PROGRESS IN ASSESSMENT OF MORBIDITY DUE TO CLONORCHIS SINENSIS INFECTION: A REVIEW OF RECENT LITERATURE

by

Minggang Chen¹, Yao Lu¹, Xiangjin Hua¹ and Kenneth E. Mott²

¹ Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai, China
² Chief, Schistosomiasis and other Trematode Infections, Division of Control of Tropical Diseases, World Health Organization, Geneva, Switzerland

TABLE OF CONTENTS

1. Introduction .................................................. 3

2. Geographical distribution of C. sinensis in Asia .............. 3
   2.1 China (including Taiwan) .................................. 3
   2.2 Korea .......................................................... 5
   2.3 Japan .......................................................... 6
   2.4 Hong Kong .................................................. 6
   2.5 Vietnam .................................................... 7
   2.6 Russia (Far East part) ...................................... 7
   2.7 Other countries ............................................ 7

3. Parasitological characteristics .................................. 8
   3.1 Life cycle .................................................. 8
   3.2 The first intermediate hosts ................................ 8
   3.3 The second intermediate hosts ............................... 9
   3.4 Animal reservoir hosts ...................................... 11
   3.5 Longevity of the parasite .................................. 13

4. Epidemiology .................................................. 13
   4.1 Modes of transmission ....................................... 13
      4.1.1 Eating habits ........................................... 13
      4.1.2 Freshwater contamination ............................... 14
   4.2 Age and sex distribution ................................... 15
   4.3 Seasonal variation .......................................... 16
   4.4 Familial aggregation ...................................... 17

5. Pathogenesis .................................................. 17

6. Pathology .................................................... 17
   6.1 Animal experiments ....................................... 17
   6.2 Human pathology ........................................... 18

This document is not issued to the general public, and all rights are reserved by the World Health Organization (WHO). The document may not be reviewed, abstracted, quoted, reproduced or translated, in part or in whole, without the prior written permission of WHO. No part of this document may be stored in a retrieval system or transmitted in any form or by any means - electronic, mechanical or other - without the prior written permission of WHO.

The views expressed in documents by named authors are solely the responsibility of those authors.
7. Clinical presentations
   7.1 Incubation period
   7.2 Acute phase
   7.3 Chronic phase
   7.4 Laboratory findings
      7.4.1 Haematology
      7.4.2 Liver function tests
      7.4.3 Immunoglobulins
   7.5 Radiography, ultrasonography and computed tomography
      7.5.1 Radiography
      7.5.2 Ultrasonography
      7.5.3 Computed tomography (CT)
   7.6 Complications
      7.6.1 Secondary bacterial infections of the hepatobiliary system
      7.6.2 Cholelithiasis
      7.6.3 Pancreatitis
      7.6.4 Liver cirrhosis
      7.6.5 Cholangiocarcinoma

8. Diagnosis
   8.1 Parasitological examinations
      8.1.1 Formaldehyde ether concentration technique
      8.1.2 Kato faecal thick smear technique
      8.1.3 Stoll's dilution egg counting technique
      8.1.4 Duodenal drainage
      8.1.5 Egg identification
   8.2 Immunological tests
      8.2.1 Intradermal tests
      8.2.2 Serological tests
         8.2.2.1 Antibody detection
            i. Enzyme-linked immunosorbent assay (ELISA)
            ii. Indirect fluorescent antibody test (IFAT)
            iii. Indirect haemagglutination test (IHA)
               iv. Complement fixation test (CFT)
               v. Counter-immunoelectrophoresis (CIEP)
               vi. Other serological tests
      8.2.2.2 Circulating antigen detection
   8.2.3 Stool antigens detection
   8.2.4 Urinary antibody detection

9. Effects of treatment on disease
   9.1 Parasitological effects
      9.1.1 Praziquantel
         9.1.1.1 Animal experiments
         9.1.1.2 Human treatment
      9.1.2 Albendazole
1. Introduction

Clonorchis sinensis infection is endemic in China including Taiwan, South Korea, Japan and northern Vietnam, and isolated, small foci have also been reported from the Far East part of Russia. The traditional habit of eating raw or undercooked freshwater fish keeps the disease endemic in a large area of several countries. The morbidity and mortality caused by the infection are important public health problems in endemic areas. Morbidity due to the infection has been a topic of many experimental and clinical studies and considerable progress has been made. The scientific literature published mainly between 1980 and 1992 including Chinese and Korean literature, a part of which has not been previously reviewed elsewhere in English publications, has documented the progress and is reviewed herein.

