Journal of Helminthology (2005) **79,** 269–281 DOI: 10.1079/JOH2005300

Clonorchiasis: an update

H.-J. Rim*

Department of Parasitology, College of Medicine, Korea University, Seoul 136-705, Korea

Abstract

Clonorchis sinensis, the Chinese or oriental liver fluke, is an important human parasite and is widely distributed in southern Korea, China (including Taiwan), Japan, northern Vietnam and the far eastern part of Russia. Clonorchiasis occurs in all parts of the world where there are Asian immigrants from endemic areas. The human and animal reservoir hosts (dogs, pigs, cats and rats) acquire the infection from the ingestion of raw fish containing infectious metacercariae. The first intermediate snail hosts are mainly species of *Parafossarulus* and *Bithynia*. Numerous species of freshwater fish serve as the second intermediate hosts of *C. sinensis*. Extensive studies of clonorchiasis during several decades in Japan, Korea, China and other countries have shown much progress in proving its morphological features including ultrastructure, biology, pathogenesis, epidemiology, clinical manifestations and chemotherapy. The present review deals with mainly current results obtained on the epidemiological, pathological and clinical aspects, as well as control measures in endemic areas. As for the complications of clonorchiasis, formation of calculi in the intrahepatic biliary passages is one of the most characteristic pathological features. It is sometimes accompanied by suppurative cholangitis, cholecystitis, cholangiohepatitis and ultimately can cause cholangiocarcinoma. Experimental results on the relationship to the occurrence of cholangiocarcinoma are presented. Clinical diagnosis by radiological findings including cholangiography, sonography and computerized tomography as well as magnetic resonance imaging for biliary or pancreatic ducts are outlined. Current studies on immunology and molecular biology of *C. sinensis* were introduced. Praziquantel is the drug of choice for clonorchiasis. The most effective regimen is 25 mg kg^{-1} three times daily (total dose, 75 mg kg^{-1}) administered orally at 5- to 6-h intervals over a single day. Prevention and control measures are also discussed.

Introduction

Clonorchiasis is an infectious disease of the biliary passages caused by the Chinese or oriental liver fluke, *Clonorchis sinensis,* the most important liver fluke of humans. This infection is common in the Far East, especially in southern Korea, China (including Taiwan), Japan, northern Vietnam and the far eastern part of Russia where the eating of raw fish containing infectious metacercariae is commonplace. Clonorchiasis occurs in all parts of the world where there are Asian immigrants from endemic areas. Dogs, pigs, cats and rats are reservoir hosts.

Clinical manifestations of the disease range from asymptomatic in light infections to severe symptoms, including marked gastrointestinal disturbances with a syndrome associated with biliary obstruction. Generally, chronic heavy infections cause damage to the bile duct epithelium, eliciting gastrointestinal symptoms, jaundice, cholangitis, biliary stones and possibly cholangiocarcinoma.

The human and animal reservoir hosts acquire the infection from the ingestion of raw, inadequately cooked freshwater fish. The flukes reach maturity with 16 to 25 days after encysted larvae are ingested. The adult worm of *C. sinensis* measures 8 to 15 mm long by 1.5 to 4.0 mm

*Fax: +(822) 780 6251
E-mail: hjrim@korea.ac.kr

wide and about 1.0 mm thick. Infected individuals may pass viable eggs for as long as 30 years. It is not directly transmitted from person to person. This infection is one of fish-borne parasitic infections linked with poverty, poor sanitation, rampant environmental pollution and population growth.

Extensive studies of clonorchiasis during several decades in Japan, Korea, China and other countries have shown much progress in proving its morphological features including ultrastructure, biology, pathogenesis, epidemiology, clinical manifestations and chemotherapy (Komiya, 1966; Rim, 1986; Chen *et al.*, 1994).

The present review deals with mainly current results obtained on the epidemiological, pathological and clinical aspects, as well as control measures in endemic areas.

Epidemiology

Occurrence and geographic distribution

The infection of *Clonorchis sinensis* is common in the Far East, and seven million people are infected with this fluke. Endemic areas include Japan, southern Korea, essentially all of mainland China except the northwest, Taiwan, northern Vietnam and the far eastern part of Russia. However, clonorchiasis occurs in all parts of the world where there are Asian immigrants from endemic areas (WHO, 1995).

The geographical distribution of clonorchiasis closely parallels the distribution of the snail intermediate host, although infected fish can be found far away from the location of the snail's habitat. However, the infection occurs because of the habit of eating raw fish. The incidence of clonorchiasis varies considerably from one district to another, even within small regions whether the inhabitants are living near streams, ponds or canal irrigations connected to nearby rivers.

Heavily endemic areas of clonorchiasis in Korea are scattered throughout the country, with the most extensive and intensive endemic regions found mainly along the Nakdong River and the lower reaches of other rivers. Generally, inhabitants living along riversides showed relatively high rates (8.0 to 40.2%), whereas those living in regions which are not connected with the rivers or in places near the seashore rarely showed the infection. According to the results of nationwide surveys, the prevalence of *C. sinensis* infection was 4.6% in 1971, 1.8% in 1976, 2.6% in 1981, 1.7% in 1986, 2.1% in 1992 and 1.5% in 1997. Therefore, the infection status of *C. sinensis* has shown little change over 25 years despite the development of effective anthelmintics such as praziquantel (Rim, 1997).

The southern part of China, particularly Guangdong Province and Guangxi Zhuang Autonomous Region are the most seriously affected. Heilongjiang, Jilin and Liaoning provinces in the northeast are also the most severely affected endemic areas (Chen *et al.*, 1994). Prevalence rates vary between 1 to 57% as was reported in a total of 24 provinces, municipalities and autonomous regions in mainland China (Yu *et al.*, 1994). In Guangdong Province, Fang (1994) reported prevalence rates of 21.1% in the Zhu Jiang delta, 4.4% in the upper reaches of the Zhu Jiang and 5.1% in Han Jiang valley after carrying out epidemiological surveys in 95 counties and cities. Recently Rim (2003) reported the results of field surveys under the Korea–China collaborating project during the years of 1995 to 1999, the mean infection rate of clonorchiasis was 7.5% with the range of 0.2% (Anhui and Jiangxi provinces) to 36% in the villages in Guangxi Province. In the northeastern region of China, 15.2% of Korean minority were infected with *C. sinensis* in the villages of Liaoning Province. On the other hand, the prevalence rate of *C. sinensis* in six villages of Zhaoyuan County in Heilongjiang Province was 44.5 to 68.8%.

In Taiwan, there are three highly endemic areas: Miao-li in the north, Sun-moon Lake in the middle and Mei-nung in the south of Taiwan. The infection rate varies greatly from 0 to 57% (Chen, 1991), but the prevalence rate was estimated at 0.012 to 1.5% in the whole population of Taiwan (Cross, 1984).

According to data of IMPE (Institute of Malaria, Parasitology and Entomology) in Vietnam, the parasite was found mainly in northeastern provinces of the Red River Delta. The prevalence rates ranged from 0.2% to 36.9% in 11 provinces (Nontasut *et al.*, 2003). The heaviest infections occurred among the inhabitants in and around Haiphong and Hanoi.

According to Komiya & Suzuki (1964) in Japan, a century ago *C. sinensis* infection was widely distributed except in Hokkaido, the northern part of Japan. At present, however, the infection rate is markedly low, which is attributed to the decreased density of the snail population in the endemic area, due to water pollution from factories, insecticides, land reclamation, as well as the rise of living standards and environmental changes (Rim, 1986).

In the far eastern part of Russia, *C. sinensis* was endemic in the Amur River territory where it is connected with the Sunghwajiang River in China which is incriminated with *Clonorchis* infection. Considerable numbers of clonorchiasis cases are presented in the region of the Upper Amur and its tributaries (Figurnov *et al.*, 2002).

Definitive and reservoir hosts

Clonorchis sinensis inhabits the bile ducts in the liver and occasionally the pancreatic ducts in humans. The natural definitive hosts other than humans are the dog, pig, cat, wild cat, marten, badger, mink, weasel and rat. Experimentally susceptible animals were demonstrated as cats, dogs, rabbits and guinea-pigs. A survey on reservoir hosts was conducted in Korea by Kim (1974) who examined the prevalence of *C. sinensis* infection in animals (pigs, dogs and house rats) in high- and low-endemic area in Korea. The prevalence rate of infection among animals in the high endemic locality was significantly higher than in the low endemic locality. Therefore, reservoir hosts such as pigs and rats may play a significant role in transmitting the eggs of *C. sinensis*. However, in endemic areas, more humans are infected than reservoir animals. This indicates that infected humans rather than reservoir hosts play a major role in the epidemiology of the disease.

https://doi.org/10.1079/JOH2005300 Published online by Cambridge University Press

Intermediate hosts

First (snail) intermediate hosts

The first intermediate snail hosts are species of *Parafossarulus* and *Bithynia.* The important species in China, Korea and Japan is *Parafossarulus manchouricus* (i.e. *P. striatulus* and *P. striatulus japonicus*). The other susceptible snails in China are reported as *Bithynia fuchsiana, Bithynia (Alocinma) longicornis, Assiminaea lutea*, and *Melanoides tuberculata* (Gibson & Sun, 1971). Some other species of snails were reported from limited areas.

