·7%
DNA vaccine (pcDNA3·1)[115]	Fatty-acid-binding protein	40·9%	27·5%
Bacillus subtilis spore-based vaccine[116]	Tegumental protein 22·3 kDa	44·7%	30·4%
Protein vaccine[117]	Rho GTPase	60·5%	68·8%
Protein vaccine[118]	14–3-3 epsilon	45·4%	37·9%
Protein vaccine[119]	Paramyosin	54·3%	50·9%
DNA vaccine (pcDNA3·1)[119]	Paramyosin	36·1%	38·8%
Protein vaccine[120]*	Enolase	56·3%	NK
Protein vaccine[120]†	Enolase	15·4%	NK
DNA vaccine (pcDNA3·1)[120]	Enolase	37·4%	NK
Bacillus subtilis spore-based vaccine[120]	Enolase	61·1%	80·7%
Protein vaccine[121]	Cathepsin B cysteine protease (CB2)	41·0%	33·6%
Protein vaccine[121]	Cathepsin B cysteine protease (CB3)	67·2%	57·7%

NK=not known. *Subcutaneously. †Intraperitoneally.

Table: **Vaccine candidates tested in the rat model against *Clonorchis sinensis* infection**

precipitation, agglutination, indirect haemagglutination, indirect fluorescent antibody, and immunoelectrophoresis, but they are seldom used in epidemiological studies.[26,85]

Different PCR methods have been developed in the past few years, which have two major advantages.[64,89,90] First, PCR shows high performance in the diagnosis of low-intensity infection. Second, the technique allows *C sinensis* to be distinguished from other trematode species. A loop-mediated isothermal amplification (LAMP) technique has been developed to detect *C sinensis* infection in intermediate hosts.[91,92] Studies to diagnose human *C sinensis* infection with LAMP are warranted in view of the simplicity of this technology compared with PCR.

Imaging diagnosis is used as an auxiliary method in clinical settings.[43] Intrahepatic bile duct dilatation, increased periductal echogenicity, and gallbladder sludge in sonography are important diagnostic indicators in clonorchiasis.[93] However, the diagnostic accuracy of imaging is dependent on the infection intensity. Furthermore, some indicators will persist for several years after treatment, which renders differentiation between active and past infections difficult.[94]

Treatment

Before the advent of praziquantel in the late 1970s, many drugs, including chloroquine, furapromide, bithionol, dithiazanine iodide, hexachlorophene, niclofolan, amoscanate, hexachloroparaxylene (hexachloroparaxylol), and metronidazole had been tested against clonorchiasis.[95] However, these drugs were only moderately efficacious, and many of them were toxic. Praziquantel, a broad-spectrum trematocidal and cestocidal drug developed in Germany, was first tested by scientists in China and South Korea towards the end of the 1970s to treat clonorchiasis.[96,97] At present, praziquantel is the only recommended drug for treatment of clonorchiasis.[98] The efficacy of this drug depends on the treatment schedule used and infection intensity. For an individual treatment, 25 mg/kg praziquantel thrice daily for 1 or 2 consecutive days is usually given, which can be highly efficacious.[96,99,100] In more detail, a total dose of 150 mg/kg resulted in cure rates of more than 90% and egg reduction rates of nearly 100%.[96,99] At half this dose, with a 1-day treatment schedule of three doses of 25 mg/kg praziquantel, high egg reduction rates were recorded. However, cure rates were low, especially in the treatment of heavy infections.[96,99,100] Additionally, a single dose of 40 mg/kg praziquantel, widely used in preventive chemotherapy programmes against schistosomiasis, resulted in a cure rate of 95% in light infections but only 89% and 69% in moderate and heavy infections, respectively.[101] Mild and transient adverse events are usually reported with this drug, such as dizziness, headache, and abdominal pain.[96,97,99–101] Severe adverse events (eg, anaphylactic reaction) occur occasionally.[102,103] Another broad-spectrum anthelmintic drug, albendazole, is also efficacious against *C sinensis* infection.[104,105] When given for several days (usually 5 or 7 days), albendazole achieves high cure rates and is well tolerated.