2. Geographical distribution of C. sinensis in Asia

2.1 China (including Taiwan)

An ancient corpse buried in the West Han Dynasty (278 B.C.) and unearthed in 1982 in Jiangling County, Hubei Province showed C. sinensis ova in the residual materials of the visceral contains, which provided so far the earliest record of C. sinensis infection in the history of parasitology in the world (110, 230, 261). At present time, in the mainland of China, clonorchiasis is widely distributed in a total of 24 provinces, municipalities and autonomous regions the prevalence rate ranging from 1-57% (127, 230). Guangdong Province and Guangxi Zhuang Autonomous Region in the south, Heilongjiang, Jilin and Liaoning provinces in the north, among them, are the most severely affected endemic areas. By ethnic group, the highest prevalence is reported among the Korean national minority in China (10, 21, 29, 36, 51, 52, 126).

Southern China has long been known as a heavily endemic area for
clonorchiasis, but there are many endemic foci reported from the middle, southwestern, northern, northeastern China. Guangdong is the most heavily endemic province in China where approximately three million persons are infected due to the general habit among Cantonese (people from Guangdong) of eating raw fish (127). Clonorchiasis is rampant in 37 of 109 counties/cities (36), especially in the watershed of the Pearl River Delta, along the Pearl River and the Han River (average prevalence 15.7% during 1973-1983) (29), in Chaozhou (5.6-12.2% in 1987) (52), Shantou (6.4% during 1973-1983) (51), and Foshan (22.8% during 1973-1983) (237). In 1980, in a province-wide sample survey, 19 out of 22 counties/cities were found to have C. sinensis infection with an overall infection rate of 17.3%. 1.4% and 1.4% of the first intermediate hosts, Parafossarulus manchouricus and Alocinna longicornis, were found to be infected and 253 freshwater fish of seven different species out of 1641 fish examined from 20 species served as the second intermediate hosts. The main animal hosts in these areas were cats (infection rate: 47.2%) and dogs (infection rate: 23.7%) (36).

The prevalence of C. sinensis is high in areas along Youjiang River in Guangxi Zhuang Autonomous Region such as, Baise District (21). Between 1986 and 1991, eight counties/cities in Baise District were investigated using both intradermal test and stool examination. 1424 (7.3%) out of 19,578 persons examined were found positive in intradermal tests or faecal eggs (21). It was estimated that the number of infected persons in the whole district was 80,000 (21), and one million in the whole Autonomous Region (127).

High prevalences of C. sinensis were seen in three northeast provinces of China, namely, Heilongjiang, Jilin and Liaoning, where most of the Korean national minority who eat raw fish reside (10, 53, 126). In Songhua River Basin with an overall infection rate of 34% by intradermal test (123), the prevalence of Clonorchis in Korean national minority was 2.7 times as high as that in the Hans, the largest ethnic group in China. The infection rate was 24.6% by stool examination in these areas investigated between 1966 and 1980 covering a population of 20,396 persons of Korean national minority (126). In 1986, four outbreaks of clonorchiasis arose in Heilongjiang due to eating raw fish in dinner parties (233, 234). Seventy people attending the dinner all had clinical symptoms and signs. Positive reactions were shown in a total of 64 persons tested intradermally. In Jilin and Liaoning provinces, the prevalence was also high (10, 52, 123). The highest infection rate, 45.8%, was found in a village in Jilin (10, 123).

In the periurban plains around Beijing, transmission of C. sinensis occurs widely in the canals. In 1982, 14,636 (12.9%) of 113,399 persons examined had C. sinensis eggs in the stool (218).