Second intermediate hosts

Numerous species of freshwater fish serve as the second intermediate hosts of *C. sinensis.* The majority of fish intermediate hosts are confined to the family Cyprinidae. There are 13 families involving at least 113 species of fish that act as the second intermediate host of *C. sinensis* (Chen *et al.*, 1994) Among them 95 species belong to the family Cyprinidae, five to the Eleotridae, two to the Ophiocephalidae, and one to each of the families Bagridae, Cichlidae, Clupeidae, Cobitidae, Cyprinodontidae, Gobiidae, Oncocepharidae, Osmeridae and Siluridae. In addition to these fish, certain freshwater shrimps of one family involving three species of freshwater shrimp (*Caridinia nilotica gracilipes, Macrobrachium superbum* and *Palaemonetes sinensis*) have been incriminated as a source of infection in children in Fukien Province, China. Overall, 76 different species of fish were found to be naturally infected with metacercariae of *Clonorchis* in China, 42 in Korea, 25 in Japan and nine in Russia (Chen *et al.*, 1994). Generally, small-sized fish such as *Pseudorabora parva, Acanthorhodeus, Rhodeus* and *Hemiculter* are more frequently and more intensely infected with metacercariae of *C. sinensis* than large-sized fish, such as *Ctenopharyngodon, Hypophthalmichthys, Carassius* and *Cyprinus.* However, *Ctenopharyngodon idellus, Mylopharyngodon aethiops,* and *Culter alburnus* are commonly eaten raw by Chinese people. In Korea and the northern part of China, *Pseudorabora parva* shows an extremely high degree of infection with *C. sinensis* metacercariae. Occasionally, small fish are eaten raw by people who live near the water, but normally they are eaten only after cooking. On the other hand, large fishes such as *Cyprinus carpio* and *Carassius carassius*, which have an extremely low rate of infection, are frequently eaten raw by the inhabitants of endemic areas (Rim, 1986).

Mode of human infection

Clonorchis infection is acquired by the ingestion of raw, inadequately cooked, or even dried, salted, or pickled flesh of infected freshwater fish. The intensity of human infection depends on the eating habits of the population. It is well known that people in endemic countries have a custom of eating raw fish at drinking parties. Therefore, in endemic areas, more men are infected than women. The incidence in children is low, but from 20 years of age onwards the incidence increases, being the highest at 40 to 50 years of age.

In Hong Kong and South China, people with a marked preference for raw fish in their diet are notably infected with *C. sinensis*. Fish is served raw in thin slices either with vegetables and condiments or with hot rice gruel. In the endemic area of South Fukien Province, residents eat raw shrimps, which are considered to be the only source of infections.

Most Korean people like to eat raw fish. In endemic areas, heavily infected small fish such as *Pseudorasbora parva* are not generally eaten raw, but may be eaten undercooked and thus transmit infection. The large fish *Cyprinus carpio* and *Carassius carassius*, which are frequently eaten raw, have a low rate of infection. However, the repeated consumption of these raw fish ultimately leads to heavy infections in individuals and a high incidence in the population (Rim, 1986).

Some workers have investigated whether humans can be infected by drinking water from a stream contaminated by infected flesh of dead fish. Infection may occasionally be transmitted in this way, but this has not been proved to occur under field conditions. Another possible mode of human infection is the accidental transference of encysted metacercariae to the mouth during the handling of fish (Komiya, 1966).

Quantitative epidemiology

In correlating *C. sinensis* infections with epidemiological and clinical significance, quantitative examination of stool samples is essential. Kato-Katz or Stoll's egg counting method is especially suitable for field work. Egg counts in terms of numbers of eggs per gram of faeces (EPG) is important in estimating worm load and are applicable in the analysis of infection as well as in assessing the efficacy of treatment.

In order to observe the endemicity of *C. sinensis* infection, Rim (1984) attempted an epidemiological analysis on the levels of intensity or endemicity, host age and sex distribution, the features of transmitting *C. sinensis* infection, together with changes in familial aggregations of clonorchiasis in highly endemic areas. The degrees of intensity or endemicity of *C. sinensis* infections in endemic areas were compared using regression analyses, which were calculated by cumulative percentages of EPG of *C. sinensis* in the surveyed areas.

Quantitative analysis of host age and sex distribution and the features of transmitting infection as well as epidemiological changes in endemic areas were applied by Muench's catalytic model. It was noted that the theoretically obtained simple and two-stage catalytic curves fitted well with observed data and that this model was found useful in understanding the characteristics of the transmission of *C. sinensis* infection (Park *et al.*, 1984; Rim, 1986).

Pathogenesis and pathology

Pathogenesis

The following factors are considered responsible for the pathogenesis of *C. sinensis:* (i) mechanical action in which ducts are blocked, caused by numerous *C. sinensis* living in the bile ducts in heavily infected cases; (ii) destructive action in which partial damage and desquamation of mucosa of the bile ducts occurs, caused by adult parasites sucking the blood of the host through the mucosa;

https://doi.org/10.1079/JOH2005300 Published online by Cambridge University Press

(iii) chemical action caused by the parasite's metabolites; (iv) inflammatory lesions of the bile ducts caused by irritation of worms as well as bacterial infection; and (v) reaction of the host affected by such factors as age, nutrition, resistance, and the existence of concomitant diseases.

The main causes of dysfunction are a mechanical obstruction of the biliary tract by the worms, congestion of bile, and the effects of soluble substances of metabolites released from the flukes into the ducts and surrounding tissues. The severity of the dysfunction depends on the number of worms present and length of infection (Rim, 1986).

Pathology

The liver appears grossly normal in light infections, but in heavy infections there is localized dilation of the slightly thickened peripheral bile ducts, which can be seen on the surface beneath the Glisson's capsule as pale blue or greenish blue blobs, and some atropy of parenchymal cells. The major microscopic findings in the earlier stage of clonorchiasis are periductal oedema and acute inflammatory cellular reponses in the bile duct walls. The bile duct shows not only desquamation but also marked hyperplasia of epithelial cells. Subsequently, marked goblet cell metaplasia of ductal epithelial cells and remarkable adenomatous hyperplasia in the mucosa appear. Together with bile duct epithelial changes, periductal connective tissue is increased around the biliary passages and the portal tract. In the chronic stage of infection, adenomatous tissue is gradually replaced by fibrous tissue, thus causing thickening of the bile duct wall.

Extrahepatic involvement by *C. sinensis* infection is rather common. Adult fluke invasion into the pancreatic duct occurs most frequently in heavy infections, but the pathological changes are usually less extensive than those in the intrahepatic bile ducts. The flukes reside in the main pancreatic duct and its tributary ducts. The changes are similar to those seen in the hepatic lesions, namely adenomatous hyperplasia of ductal epithelium, and sometimes, squamous metaplasia (Chen *et al.*, 1994).

Complications

The formation of calculi in the intrahepatic biliary passages is one of the most characteristic pathological features of clonorchiasis. This is sometimes accompanied by suppurative cholangitis, cholecystitis, and biliary abscesss or so-called cholangiohepatitis and ultimately can cause primary liver cancer, especially cholangiocarcinoma.

The occurrence of calculi in clonorchiasis is caused by bile stagnation due to mechanical obstruction and the presence of worms and eggs becoming nuclei for hepatolithiasis. Chemically, such calculi in the intra- and extra-hepatic bile ducts are composed almost entirely of bilirubin carbonate. According to Chen *et al.* (1994), the formation of pigment stones in clonorchiasis may be attributed to changes in the concentration of bilirubin, cholesterol, phospholipid, bile acid and the activity of bacterial glucuronidase in bile stagnation caused by mechanical obstruction. In relation to goblet cell metaplasia of the bile duct epithelium, the bile contains a high content of mucous secretion. The mucin-rich bile and the presence of worms and eggs in the bile duct cause cholestasis and furnish a favourable environment for secondary bacterial infection, the most frequent being *Escherichia coli*-induced ascending cholangitis from the intestine.

The most serious consequence of clonorchiasis is cholangiocarcinoma. The causative role of clonorchiasis in the development of this tumour is supported by several epidemiological studies. Cholangiocarcinoma is seen more frequently in the endemic area of clonorchiasis than non-endemic areas, and *C. sinensis* infection is seen in 94.7% of cholangiocarcinoma cases as compared to 31.7% in a control population in Hong Kong (Belamaric, 1973). Furthermore, morphological studies in Hong Kong (Hou, 1956; Chou & Gibson, 1970) and Korea (Kim *et al.*, 1974) indicated that carcinomas occur most frequently in association with pre-existing epithelial dysplastic change or adenomatous formation of the secondary order of intrahepatic bile ducts. The mucin-producing activity is also a frequent manifestation of this neoplasm which suggests the neoplastic transformation or participation of pre-existing metaplastic goblet cell changes in the lining cells of the bile duct (Kim, 1984).