The anthelmintic drug tribendimidine was approved by Chinese authorities for treating soil-transmitted helminthiasis in 2004.[106] Recently, inspired by promising results recorded in a series of in-vivo and in-vitro studies, two randomised controlled trials have been done in China to assess the efficacy and safety of tribendimidine against *C sinensis*.[100,107] A dose of 400 mg tribendimidine achieved cure rates of 44–50%. Furthermore, tribendimidine given at 400 mg divided in two doses led to a cure rate of 33%,[107] whereas a 3-day regimen of 400 mg tribendimidine once daily resulted in a cure rate of 58%.[100]

Immunology and vaccines

High concentrations of IgG have been measured in serum and bile from patients infected with *C sinensis*, whereas concentrations of serum IgA and IgE and secretory IgA in bile are only moderately raised.[108] Bile and serum concentrations of IgG4 are correlated with infection intensity, which indicates the importance of the T-helper-2 (Th2) immune response. These findings are consistent with the changes in cytokine concentrations in infected patients—namely, a decrease in interleukin 2 and an increase in interleukin 4.[109] However, high infection intensity and reinfection rates in populations in endemic areas suggest that these immune responses have little protective effect. Notably, mice exhibit only low susceptibility to *C sinensis* infection, which may be related to both Th1 and Th2 responses, as suggested by raised concentrations of IgE, interferon γ, and interleukin 13.[110] Although rats are susceptible to *C sinensis* infection, protective immunity appears in reinfection.[111,112] This resistance to reinfection is related to the worm burden,

duration of primary infection, and time since treatment; it might also be driven by raised concentrations of serum IgE and secretory IgA.[113]

All vaccine candidates against clonorchiasis are in the preclinical phase, some of which show promising results in the rat model (table).[114–121] Most proteins within the vaccine candidates belong to tegumental proteins or ESPs. Both protein-based and nucleic acid-based vaccines have been tested, with *Bacillus subtilis* spore-based vaccine offering the key advantage of oral administration. Some DNA vaccines induce a Th1-dominated immune response, whereas others induce combined Th1 and Th2 immune responses. By contrast, protein vaccines induce combined Th1 and Th2 immune responses. Finally, *B subtilis* spore-based vaccine of tegumental protein 22·3 kDa can induce a secretory IgA mucosal response. In terms of protective efficacy, none of the candidates achieved a worm reduction rate of more than 70%. In view of the differences in immune response and resistance to reinfection between human beings and rodents, we are still a long way from a human vaccine for clonorchiasis. However, another avenue of vaccine research is worth a mention—namely, vaccination of the second intermediate host, freshwater fish, with feed probiotics. Indeed, an oral vaccine based on *B subtilis* expressing enolase is being tested in freshwater fish.[120]

Control

At present, management of clonorchiasis focuses on morbidity control with praziquantel.[98] In high-endemicity areas, prevalence and infection intensity decreased substantially within 3 years when praziquantel (75 mg/kg) was given in selective chemotherapy twice per year or through mass drug administration once per year.[122] In moderately endemic areas, yearly administration of praziquantel under selective chemotherapy also resulted in a substantial decrease in terms of prevalence and intensity of infection within 3 years.[122] Information, education, and communication (IEC) is usually combined with chemotherapy to enhance sustainability.[123] Overall, disease knowledge is insufficient and unequal in different population groups.[69,124] Men often have a better knowledge of clonorchiasis as a result of high literacy, but because of their strong belief in tradition, to dissuade them from eating raw fish is difficult; hence, men often show higher prevalence and infection intensity than women. Hospital-based IEC should therefore be combined with traditional community-based approaches to increase persuasiveness. Additionally, misconceptions need to be challenged, such as the belief that consumption of alcohol or spicy food can prevent infection.[77] By contrast, despite poor knowledge, the habit of eating raw fish is less deeply rooted in women and children.[69] Therefore, IEC tailored to children might promote control in the long term.[125] Additionally, awareness of the disease, particularly clinical-diagnostic algorithms, should be raised in medical workers.[124]

Environmental modification should be adopted to prevent the contamination of water by faeces. Removal of toilets over fish ponds leads to a substantial decrease in the number of snails and fish infected with *C sinensis*.[126] However, complete removal of faecal contamination is not feasible at present. Many fish are produced in large bodies of water, including rivers, lakes, and water reservoirs, where control of contamination with faeces from animal reservoirs and people is difficult to achieve.[73,74,127,128] Control of snails with molluscicides is not recommended because of the wide distribution and low rate of fluke infection of snails, and the potential toxic effects to fish.[129] Biological control, with predator fish that feed on snails, needs further investigation.[130]