Since 1958, several surveys have been carried out in the provinces along the Yangtze River. Most of them showed low prevalences of human infection with the exception of part of Jiangsu Province (33.6% in a county) (51), although high or moderate infection rates were found in snails, fish and animal reservoir hosts. The fact that few people there
have the habit of eating raw fish may explain this observation (51, 52, 123, 211, 270, 277).

Clonorchiasis was first reported in Taiwan in 1915. The infection of C. sinensis is found in almost every city/county on the island with the infection rate varying from 0-57% (17, 181). The human prevalence is currently about 20-50% in some of the traditionally heavily endemic areas and 10-20% in newly endemic localities (22). Three areas, Mei-Nung in the south, Sun-Moon lake area at the centre and Miao-Li in the middle, have been affected seriously by C. sinensis and the respective high prevalences of 52%, 52% and 57% were recorded. As for the whole population in the island, the prevalence was estimated about 0.012% to 1.5% in 1984 (46, 136). The first intermediate host of C. sinensis in Taiwan is P. manchouricus. Other species, Semisulcospira libertina and Thiara granifera, may also serve as intermediate hosts. Fifteen species of freshwater fish are known as the second intermediate hosts of the parasite. Of them, Mugil cephalus and Ctenopharyngodon idellus are the most important ones with above 80% infected and they are frequently consumed raw by the inhabitants in endemic areas. Both intermediate hosts are distributed in the lakes, ponds and small streams throughout Taiwan (17, 22, 46). Rats, cats, dogs and pigs are the main reservoir hosts on the island. Among those, the infection rate in pigs is the highest. Recent studies have shown that the geographical limits of the disease area are extending (22).

2.2 Korea

Clonorchiasis has long been known endemic in the Republic of Korea with its first report in 1915, because eating raw freshwater fish is the habit of the local residents (181). Endemic areas of clonorchiasis are scattered all over the country. In 1958, 1701 (11.7%) of 14,519 fecal samples were found egg positive in stool examination (181). In a nationwide skin test survey in 1959, 4.5 million (15%) of 30 million Koreans were positive (44, 200).

Clonorchiasis is mainly distributed in areas near seven large rivers, i.e., Han, Kun, Nakdong, Mangyong, Yeongsan, Seonjin and Tamjin. Since 1958, several nationwide and local surveys have been conducted among the populations in these river basins and other areas. The most extensive and intensive endemic regions were located along the Nakdong River and the lower reaches of other rivers (181, 193, 203). Song (203) reported that in Bug Gu, an area of the lower Nakdong River, the positive rate was 38.4% by Kato faecal thick smear method, although 457 (89.1%) of 513 infected persons showed less than 10,000 eggs per gram (EPG). In certain areas of these regions, the prevalence reached as high as 82.9% (181).

From 1979 to 1980, a survey was carried out by Seo et al. (193) among a total of 13,373 inhabitants living in 40 villages using both the Kato and Stoll's stool examination techniques. The positive rate of each river basin differed significantly, 40.2% in the Nakdong River, 30.8% in the Yeongsan River, 17.3% in the Seonjin River, 15.9% in the Tamjin River, 15.7% in the Han River, 12.0% in the Kun River and 8.0% in the Mangyong
River, with an overall prevalence of 21.5%. According to their estimates, about 830,000 to 890,000 of the total of 4 million inhabitants living along these rivers were infected. Among those infected, 6.8% were heavy infections (EPG>10,000); 28.6%, moderate infection (EPG 1000-9900); and the largest proportion (64.7%) had subclinical or, light infections (EPG<900).

Song et al. (202) reported another survey performed between 1973 and 1982 in inhabitants residing along six rivers, i.e., Han, Kum, Nakdong, Mangyong, Yeongsan and Seomjin. 19,758 stool samples were collected and examined. The results showed that the prevalence rates among the residents living in the river basins were 7.8%, 15.2%, 25.3%, 3.0%, 20.1% and 1.3%, respectively. Prevalence rates in residents living in the river basins were higher than those in the areas far away from the riversides (40, 193). The mean egg counts were also higher in the areas with higher prevalence.