Human cholangiocarcinoma can be divided into two macroscopic types according to the site of involvement, i.e. hilar and peripheral types. The peripheral type has multicentric growth as seen most frequently in *Clonorchis*-related neoplasms. Most peripheral cholangiocarcinoma develop in the small ducts and grow in a nodular, massive or diffuse pattern with early infiltration into the hepatic parenchyma. In rare instances, the hilar (large duct) type of cholangiocarcinoma may arise in the right or left hepatic ducts or in a major branch, and remain localized within the ducts (Kim *et al.*, 1989). According to Chou & Chan (1976), only four of 50 autopsy cases of mucin-secreting cholangiocarcinoma in Chinese patients were found to be the hilar type of tumour. These hilar cholangiocarcinomas took the form of mural thickenings of the main hepatic ducts at the hilum of the liver, which were obstructed by stones, and bile ducts were dilated by heavy infections with *C. sinensis* (Parkin *et al.*, 1993).

Several cases of hepatocellular carcinoma have been associated with *Clonorchis* infections, but this seems more likely to be a coincidental association, especially in areas where both hepatocellular carcinomas and clonorchiasis are common. The major difference between patients with cholangiocarcinoma and hepatocellular carcinoma was cirrhosis. Of 38 subjects from Hong Kong with chronic infections with *C. sinensis*, only one patient with cholangiocarcinoma had cirrhosis, whereas all but one patient with hepatocellular carcinoma had cirrhosis (Purtilo, 1976).

According to an IARC (International Agency for Research on Cancer) monograph (1994, Vol. 61), two case-control studies of the relationship between *C. sinensis* and liver cancer have been carried out in Korea. Significantly increased estimated relative risks of 6.5 and 6.0 were seen with cholangiocarcinoma, but no significant association was seen with the occurrence of hepatocellular carcinoma. In a third case-control study, in

https://doi.org/10.1079/JOH2005300 Published online by Cambridge University Press

Hong Kong, the estimated relative risk for cholangiocarcinoma was 3.1, while that for hepatocellular carcinoma was 0.7. In view of similarity to the biological and pathological characteristics of *Opisthorchis* and *Clonorchis*, IARC (1994) noted that experimental and epidemiological studies on *C. sinensis* confirmed the occurence of cholangiocarcinoma in infected animals, especially when infection is combined with administration of known carcinogens, and that the relative risks for cholangiocarcinoma, not for hepatocellular carcinoma, is consistently increased in people infected with *C. sinensis*. In making the overall evaluation, IARC (1994) concluded that an infection with *C. sinensis* is probably carcinogenic to humans.

Experimental induction of cholangiocarcinoma by C. sinensis

Experimental animals such as rats, rabbits, guinea pigs and hamsters infected with *C. sinensis* alone have never developed bile duct tumours (Lee & Rim, 1996). Bile duct hyperplasia which was irritated by *C. sinensis* infection underwent a carcinomatous transformation through a stage of dysplasia (Kim, 1984). Lee *et al.* (1993) conducted animal experiments for the induction of cholangicarcinoma in hamsters infected with *C. sinensis*. Tumours were produced in 8 of 11 animals at 11 weeks after *C. sinensis* infection and 0.0015% dimethylnitrosamine (DMN) treatment. On the other hand, no tumour was produced in *C. sinensis*-infected or DMN-treated hamsters, respectively. This study suggested that *C. sinensis* infection and DMN could synergically induce cholangiocarcinoma in hamsters. To evaluate the role of *C. sinensis* infection during two-step carcinogenesis, an experiment was designed to determine whether *C. sinensis* could act as a potential promoter or initiator (Lee *et al.*, 1994). In this experiment, cholangiocarcinoma was produced only in the promoting group of *C. sinensis*, not in the initiating group of *C. sinensis* infection (Lee *et al.*, 1995). During cholangiocarcinogenesis chronic clonorchiasis has stimulated bile duct cells to proliferate continuously and promote transformed bile duct cells (Oshima & Bartsch, 1994). The fact that biliary cells were stimulated to proliferate by *C. sinensis* is thought to be important in cholangiocarcinogenesis, since a proliferation mechanism might play an important role in inducing cholangiocarcinoma. The study for proliferating cell nuclear antigen (PCNA) and apoptosis in *C. sinensis*-infected hamsters showed that the infection had an effect on hepatocytes in periportal zones as well as biliary cells. It has been suggested that oval cells represent hepatic stem cells which might play an important role in the histogenesis of cholangiocarcinomas (Lee & Rim, 1996). To study oval cell participation in cholangiocarcinoma caused by *C. sinensis* infection, hamster livers were examined immunohistochemically and electron-microscopically. Although it is not clearly defined where oval cells originated from, hamster livers showed three types of cells during cholangiocarcinogenesis by DMN and *C. sinensis*: the small periductal cells, ductular-like oval cells and hepatocyte-like oval cells (Lee *et al.*, 1997). These studies revealed in hamsters that cholangiocarcinogenesis associated with *C. sinensis* infection might begin from oval cells which develop through dysplasia in portal areas.

Clinical aspects

Clinical manifestions

Clinical manifestations are related to the number of worms present, the infection period, the condition of the worms and complications. In the early stage of infection or when the number of worms is small, symptoms are almost nil. However, the symptoms are aggravated in accordance with the intensity and the duration of infection. The clinical manifestations of clonorchiasis may be conveniently classified as follows.

Invasion by immature worms

The earliest indications of illness are usually general malaise, abdominal discomfort, and occasional diarrhoea. These symptoms are caused by the mechanical and toxic irritation of biliary tract for 3 to 4 weeks during invasion by immature worms. This stage is characterized by significant digestive disturbances and general symptoms associated with marked eosinophilia. Earlier heavy infections produce sudden effects with chills and fever up to 40°C, followed by liver pain and enlargement and jaundice. The jaundice is caused by obstruction of intrahepatic biliary trees by worms or inflammatory changes. However, in light infections, an acute stage cannot usually be observed.

Infection by mature worms

In light and early infections, infected persons usually show no symptoms. In progressive cases, symptoms include indigestion, fullness of the abdomen, loss of appetite, epigastric distress unrelated to meals, and weakness. In moderate infections, clinical symptoms include loss of appetite, indigestion, fullness of the abdomen, epigastric distress unrelated to meals, discomfort in the right upper quadrant, diarrhoea, oedema, and some hepatomegaly. In heavy infections, symptoms are weakness and lassitude, epigastric discomfort, paresthesia, loss of weight, palpitation of the heart and tachycardia, diarrhoea, vertigo, titanic cramps and tremors, and toxaemia from liver impairment. In the later stages of heavy infections or severe cases, symptoms include marked gastrointestinal disturbances, with a syndrome associated with portal hypertension, splenomegaly, ascites and oedema.

Diagnosis

Clinical diagnosis is suggestive in patients with an enlarged liver and symptoms of hepatitis in endemic areas where uncooked fish is eaten. Advanced infections require differentiation from malignancy, cirrhosis of the liver, and other causes of hepatic enlargement.

Parasitological examination

Definitive diagnosis of *C. sinensis* infection is based on the finding of eggs either in faeces or duodenal fluid, or adult flukes during laparatomy. Examination of duodenal drainage fluid for eggs is very sensitive but inconvenient and not readily accepted by patients.

https://doi.org/10.1079/JOH2005300 Published online by Cambridge University Press

No significant progress has been made in the past decade or so on parasitological diagnosis using faecal examination techniques. The formalin-ether sedimentation technique, Kato faecal thick smear technique or Kato-Katz technique and Stoll's dilution egg counting technique are currently being used in endemic areas. The Kato-Katz technique in particular has been widely used in field surveys in many countries, because of its simplicity, low cost, and reproducibility of results between investigators. The Stoll's dilution egg counting technique uses sodium hydroxide which faciliatates identification of the eggs. The detection rate is higher than the direct smear technique and the zinc sulphate flotation technique, but not as high as the formalin-ether concentration and Kato techniques for *C. sinensis* infection (Min, 1984).

Regarding egg identification, there are many species of trematodes that can parasitize the human intestine, and the morphology of their eggs is similar to that of eggs of *C. sinensis*. These trematodes belong to the families Opisthorchiidae and Heterophyidae. It is difficult to identify eggs of *Metorchis orientalis, Haplorchis* spp., *Heterophyes heterophyes* and *Metagonimus yokogawai* based only on measurement of egg size. Egg differentiation should consider the width and height of the operculum, and the significance of a shoulder-like rim of the narrow anterior end of the shell. A comparative study between eggs of Heterophyidae and *C. sinensis* in Korea was conducted by Lee *et al.* (1984), who concluded that despite similar morphological characteristics of these eggs, differential diagnosis of human infection by faecal examination is inconclusive and thus isolation of adult worms is required to determine the exact species. It is also useful to note that immature *Clonorchis* eggs may not be distinguishable from *Metagonimus* eggs (Kammerer *et al.*, 1977).

Immunological tests

Immunological tests have a complementary role in the diagnosis of hospital patients and in epidemiological surveys. During the past years, immunodiagnosis has been developed rapidly and the sensitivity and specificity of the tests have improved.