Although the burden of clonorchiasis is substantial, technical capacities in combating this liver fluke disease are still insufficient in many endemic settings. In China, since 2010, a national technique competition has been in place with the objective to enhance the accuracy of the diagnosis of parasitic infections, including *C sinensis* infection.[131] This initiative promotes the implementation of widespread training at a provincial level.[132] Under-diagnosis of *C sinensis* infection is common because of the absence of a highly sensitive detection method and the paucity of clinical-diagnostic algorithms.[133] *C sinensis* infection might be misdiagnosed as hepatitis B because of the geographical overlap and similar clinical manifestations of these diseases.[133] Co-infection with hepatitis B virus is a challenge not only for diagnosis but also for treatment of clonorchiasis.[134] Thus, specialised training for clinicians and laboratory technicians on how to diagnose *C sinensis* infection warrants high priority in hospitals in clonorchiasis-endemic areas.

Conclusions

The public health importance of liver fluke infections, especially clonorchiasis, has been neglected for decades, which might explain the persistence and growing number of infections and corresponding disease burden.[135] Clinical and epidemiological research into clonorchiasis over the past 140 years has contributed to a deeper understanding of the parasite, intermediate hosts, and disease. However, research into the biology of *C sinensis* and control of clonorchiasis lags behind that of opisthorchiasis and many other parasitic diseases.[58]

Although the association between *C sinensis* infection and cholangiocarcinoma has been firmly established in past years, underlying mechanisms have not been fully elucidated, which hampers accurate diagnosis and prevention. Additionally, currently available local, national, regional, and worldwide burden estimates for clonorchiasis are guesses at best. For example, no national survey has so far been done in Vietnam, and thus the estimated number of people infected with *C sinensis* in this country—and the world—is inaccurate. National sampling surveys calculate prevalence rates only at a provincial level in China, whereas data for

Panel: Research priorities for clonorchiasis

- Establish a global database for *Clonorchis sinensis*—including behavioural, climatic, demographic, ecological, and socioeconomic factors—to help with global disease burden estimates, risk mapping, and prediction
- Assess morbidity due to *C sinensis* infection and drivers of carcinogenesis caused by chronic infection; discover biomarkers for early diagnosis, especially those differentiating the disease from hepatitis B virus infection
- Develop rapid immunological techniques to be used in moderate-endemicity and low-endemicity areas where research into control and elimination of clonorchiasis is limited
- Set up large-scale trials to assess the efficacy and safety of tribendimidine and combination therapies
- Put forward a research and development portfolio for vaccines, including vaccines targeting the intermediate hosts
- Use mathematical modelling to assess different tools and strategies for large-scale control of clonorchiasis

counties and villages are absent, thus hampering control activities. More emphasis should be placed on surveillance, so that control interventions can be tailored to specific settings. Furthermore, hepatitis B infection is highly prevalent in many clonorchiasis-endemic areas, and the potential interaction between the two diseases has not gained wide attention.[136] The panel shows research priorities that should guide future studies.

In the long term, new control strategies might emerge, which should include chemotherapy, IEC, environmental modification, and capacity building through intersectoral collaboration (eg, health, education, agriculture, water resources, and publicity). Collaboration should also be encouraged within health agencies, such as disease control departments and hospitals.

Contributors

M-BQ, JU, JK, and X-NZ conceived the paper. M-BQ, JU, JK, and X-NZ performed the literature search, prepared the figures, and interpreted the data. M-BQ wrote the first version of the manuscript. JU, JK, and X-NZ assisted in the restructuring and revision of the manuscript. All authors read, contributed to, and approved the final version.

Declaration of interests

We declare no competing interests.

Acknowledgments

We thank Ying-Dan Chen, Cheng-Wen Gu, Ting-Jun Zhu, Robert Bergquist, and Shang Xia for their assistance in the accompanying video project. This investigation was supported by the International Development Research Center (IDRC), the Canadian International Development Agency (CIDA), and the Australian Agency for International Development (AusAID) in partnership with the Global Health Research Initiative (grant number 105509–00001002–023). JK is grateful to the European Research Council for financial support (ERC-2013-CoG 614739-A_HERO). The funders of this work had no role in design, data collection, data analysis, the decision to publish the report, or preparation of the manuscript. M-BQ and X-NZ had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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