A new endemic area for C. sinensis along the Nam River in Gyeongsangnam-do was reported by Bae et al. (8) in 1983. The infection rate was 38.7% among 5291 examined: light infections (EPG<4000), 53.6%; moderate infections (EPG 4001-10,000), 30.3%; and heavy infections (EPG>10,001), 16.1%.

In recent years, the overall prevalence of C. sinensis has been decreasing in Korea. In Kimhae City, a high endemic area in Korea, the infection rate was reduced from 68.3% (1973) to 45.6% (1983) and the mean egg count was also on the wane, from 10,846 (1973) to 4,858 (1983) (162). Since 1984, the government launched a mass treatment programme for clonorchiasis in endemic areas. Between 1984 and 1990, a total of 3,009,166 persons were screened by Kato technique and those with eggs in their stools were treated with praziquantel. As a result, the prevalences in inhabitants of the programme areas were: 13.3% of 168,877 in 1984, 7.0% of 477,237, 2.2% of 496,835, 1.8% of 502,026, 1.2% of 488,553, 0.9% of 496,361 and 0.9% of 409,277 from 1985 through 1990, respectively, showing a sharp decrease (183). There was a similar trend in the reduction of the prevalence in other areas (40).

2.3 Japan

The first case in Japan was found in 1878 (96). Many early studies revealed that C. sinensis infection was once widely distributed in Japan except in the north. In 1960s, the basins of rivers Kitagami, Tone, Ooe and Yoshino, the Biwa Lake and its vicinity and the coastal area of Kojima Bay were heavily endemic for clonorchiasis. In these areas, P. manchouricus snails and freshwater fish were found to be infected (96). However, most infected cases in these areas showed light infections with less than 1000 EPG of faeces and without clinical manifestations (181). Owing to the rise of living standard and environmental changes, at present, the infection of C. sinensis is markedly decreased (96, 181).

2.4 Hong Kong

Clonorchiasis is the most prevalent parasitic disease detected by
stool examination in Hong Kong especially among Cantonese population. Among Chinese in Hong Kong, 23% were shown to have C. sinensis at autopsies in 1973 (7). According to Duchastel’s report, in 1984, 13.4% of 25,095 Hong Kong residents applying for the emigration for Canada during 1979-1981 were found to be infected on a single stool smear (97). The flukes are encountered frequently by surgeons during routine gall-bladder operations. They are also the proven cause of pancreatitis of unknown etiology in 83% of patients in Hong Kong (97, 208). Even with such a high prevalence of clonorchiasis, no susceptible snails have been found in Hong Kong and the infection is acquired from infected fish from the mainland of China (7). Clonorchiasis is more common in wealthy people than the low-wage earners since these imported fish are usually expensive. However, it seems that clonorchiasis is now decreasing in Hong Kong due to control measures implemented in China (16, 46).

2.5 Vietnam

According to early studies, clonorchiasis was mainly endemic in the northern part of Vietnam such as the Red River Delta. In this endemic area, 28.4% of the residents were infected; higher than 40% in adults and 8% in children, and highest among males (86, 181). Two endemic foci within the Delta, Haiphong and Hanoi, were reported to have the highest infection rate (73%), whereas in the western and northern parts of this region the infection rates were low. A few cases recorded clinically in southern Vietnam were all among the northerners who got the disease in original places. At present, the infection rate is much lower than before (181).

Several parasitological surveys among Indochinese refugees in non-endemic countries have been reported. Among Indochinese refugees in the United States including Vietnamese, Laotians, and Cambodians, the infection rate of C. sinensis in adults between 19 and 54 years of age was 68% (15). Some of these infections were due to Opisthorchis. Screening of Indochinese refugees for parasitic and other diseases carried out at the Mayo Clinic in USA revealed that two of 100 refugees had Clonorchis infection (80). Among 444 Vietnamese refugees living Okinawa only one was found to have C. sinensis infection (6).