The intradermal test has been widely used in Korea for the past four decades for the purpose of epidemiological studies on clonorchiasis. An adult worm antigen preparation of *C. sinensis* has been used in a Veronal-buffered saline extract (VBS antigen, 1:10,000 dilution). Although the intradermal test is a rapid, sensitive, and useful epidemiological tool for studying the prevalence of *Clonorchis* infection, cross-reactions with other trematode infections and moderate false-negative reactions in light infections are the main disadvantages in its practical application (Rim, 1986)

For antibody detection, enzyme-linked immunosorbent assay (ELISA) has been widely used since its introduction to China and Korea in the late 1970s. This technique, which has the advantages of higher sensitivity and specificity, ease of use and reading and convenience for field work, is the immunological test of choice in field surveys for *C. sinensis.* The sensitivity of ELISA was reported to be higher than that of the indirect haemagglutination test (IHA), counter-immunoelectrophoresis (CIEP), complement fixation test (CFT) and indirect fluorescent antibody test (IFAT). The antibody titre detectable by ELISA was higher than CFT and CIEP. Cross-reactions with other trematode infections were reduced by purification of the crude antigen (Chen *et al.*, 1994). A monoclonal antibody reactive to a species-specific carbohydrate antigen was used in the ELISA-inhibition test, which showed a higher sensitivity and specificity in the immunodiagnosis of clonorchiasis than the conventional ELISA (Yong *et al.*, 1991, 1996). However, immunodiagnosis is still regarded as a supplementary diagnostic method.

Laboratory findings

Haematological values are within the normal range except for eosinophil counts. In a very heavily infected group, however, the mean values of haemoglobin, haematocrit and red blood cell counts were somewhat higher (Kim *et al.*, 1982a). On the other hand, the mean values of MCH, MCV and MCHC did not show any difference. Eosinophilia is the most obvious change in the blood picture in *C. sinensis* infections. As the degree of infection becomes higher, eosinophil counts also become higher. Experimental studies have shown that the changes in eosinophils are somewhat related to repeated infections except for the degree and the duration of infection (Joo & Rim, 1982). Usually, in endemic regions most patients have a habit of eating raw freshwater fish and thus the increase in eosinophils is thought to arise from repeated infections.

With reference to blood biochemistry, the mean values of serum alanine transaminase (ALT) and aspartate aminotransferase (AST), bilirubin, and cholesterol increased with the intensity of infection, while the total protein, and albumin, A/G ratio tended to decrease. In general, serum albumin, globulin, and A/G ratio, bilirubin, thymol turbidity, etc. were normal, and no significant relationship was shown among groups with different intensities of infection (Chen *et al.*, 1994).

Radiological images

Plain abdominal radiography may show gallstones in the biliary tract. Cholangiography, which has been used for several decades, once played a major role in the assessment of the biliary tract, especially in heavily infected cases with obstructive jaundice, or in patients with clonorchiasis complicated by recurrent pyogenic cholangitis or cholangiocarcinoma. Cholangiographic findings show the flukes within the lumen of the bile ducts, gallbladder, and pancreatic ducts as well as ductal dilation and periductal tissue reaction. Defects occur mostly within small intrahepatic bile ducts and their peripheral branches, whereas the extrahepatic bile ducts are normal or only slightly dilated (Lim, 1990).

Among the types of cholangiography used, retrograde endoscopic cholangiopancreatography (ERCP) is the most useful method to locate pathological changes in biliary and pancreatic systems. Filamentous filling defect of adult worms in the bile ducts is a pathognomonic finding on ERCP examination and is seen obviously as the most important diagnostic criterion (Leung *et al.*, 1989). Significant dilation of the secondary and tertiary

https://doi.org/10.1079/JOH2005300 Published online by Cambridge University Press

intrahepatic bile ducts, and dilation and blunting of the terminal branches of the biliary tree are also common findings. In *C. sinensis* infections in the pancreatic system, diffuse irregular dilation of tributaries and the main duct in the tail of the pancreas was shown, but the main pancreatic duct and tributaries in the body and head were normal (Lim & Ko, 1990).

Recently, however, ultrasonography and computed tomography (CT) have been introduced in the diagnosis of hepatobiliary diseases. Ultrasonography is a useful and simple technique to assess disease of the hepatobiliary tract, to identify flukes in the bile ducts, and to evaluate the effect of a specific treatment. It is now considered a good tool for use in the clinical diagnostic procedure for clonorchiasis, particularly in individuals with a moderate or heavy infection. The walls of dilated bile ducts showed thickening and were highly echogenic, whereas the common bile duct showed only mild dilation and slightly thickened walls. Adult flukes in the gallbladder are demonstrated by the finding of floating or dependent, decreased echogenic foci in the lumen (Lim *et al.*, 1989). The effectiveness of treatment was assessed by the cessation of movement of flukes and a reduction in the number of small echogenic areas in the bile ducts four days after treatment with praziquaqntel, although the thickness of dilation of the bile ducts remained unchanged. Dilation of the peripheral intrahepatic bile ducts with increased echogenicity of the duct walls, in the absence of dilation of the extrahepatic bile ducts by ultrasonography, is highly suggestive of a diagnosis of clonorchiasis (Lim, 1990; Lim *et al.*, 1989),

Choi *et al.* (2004) reported recently the characteristic sonographic findings of clonorchiasis for the diagnosis of active infections in an endemic village in northeastern China. Among 457 subjects, 316 positive and 141 negative for eggs of *C. sinensis* were examined using ultrasonography. Four sonographic findings differentiating subjects with active clonorchiasis from controls were recognized: increased periductal echogenicity, floating echogenic foci in gallbladder, diffuse dilation of the intrahepatic bile ducts and gallbladder distension. Of these findings, increased periductal echogenicity and floating echogenic foci in the gallbladder were more significantly associated with active infections of *C. sinensis*.

Biliary stones can be identified by ultrasound. An ultrasound survey for gallbladder disease among 947 Hakkanese people with *C. sinensis* infections in Taiwan was reported by Hou *et al.* (1989). Among them, 89 persons were found to have stones in the biliary system including gallbladder stones (85 cases), common bile duct stones (three cases) and intrahepatic duct stone (one case) with an overall prevalence of 9.4%.

Computed tomography detects the same pathological processes as cholangiography and ultrasonography. The CT features of clonorchiasis were typically diffuse, uniform, and minimal or mild dilation of intrahepatic bile ducts, particularly distally, without any evidence of extrahepatic biliary dilation (Choi *et al.*, 1988, 1989). Cholangiocarcinoma associated with *C. sinensis* has characteristic CT findings, including a large, irregular mass with low-density, stippled areas or aggregated, powder-like, high-density areas within it on precontrast CT scans; on postcontrast scans, there is slight enhancement of the mass, usually at the periphery (Choi *et al.*, 1988).

Recently the usefulness of magnetic resonance imaging (MRI) has been extended for imaging clonorchiasis and cholangiocarcinoma of the liver. Although sonography, CT and cholangiography have been used traditionally to diagnosis these diseases, the role of MRI has increased rapidly (Choi *et al.*, 1998).

Immunology and molecular biology

Elevated serum levels of IgM antibody were shown in cats seven days after the infection, followed by elevation of IgA and IgG (Chen *et al.*, 1989). In patients with chronic infection, serum IgA was normal, whereas IgG and IgM were still elevated (Liu *et al.*, 1989). Serum levels of total IgE in clonorchiasis patients were several times higher than in normal patients, and *Clonorchis*-specific IgE was detected in 44.8% of 38 clonorchiasis patients, but none was detected in controls (Zhou & Zhao, 1981). According to Yen *et al.* (1992), IgG and IgE antibody levels were elevated in individuals infected with *C. sinensis*. Elevated levels of IgG and secretory IgA in bile from infected patients indicate that there are local as well as peripheral immunological responses to infection. Therefore, increased levels of IgG and IgA secreted into the duct may promote protective immunity against *C. sinensis* infection.

Quan *et al.* (2004) examined the correlation between immunoglobulin isotype levels and resistance to reinfection of *C. sinensis.* Rats were infected with *C. sinensis* (primary infection), and then treated with praziquantel on days 1, 3, 7, 14 and 28 post infection. To measure resistance, rats were reinfected with *C. sinensis* at 2 weeks after treatment (secondary infection) and worms were recovered 4 weeks later. Significant differences of worm number between the infected and control group was found on days 14 and 28 ($P < 0.001$). IgG was observed on days 14 and 28 during the primary infection. During the secondary infection, significantly increased levels of IgG were found from days 3 to 28 and IgE on days 7 and 14, while significant levels of IgM were found on days 3 and 14. Changes in IgG, IgM and IgE levels were shown in rats infected with *C. sinensis* in primary and secondary infection, and a correlation was observed between antibody (IgG, IgM and IgE) levels and the resistance to reinfection ($P = 0.004$).

Flukes generate energy through a glycolytic pathway utilizing external glucose and provide metabolic intermediates for physiological maintenance. The enzyme phosphoglycerate kinase was abundant in physiologically active tissues such as muscle fibres of all organs and the tegument (Hong *et al.*, 2000, 2003). Anti-oxidant enzymes of parasites defend against offensive immunological and physiological attacks. Glutathione S-transferases (GSTs) catalyse the conjugation of reduced glutathione to bioreactive compounds and to exogenous toxins. Glutathione S-transferases cloned from *C. sinensis,* with molecular masses of 28 kDa and 26 kDa, were classified as class sigma and class mu GSTs, respectively. The two GSTs revealed enzymatic activity against

https://doi.org/10.1079/JOH2005300 Published online by Cambridge University Press

substrates and sensitivity to inhibitors. The enzymes were localized in tegumental and mesenchymal tissues (Hong *et al.*, 2001, 2002b, 2002c), and were, therefore, likely to function as a second defence line in *C. sinensis*. Recombinant 28 and 26 kDa GST proteins were antigenic to IgG and IgE antibodies from clonorchiasis patients' sera (Kang *et al.*, 2001; Hong *et al.*, 2002a). Cysteine proteinases from parasitic helminths have been recognized as important for parasite development and pathogenic damage to the hosts. From adult *C. sinensis*, a cysteine proteinase with molecular mass 24 kDa was cloned. The recombinant enzyme produced in yeast exhibited activity close to cathepsin L-like cysteine proteinase (Park *et al.*, 2001). The second cysteine proteinase cloned had a molecular mass of 28.5 kDa and the bacterially-produced recombinant protein revealed enzymatic activity (Na *et al.*, 2002). The third cysteine proteinase was cloned as 37 kDa protein and localized in intestinal epithelium and intra-uterine eggs (Nagano *et al.*, 2004). In the metacercariae, the cysteine proteinase, as an intrinsic factor, facilitated excystation in coordination with extrinsic factors such as bile and trypsin (Li *et al.*, 2004). The recombinant cysteine proteinases were evaluated for specific serodiagnostic antigens for human clonorchiasis (Na *et al.*, 2002; Nagano *et al.*, 2004).

Ubiquitination is an essential process for protein degradation. An ubiquitin-conjugating enzyme (E2) cloned from *C. sinensis* catalysed conjugation of ubiquitin to histone H2A (Song *et al.*, 2004). In the membrane proteins, clonorin is a pore-forming peptide of *C. sinensis*, expressed stage-specifically in juvenile and adult flukes, and localized in the intestinal epithelium. Native and recombinant clonorins exerted lytic activity in a dose-dependent manner on erythrocytes. It was, therefore, suggested that clonorin enhances proteolytic digestion of cellular contents in the intestine (Lee *et al.*, 2002). The clonorin-specific IgG antibodies increased in experimental rabbits 8 weeks after infection. The recombinant clonorin protein was evaluated to be a specific but not sensitive serodiagnostic antigen for human clonorchiasis (Lee *et al.*, 2003b). A pore-forming subunit of ATP-sensitive potassium channel was cloned from *C. sinensis*, and named CsKir6.2 (Hwang *et al.*, 2003).

Molecular biological techniques have been employed in the search for useful serodiagnostic antigens for clonorchiasis. Recombinant proteins were produced from cloned cDNAs in bacterial and yeast systems and evaluated against helminth-infected human sera. Glycine-rich *C. sinensis* protein (GRCSP), localized in vitellaria, appeared specific for clonorchiasis (Yong *et al.*, 1998; Yang *et al.*, 2000). The proline-rich antigen (CsRPA), containing a repetitive peptide of GPDAPVPKSG, revealed a high sensitivity and specificity to sera of patients with clonorchiasis (Kim *et al.*, 2001). The 7-kDa protein of excretory–secretory products of adult *C. sinensis* was a highly specific antigen (Lee *et al.*, 2002). Myoglobin is an abundant protein and localized throughout the parenchymal tissues of adult *C. sinensis* (Chung *et al.*, 2003; Sim *et al.*, 2003). The fatty acid-binding protein mediates intracellular transport of long-chain fatty acids. The recombinant *C. sinensis* fatty acid-binding protein is cross-reactive to sera from patients with paragominiasis or fascioloiasis (Lee & Yong, 2004).

A *C. sinensis* adult cDNA library has been constructed and EST (expressed sequence tags) were analysed from 450 clones (Lee *et al.*, 2003a). The dataset showed genes encoding cystein proteases, a lipid binding protein, antigen proteins and other genes of interest from a diagnostic, drug or vaccine development viewpoint (Lee *et al.*, 2003a). Using the cDNA library, a myoglobin gene was immunoscreened (Sim *et al.*, 2003), and a fatty acid binding protein of *C. sinensis* (CsFABP) was cloned and expressed in *E. coli.* The CsFABP showing cross-species reactivity was suggested as a vaccine candidate molecule (Lee & Yong, 2004).

Genetic characterizations were carried out by Park *et al.* (2000) and Park & Yong (2001). A karyological study showed that the chromosome number of *C. sinensis* was $2n = 56$, which consisted of eight pairs of large and 20 pairs of small chromosomes (Park *et al.*, 2000). Isozyme analysis and genotyping using 18S rDNA, ITS2 and mtCOI genes to reveal geographical variations of *C. sinensis* showed a high homogeneity among populations of *C. sinensis* from Korea and China (Guangxi and Liaoning Provinces) (Park & Yong, 2001).

Treatment, prevention and control

Treatment

Praziquantel is the drug of choice for treating clonorchiasis. The most effective regimen is 25 mg kg^{-1} three times daily (total dose, 75 mg kg^{-1}) administered orally at 5- to 6-h intervals over a single day. For large scale treatments, a single dose of 40 mg kg^{-1} is recommended. In cases of heavy infection, however, a dosage of 25 mg kg^{-1} three times daily for 2 consecutive days is needed for a complete cure. Praziquantel appears to be well tolerated, and side effects (headache, dizziness and abdominal distress) are mild and transient (Rim *et al.*, 1981). However, it should be noted that when clonorchiasis patients with concomitant disease such as cerebral cysticercosis are given praziquantel, intracranial hypertension with coma is occasionally observed. Allergic shock and *grand mal* seizures appearing after drug administration have been reported (Su & Mon, 1983; Li *et al.*, 1990; Shu *et al.*, 1992).

In vivo studies of praziquantel have shown that the drug acts on all organs of *C. sinensis* inducing degenerative changes. After treatment with praziquantel, flukes in rats became contracted and deformed and blebs of various sizes appeared on the surface. Using electron microcopy, bleb-like structures were observed on the sensory papillae around the oral and ventral suckers of the flukes at an early stage following praziquantel treatment (Lee *et al.*, 1983). Upon reaching the surface, the vacuoles were fused, leading to disruption of the apical region of syncytial tegument along the basement layer. Finally the basement layer was dislocated and appeared balloon-like (Kim *et al.*, 1982b; Mehlhorn *et al.*, 1983). However, the worms in this stage were still alive in bile ducts and are washed out by bile fluid due to the detachment from the duct following the destruction of sensory papillae. The bleb-like and balloon-shaped structures on the tegumental surface led to the death of

https://doi.org/10.1079/JOH2005300 Published online by Cambridge University Press

worms by the bursting of the tegument a few days later (Rim, 1986).

Antibody levels

In some follow-up studies, serological tests were performed to determine changes in antibody levels as an indication of cure. Antibody levels decreased significantly three months after treatment with praziquantel, and at 6 month follow-up, 51.8% (Qu *et al.*, 1983) and 60.8% (Chen *et al.*, 1988) of patients showed a negative conversion by ELISA. Wang *et al.* (1985) reported that 36.4–40.4% of infected subjects became negative by ELISA four months after treatment with praziquantel. Serum specific IgG levels decreased six months after treatment and the decrease was more significant in the group with the highest pre-treatment intensity of infection (Lee *et al.*, 1986). Hong (1988) reported that the period for serological negative conversion after praziquantel treatment was between nine weeks and seven months in human clonorchiasis. Absorbance values of ELISA in serum and urine decreased gradually after drug medication. Even in parasitologically cured patients, the values at 18 months after treatment were only reduced by about one-half compared with pre-treatment levels, (Kim *et al.*, 1987). However, in the author's experience on clonorchiasis in patients treated by mass chemotherapy, complete negative sero-conversion in ELISA was observed at 48 months after treatment.

Prevention and control

Clonorchiasis is acquired through ingesting raw or improperly cooked or processed freshwater fish. This disease may cause direct or indirect economic losses. Medical costs include case detection, treatment and possible hospitalization. Loss of income and productivity because of illness can be a significant burden to the economy of the endemic country. People who live in poor villages in the endemic areas catch freshwater fish easily in the streams near their villages. Eating raw fish is one of the major sources of protein for the villagers. The majority of the people have contracted *Clonorchis* infection, but they are ignorant of this disease. In most endemic developing countries, raw fish preparation is influenced by their own cultural tradition. Education is a useful means of changing the traditional ways of preparing and processing raw fish.

The most practical method of preventing human infection is to avoid eating raw, freshly pickled, or undercooked freshwater fish. However, it is exceedingly difficult to carry out these simple measures in the face of centuries-old traditions. Nevertheless, in the districts where it is customary to eat raw fish, educational propaganda stressing the importance of thoroughly cooking all freshwater fish appears to be most effective means of preventing clonorchiasis (Rim, 1986).

The first approach to be implemented may be the construction of enough hygienic toilets in most endemic areas; however, this approach must be linked to general development of infrastructure. In the control of clonorchiasis in Korea, practical control measures were not attempted until 1981 when praziquantel was found as a safe and effective drug against *C. sinensis*. The Ministry of Health and Social Affairs (MHSA) and the Korea Association for Parasite Eradication (KAPE) conducted a pilot project of treatment for clonorchiasis using praziquantel in 1982. The most convenient and effective dosage schedule of the drug for mass chemotherapeutic control was recommended as a single dose of 40 mg kg^{-1} of praziquantel under field conditions (MHSA and KAPE, 1982; Lee, 1984).

The Korean Government launched a mass treatment project of clonorchiasis in 1984 and, from 1984 to 1990, a total of 3,009,166 people living in endemic areas were examined microscopically by the Kato-Katz method. Each egg-positive case was treated with praziquantel in a single dose of 40 mg kg^{-1} of body weight at local health centres under the supervision of physicians (MHSA and KAH, 1992). Egg positive rates in inhabitants from the endemic areas were 13.3% of 168,877 people examined in 1984; 7.0% of 447,237 in 1985; 2.2% of 496,835 in 1986; 1.8% of 502,026 in 1987; 1.2% of 488,553 in 1988; 0.9% of 496,361 in 1989 and 0.9% of 409,277 in 1990. Following this mass treatment from 1984 to 1990, the status of clonorchiasis significantly improved. It was recognized that not only were the infection rates reduced in the previously endemic areas, but also the proportion of heavy infections of clonorchiasis decreased from 11.9% to 3.6%.

Recently, in China, a control strategy on clonorchiasis was tested at nine pilot villages in Zhaoyuan, Hailin and Ningan counties of Heilongjiang Province from 2001 to 2004 under the KOICA (Korea International Cooperation Agency) project. After four years of intervention using different strategies, the *Clonorchis* infection rate decreased in all pilot areas. Praziquantel was given orally three doses of 25 mg kg^{-1} twice a year, once in a year, or once in two years in heavy and moderate endemic areas. The rate decreased remarkably in heavy and moderate endemic areas, with prevalence decreasing by 71.8% to 96.1% (Ji *et al.*, 2004).

The control of clonorchiasis is fundamentally directed at reducing or eliminating disease transmission, so that no more new infections, reinfections or superinfections occur. There are several approaches that can be used for the control of *C. sinensis* infection: (i) promotion of diagnostic techniques; (ii) chemotherapy; (iii) provision of satisfactory sanitary facilities; (iv) environmental control; (v) avoidance of capture, culture, harvest, or selling of fish from areas known to be endemic; (vi) the application of HACCP (the Hazard Analysis and Critical Control Point); and (vii) health education.

The control of clonorchiasis is theoretically straightforward, because the infection can only be contracted by way of encysted metacercariae, which are introduced when the intermediate host (fish) is eaten. Measures to prevent stools containing viable eggs from reaching bodies of water containing snail intermediate hosts would apply to the human population, although contamination from reservoir animal hosts cannot be controlled, so this method alone would not be sufficient to produce tangible benefits. Together with mass treatment, voluntary treatment with praziquantel of infected individuals is popular in Korea. In addition to chemotherapy, reinfection seemed to be blocked in many previous endemic areas because of environmental changes due to

https://doi.org/10.1079/JOH2005300 Published online by Cambridge University Press

mechanization of farms, urbanization, industrialization, water pollution, reduced population of freshwater fish caused by the use of chemical fertilizers and pesticides, and the changing attitudes of the local people towards eating raw freshwater fish. However, many people who enjoy eating raw fish still remain in certain areas, especially in the basins of rivers. At present, chemotherapy has proved to be the most rapid and effective method of control. Therefore, a combination of health education and mass treatment with praziquantel coupled with governmental aid could significantly reduce the disease.

References

Belamaric, J. (1973) Intrahepatic bile duct carcinoma and *C. sinensis* infection in Hong Kong. *Cancer* **31**, 468–473.

Chen, C.Y., Shin, J.W., Chen, S.N. & Hsieh, W.C. (1989) A preliminary study of clinical staging in clonorchiasis. *Chinese Journal of Microbiology and Immunology (Taiwan)* **22**, 193–200.

Chen, E.R. (1991) Current status of food-borne parasitic zoonoses in Taiwan. *Southeast Asian Journal of Tropical Medicine and Public Health* **22** (Supplement), 62–64.

Chen, M., Lu, Y., Hua, X. & Mott, K.E. (1994) Progress in assessment of morbidity due to *Clonorchis sinensis* infection: a review of recent literature. *Tropical Diseases Bulletin* **91**, R7–R65.

Chen, X.B., Hu, S.F. & Shen, J.L. (1988) Detection of antibody levels in treated patients with clonorchiasis. *Acta Academiae Medicinae Bengbu* **13**, 215–217 (in Chinese).

Choi, B.I., Park, J.H., Kim, Y.I., Yu, E.S., Kim, S.H., Kim, W.H., Kim, C.Y. & Han, M.C. (1988) Peripheral cholangiocarcinoma and clonorchiasis: CT findings. *Radiology* **169**, 149–153.

Choi, B.I., Kim, H.J., Han, M.C., Do, Y.S., Han, M.H. & Lee, S.H. (1989) CT findings of clonorchiasis. *American Journal of Roentgenology* **152**, 281–284.

Choi, B.I., Kim, T.K. & Han, J.K. (1998) MRI of clonorchiasis and cholangiocarcinoma. *Journal of Magnetic Resonance Imaging* **8**, 359–366.

Choi, D., Hong, S.T., Lim, J.H., Cho, S.Y., Rim, H.J., Ji, Z., Yuan, R. & Wang, S. (2004) Sonographic findings of active *Clonorchis sinensis* infection. *Journal of Clinical Ultrasound* **32**, 17–23.

Chou, S.T. & Chan, C.W. (1976) Mucin-producing cholangiocarcinoma: an autopsy study in Hong Kong. *Parasitology* **8**, 321–328.

Chou, S.T. & Gibson, J.B. (1970) The histochemistry of biliary mucins and the changes caused by infection with *Clonorchis sinensis*. *Journal of Pathology* **101**, 185–201.

Chung, Y.B., Yang, H.J., Hong, S.J., Kang, S.Y., Lee, M., Kim, T.Y., Choi, M.H., Chai, J.Y. & Hong, S.T. (2003) Molecular cloning and immunolocalization of the 17 kDa myoglobin of *Clonorchis sinensis*. *Parasitology Research* **90**, 365–368.

Cross, J.H. (1984) Changing pattern of some trematode infections in Asia. *Arzneimittel Forschung* **34**, 1224–1226.

Fang, Y.Y. (1994) Epidemiologic characteristics of *clonorchiasis sinensis* in Guangdong Province. *China. Southeast Asian Journal of Tropical Medicine and Public Health* **25**, 291–295.

Figurnov, V.A., Chertov, A.D., Romanenko, N.A., Grigorenko, A.A., Gavrilov, V.A., Soldatkin, P.K., Prokhorov, P.P., Solozhenkin, V.G., Kalinina, V.V., Katin, I.S., Gavrilov, A.V., Mosolova, N.P. & Figurnova, E.V. (2002) Clonorchiasis in the Upper Amur region: biology, epidemiology, clinical presentation. *Medical Parasitology (Moskva)* Oct-Dec, 20–23 (in Russian).

Gibson, J.B. & Sun, T. (1971) Clonorchiasis. pp. 546–566 *in* Marcial-Rojas, R.A. (*Ed.*) *Pathology of protozoal and helminthic diseases with clinical correlation*. Baltimore, Williams & Wilkins.

Hong, S.J., Seong, K.Y., Sohn, W.M. & Song, K.Y. (2000) Molecular cloning and immunological characterization of phosphoglycerate kinase from *Clonorchis sinensis*. *Molecular and Biochemical Parasitology* **108**, 207–216.

Hong, S.J., Lee, J.Y., Lee, D.H., Sohn, W.M. & Cho, S.Y. (2001) Molecular cloning and characterization of a *mu*-class glutathione S-transferase from *Clonorchis sinensis*. *Molecular and Biochemical Parasitology* **115**, 69–75.

Hong, S.J., Kim, T.Y., Gan, X.X., Shen, L.Y., Sukontason, K. & Kang, S.Y. (2002a) *Clonorchis sinensis*: glutathione S-transferase as a serodiagnostic antigen for detection IgG and IgE antibodies. *Experimental Parasitology* **101**, 231–233.

Hong, S.J., Kim, T.Y., Kang, S.Y., Yu, J.R., Song, K.Y. & Cho, S.Y. (2002b) *Clonorchis sinensis*: immunolocalization of 26 kDa glutathione S-transferase in adult worms. *Experimental Parasitology* **102**, 191–193.

Hong, S.J., Yu, J.R. & Kang, S.Y. (2002c) Ultrastructural localization of 28 kDa glutathione S-transferase in adult *Clonorchis sinensis*. *Korean Journal of Parasitology* **40**, 173–176.

Hong, S.J., Shin, J.K., Kang, S.Y. & Ryu, J.R. (2003) Ultrastructural localization of phosphoglycerate kinase in adult *Clonorchis sinensis*. *Parasitology Research* **90**, 369–371.

Hong, S.T. (1988) Changes of anti-*Clonorchis sinensis* IgG antibody in serum after praziquantel treatment in human clonorchiasis. *Korean Journal of Parasitology* **26**, 1–8.

Hou, M.F., Ker, C.G., Sheen, P.C. & Chen, E.R. (1989) The ultrasound survey of gallstone diseases of patients infected with *Clonorchis sinensis* in southern Taiwan. *Journal of Tropical Medicine and Hygiene* **92**, 108–111.

Hou, P.C. (1956) Relationship between primary carcinoma of the liver and infestation with *Clonorchis sinensis*. *Journal of Pathology and Bacteriology* **72**, 239–246.

Hwang, S.Y., Han, H.J., Kim, S.H., Park, S.G., Seog, D.H., Kim, N., Han, J., Chung, J.Y. & Kho, W.G. (2003) Cloning of a pore-forming subunit of ATP-sensitive potassium channel from *Clonorchis sinensis*. *Korean Journal of Parasitology* **41**, 129–133.

IARC (1994) Schistosomes, liver flukes and *Helicobacter pylori*. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* **61**, 270 pp. International Agency for Research on Cancer, WHO.

Ji, Z., Li, Z., Wang, S., Yuan, R., Zhen, S., Feng, Z., Xu, L., Hong, S.T., Cho, S.Y., Choi, D.I., Hong, K.S. & Rim, H.J. (2004) Final report of China–Korea collaborative project of control strategies of clonorchiasis in Heilongjiang pilot areas (2001–2004). pp. 33–56 in *Proceedings of the 5th workshop on the Korea–China collaborative project of control strategies for helminthiasis in pilot areas*, November.

Joo, K.H. & Rim, H.J. (1982) Observation on the serum IgE level and eosinophil counts in clonorchiasis. *Korea University Medical Journal* **19**, 51–62 (in Korean).

Kammerer, W.S., Decker, J.D., Keith, T.B. & Mott, K.E. (1977) Clonorchiasis in New York City Chinese. *Tropical Doctor* **7**, 105–106.

Kang, S.Y., Ahn, I.Y., Park, C.Y., Chung, Y.B., Hong, S.T., Kong, Y., Cho, S.Y. & Hong, S.J. (2001) *Clonorchis sinensis*: molecular cloning and characterization of 28-kDa glutathione S-transferase. *Experimental Parasitology* **97**, 186–195.

Kim, D.C. (1974) Ecological studies of *Clonorchis sinensis*. *Yonsei Reports of Tropical Medicine* **5**, 3–44.

Kim, H.D., Eom, K.S. & Rim, H.J. (1987) Changes of serum and urine antibody levels by ELISA after treatment in clonorchiasis. *Korea University Medical Journal* **24**, 107–116 (in Korean).

Kim, M.S., Lee, J.S. & Rim, H.J. (1982a) Studies on the clinical aspects of clonorchiasis in Korea. *Korea University Medical Journal* **19**, 107–121 (in Korean).

Kim, S.S., Kim, S.J. & Rim, H.J. (1982b) Electron-microscopic studies on the effect of praziquantel to *Clonorchis sinensis*. *Korea University Medical Journal* **19**, 91–105 (in Korean).

Kim, T.Y., Kang, S.Y., Ahn, I.Y., Cho, S.Y. & Hong, S.J. (2001) Molecular cloning and characterization of antigenic protein with a repeating region from *Clonorchis sinensis*. *Korean Journal of Parasitology* **39**, 57–66.

Kim, Y.I. (1984) Liver carcinoma and liver fluke infection. *Arzneimittel Forschung* **34**, 1121–1126.

Kim, Y.I., Yang, D.H. & Chang, K.L. (1974) Relationship between *Clonorchis sinensis* infection and cholangiocarcinoma of the liver in Korea. *Seoul Journal of Medicine* **15**, 247–255 (in Korean).

Kim, Y.I., Yu, E.S. & Kim, S.T. (1989) Intraductal variant of peripheral cholangiocarcinoma of the liver with *Clonorchis sinensis* infection. *Cancer* **63**, 1562–1566.

Komiya, Y. (1966) *Clonorchis* and clonorchiasis. *Advances in Parasitology* **4**, 53–106.

Komiya, Y. & Suzuki, N. (1964) The distribution of *Clonorchis* infection in Japan with remarks on its epidemiology. *Progress of Medical Parasitology in Japan* **1**, 31–33.

Lee, J.H. & Rim, H.J. (1996) Cholangiocarcinoma caused by *Clonorchis sinensis* infection in laboratory animals. pp. 43–49 in *Proceedings of the 2nd Japan–Korea Parasitologists Seminar (Forum Cheju-2)*, April.

Lee, J.H., Rim, H.J. & Bak, U.B. (1993) Effect of *Clonorchis sinensis* and dimethylnitrosamine administration on the induction of cholangiocarcinoma in Syrian golden hamsters. *Korean Journal of Parasitology* **31**, 21–30.

Lee, J.H., Yang, H.M., Bak, U.B. & Rim, H.J. (1994) Promoting role of *Clonorchis sinensis* infection on induction of cholangiocarcinoma during two-step carcinogenesis. *Korean Journal of Parasitology* **32**, 13–18.

Lee, J.H., Rim, H.J. & Sell, S. (1995) Promotion of cholangiocarcinomas by *Clonorchis sinensis* infection in hamsters after induction by dimethylnitrosamine. *Journal of Tumor Marker Oncology* **10**, 7–16.

Lee, J.H., Rim, H.J. & Sell, S. (1997) Heterogeneity of the 'oval-cell' response in the hamster liver during cholangiocarcinogenesis following *Clonorchis sinensis* infection and dimethylnitrosamine treatment. *Journal of Hepatology* **26**, 1313–1323.

Lee, J.S. & Yong, T.S. (2004) Expression and cross-species reactivity of fatty acid-binding protein of *Clonorchis sinensis*. *Parasitology Research* **93**, 339–343.

Lee, J.S., Lee, J., Park, S.J. & Yong, T.S. (2003a) Analysis of the genes expressed in *Clonorchis sinensis* adults using the expressed sequence tag approach. *Parasitological Research* **91**, 283–289.

Lee, J.Y., Cho, P.Y., Kim, T.Y., Kang, S.Y., Song, K.Y. & Hong, S.J. (2002) Hemolytic activity and developmental expression of pore-forming peptide, clonorin. *Biochemistry and Biophysical Research Communications* **296**, 1238–1244.

Lee, J.Y., Kim, T.Y., Gan, X.X., Kang, S.Y. & Hong, S.J. (2003b) Use of a recombinant *Clonorchis sinensis* pore-forming peptide, clonorin, for serological diagnosis of clonorchiasis. *Parasitology International* **52**, 175–178.

Lee, K.H., Eom, K.S. & Rim, H.J. (1986) Serum IgG levels of clonorchiasis patients before and after therapy with praziquantel. *Korea University Medical Journal* **23**, 13–22 (in Korean).

Lee, M.K., Kim, S.J. & Rim, H.J. (1983) Electron-microscopic studies on the effect of various drugs to *Clonorchis sinensis*. *Korea University Medical Journal* **20**, 145–164 (in Korean).

Lee, S.H. (1984) Large scale treatment of *Clonorchis sinensis* infections with praziquantel under field conditions. *Arzneimittel Forschung* **34**, 1227–1230.

Lee, S.H., Hwang, S.W., Chai, J.Y. & Seo, B.S. (1984) Comparative morphology of eggs of heterophyids and *Clonorchis sinensis* causing human infections in Korea. *Korean Journal of Parasitology* **22**, 171–180.

Leung, J.W., Sung, J.Y., Chung, S.C. & Metreweli, C. (1989) Hepatic clonorchiasis – a study by endoscopic retrograde cholangiopancreatography. *Gastrointestinal Endoscopy* **35**, 226–231.

Li, S., Chung, Y.B., Chung, B.S., Choi, M.H., Yu, J.R. & Hong, S.T. (2004) The involvement of the cysteine proteinases of *Clonorchis sinensis* metacercariae in excystment. *Parasitology Research* **93**, 36–40.

Li, Y.J., Li, S.L. & Bao, Z.Y. (1990) Two different dosage-schedules of praziquantel in the treatment of *Clonorchis sinensis* infection. *Guangxi Medicine* **12**, 65–66 (in Chinese).

Lim, J.H. (1990) Radiologic findings of clonorchiasis. *American Journal of Roentgenology* **155**, 1001–1008.

Lim, J.H. & Ko, Y.T. (1990) Clonorchiasis of pancreas. *Clinical Radiology* **41**, 195–198.

Lim, J.H., Ko, Y.T., Lee, D.H. & Kim, S.Y. (1989) Clonorchiasis: sonographic findings in 59 proved cases. *American Journal of Roentgenology* **152**, 761–764.

Liu, H.R., Wang, H., Liu, K. & Liu, F.K. (1989) Determination of serum immunoglobulins in

https://doi.org/10.1079/JOH2005300 Published online by Cambridge University Press

Clonorchis sinensis-infected subjects. *Journal of Beijing Medical University* **21**, 185 (in Chinese).

Mehlhorn, H., Kojima, S., Rim, H.J., Ruenwongsa, P., Andrews, P., Thomas, H. & Bunnag, B. (1983) Ultrastructural investigations on the effects of praziquantel on human trematodes from Asia: *Clonorchis sinensis, Metagonimus yokogawai, Opisthorchis viverrini, Paragonimus westermani and Schistosoma japonicum*. *Arzneimittel Forschung* **33**, 91–98.

MHSA & KAPE (1982) Report on the pilot treatment project of *Clonorchis sinensis* infection with praziquantel, 1–45 (in Korean).

MHSA & KAH (1992) Evaluation of the mass-treatment project of clonorchiasis in Korea (1984–1990), 1–118 (in Korean).

Min, D.Y. (1984) Remarks on the diagnosis of *Clonorchis sinensis* infection. *Arzneimittel Forschung* **34**, 1153–1156.

Na, B.K., Lee, H.J., Cho, S.H., Lee, H.W., Cho, J.H., Kho, W.G., Lee, J.S., Lee, J.S., Song, K.J., Park, P.H., Song, C.Y. & Kim, T.S. (2002) Expression of cysteine proteinase of *Clonorchis sinensis* and its use in serodiagnosis of clonorchiasis. *Journal of Parasitology* **88**, 1000–1006.

Nagano, I., Pei, F., Wu, Z., Wu, J., Cui, H., Boonmars, T. & Takahashi, Y. (2004) Molecular expression of a cysteine proteinase of *Clonorchis sinensis* and its application to an enzyme-linked immunosorbent assay for immunodiagnosis. *Clinical and Diagnostic Laboratory Immunology* **11**, 411–416.

Nontasut, P., Thong, T.V., Waikagul, J., Fungladda, W., Imamee, N. & De, N.V. (2003) Social and behavioral factors associated with *Clonorchis* infection in one commune located in the Red River Delta of Vietnam. *Southeast Asian Journal of Tropical Medicine and Public Health* **34**, 269–273.

Ohshima, H. & Bartsch, H. (1994) Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutation Research* **305**, 253–264.

Park, G.M. & Yong, T.S. (2001) Geographical variation of the liver fluke, *Clonorchis sinensis*, from Korea and China based on the karyotypes, zymodeme and DNA sequences. *Southeast Asian Journal of Tropical Medicine and Public Health* **32** (Suppl 2), 12–16.

Park, G.M., Im, K.I., Huh, S. & Yong, T.S. (2000) Chromosomes of the liver fluke, *Clonorchis sinensis*. *Korean Journal of Parasitology* **38**, 201–206.

Park, S.B., Joo, K.H. & Rim, H.J. (1984) Studies on the epidemiological changes of clonorchiasis in the high endemic area (Kimhae City). *Korea University Medical Journal* **21**, 77–90 (in Korean).

Park, S.Y., Lee, K.H., Hwang, Y.B., Kim, K.Y., Park, S.K., Hwang, H.A., Sakanari, J.A., Hong, K.M., Kim, S. & Park, H. (2001) Characterization and large-scale expression of the recombinant cysteine proteinase from adult *Clonorchis sinensis*. *Journal of Parasitology* **87**, 1454–1458.

Parkin, D.M., Oshima, H., Srivatanakul, P. & Vatanasapt, V. (1993) Cholangiocarcinoma: epidemiology, mechanisms of carcinogenesis and prevention. *Cancer Epidemiology, Biomarkers and Prevention* **2**, 537–544.

Purtilo, D.T. (1976) Clonorchiasis and hepatic neoplasm. *Tropical and Geographical Medicine* **28**, 21–27.

Qu, Z.Q., Chen, R.X., Zeng, M.A., Li, J.Y., Zhang, C.D., Zeng, L.T., Qu, B.D. & Han, B.Y. (1983) Alteration of antibody levels in patients with *Clonorchis sinensis* after chemotherapy. *National Medical Journal of China* **63**, 241–243 (in Chinese).

Quan, F.S., Matsumoto, T., Shin, Y.O., Min, Y.K., Yan, H.M., Othman, T. & Lee, J.B. (2004) Relationships between IgG, IgM, IgE and resistance to reinfection during the early phase of infection with *Clonorchis sinensis* in rats. *Immunological Investigations* **33**, 51–60.

Rim, H.J. (1984) Studies on clonorchiasis in Korea. *Journal of Natural Academic Science Korea, Natural Science Series* **23**, 279–354.

Rim, H.J. (1986) The current pathobiology and chemotherapy of clonorchiasis. *Korean Journal of Parasitology* **24** (Supplement), 1–141.

Rim, H.J. (1997) Epidemiology and control of clonorchiasis in Korea. *Southeast Asian Journal of Tropical Medicine and Public Health* **28** (Supplement 1), 47–50.

Rim, H.J. (2003) Experiences on the epidemiology and control of clonorchiasis in Korea and China. pp. 11–15 in *Proceedings of the 4th Workshop Report on the Korea–China Collaborative Project of Control Strategies for Helminthiasis in Pilot Areas*.

Rim, H.J., Lyu, K.S., Lee, J.S. & Joo, K.H. (1981) Clinical evaluation of the therapeutic efficacy of praziquantel against *Clonorchis sinensis* infection in man. *Annals of Tropical Medicine and Parasitology* **75**, 27–33.

Shu, J., Wen, J.S. & Li, Y.H. (1992) A case of intracranial hypertension induced by treatment with praziquantel for clonorchiasis in a patient complicated with cerebral cysticercosis. *Chinese Journal of Parasitology and Parasitic Diseases* **10**, 289 (in Chinese).

Sim, S., Park, G.M. & Yong, T.S. (2003) Cloning and characterization of *Clonorchis sinensis* myoglobin using immune sera against excretory secretory antigens. *Parasitology Research* **91**, 338–343.

Song, L., Chen, S., Yu, X., Wu, Z., Xu, J., Yang, G., Zheng, N., Hu, X., Guo, L., Dai, J., Xu, J., Ji, C., Gu, S. & Ying, K. (2004) Molecular cloning and characterization of cDNA encoding a ubiquitin-conjugating enzyme from *Clonorchis sinensis*. *Parasitology Research* **94**, 227–232.

Su, Z.C. & Mon, R.K. (1983) A grand mal of epilepsy after treatment with praziquantel in a patient with *Clonorchis sinensis*. *Chinese Journal of Parasitology and Parasitic Diseases* **1**, 192 (in Chinese).

Wang, J., Lu, Z.Y., Wang, W., Wang, M.J., Chen, M.G. & Qiu, L.S. (1985) Changes in antibody levels in clonorchiasis patients before and after treatment. *Chinese Journal of Parasitology and Parasitic Diseases* **3**, 60 (in Chinese).

WHO (1995) Control of foodborne trematode infections. *WHO Technical Report Series* **849**, 1–157.

Yang, H.J., Park, S.J., Im, K. & Yong, T.S. (2000) Identification of a *Clonorchis sinensis* gene encoding a vitellaria antigenic protein containing repetitive sequences. *Molecular and Biochemical Parasitology* **111**, 213–216.

Yen, C.M., Chen, E.R., Hou, M.F. & Chang, J.H. (1992) Antibodies of different immunoglobulin isotypes in

serum and bile of patients with clonorchiasis. *Annals of Tropical Medicine and Parasitology* **86**, 263–269.

Yong, T.S., Im, K. & Chung, P.R. (1991) Diagnosis of clonorchiasis by ELISA-inhibition test using *Clonorchis sinensis* specific monoclonal antibodies. *Southeast Asian Journal of Tropical Medicine and Public Health* **22** (Supplement), 186–188.

Yong, T.S., Lee, J.S., Cho, S.N., Seo, J.H. & Park, H. (1996) A carbohydrate antigen of *Clonorchis sinensis* recognized by a species-specific monoclonal antibody. *Korean Journal of Parasitology* **34**, 279–281.

Yong, T.S., Yang, H.J., Park, S.J., Kim, Y.K., Lee, D.H. & Lee, S.M. (1998) Immunodiagnosis of clonorchiasis using a recombinant antigen. *Korean Journal of Parasitology* **36**, 183–190.

Yu, S., Xu, L., Jiang, Z., Xu, S., Han, J., Zhu, Y., Chang, J., Lin, J. & Xu, F. (1994) Report on the first nationwide survey of the distribution of human parasites in China. 1. Regional distribution of parasite species. *Chinese Journal of Parasitology and Parasitic Disease* **12**, 241–247 (in Chinese).

Zhou, Z.R. & Zhao, M.L. (1981) Measurment of IgE by RIST (radioimmunosorbent test) and IgE antibody against *Clonorchis sinensis* antigens by RAST (radioallergensorbent test). *Journal of China Medical University* **10**, 24–27 (in Chinese).

(Accepted 6 June 2005)