2.6 Russia (Far East part)

Cases of C. sinensis infection were reported in the Amur River territory, the Far East part of Russia. The first intermediate host of C. sinensis, P. manchouricus snail, and the second intermediate host, certain species of fish belonging to Cyprinidae family, were also occurred in water bodies of the Amur region. In the southern part of the Khabarovsky region, foci of the Amur River basin, high infection rates were observed among snails, fish and domestic cats, being 2.9%, 9.5% and 74.6%, respectively. Thus, even occasional consumption of raw fish carries a high risk of acquiring infection (58, 163, 164).

2.7 Other countries
In a survey on 300,000 Filipinos by Cross in 1967-1983, eggs similar to those of *Opisthorchis* sp. or *C. sinensis* were detected in 135 stool specimens from Northern Luzon, Palawan, and Mindanao (47). However, *C. sinensis* infection in the indigenous population has not been confirmed parasitologically.

Persons with *C. sinensis* infection were infrequently reported from Singapore, Malaysia and other countries. However, the cases were infected in other endemic countries or through eating imported fish.

3. Parasitological characteristics

3.1 Life cycle

The living fluke is pinkish, semitransparent, and spatulate, with attenuated anterior and round posterior ends, 8 to 25 mm long, 1.5 to 5 mm wide, and about 1 mm thick with a smooth cuticle (11, 181). However, the size of the fluke varies according to its age, species of the host, number of the parasites in a host and its location in the large vs. small bile ducts (181). The adult flukes live in the distal biliary tract in man and fish-eating mammals. The eggs are yellow in colour, 26-30x15-17 μm in size with a prominent shoulder. Eggs are passed in faeces into water where they can remain alive but do not hatch. The hatching of miracidium from egg is accomplished in a suitable vector snail which ingests the eggs. In the snail, the eggs hatch in the gut, and become miracidia. Miracidia penetrate into tissue, after sporocysts, rediae formation and develop into thousands of long-tailed cercariae. The cercariae penetrate susceptible freshwater fish beneath the scales, lose their tails and encyst and develop into metacercariae mainly in the muscles and subcutaneous tissue and less often on the fish scales themselves. When the definitive host ingests living metacercariae by eating raw or undercooked fish, young flukes excyst in the intestine, migrate and enter the main bile duct and become adult flukes. The highest number of flukes was reported to be 27,600 in a Korean who died from obstructive jaundice (181). Usually the eggs can be found in the faeces of the experimental hosts four weeks after the infection (181).

3.2 The first intermediate hosts

The development of the *Clonorchis* egg in a vector snail is greatly influenced by environmental temperature. Temperatures below 15°C or above 35°C are unsuitable for its growth. At least 80 days are required to fulfill the part of the life cycle in the vector snail under an optimal temperature of 25°C (230).

Up to the present, nine different species of snails belonging to four families, i.e., Assimineidae, Hydrobiidae, Melaniidae and Thiaridae, have been confirmed to be the first intermediate hosts involved in the life cycle of *C. sinensis*, as shown in Table 1. Among them, *Parafossarulus anomalospiralis* (Liu, 1983) is a new species recently discovered as an intermediate host of *C. sinensis* by Li (118). Of these, *P. manchouricus* is the most widespread and the only one found in countries other than China. Accounting for 0.08-3.1% of the snails examined in Korea, *P.*
manchouricus has been identified in the plain areas along the rivers Han, Rum, Mankyang, Maktong, and Yeongsan in southern Korea (40, 175, 199, 200). The rate of C. sinensis infection in the vector snails is very low, ranging from 0.09-0.6% (40, 175, 200). In China, nine snail species were found to have been infected with miracidia of C. sinensis, the most frequent being P. manchouricus and A. longicornis. In Guangdong Province, 8% of P. manchouricus and 27.5% of A. longicornis were found naturally infected (127) and in Liaoning, the infection rate of P. manchouricus reached 15.8% (113). Since C. sinensis has a strict selection of the first intermediate snail host, the presence of a specific snail is an important risk factor in the identification of endemic foci for clonorchiasis.

Usually the vector snail does not dominate any habitat. Other freshwater snails tend to predominate. On the other hand the infection rates are inversely proportional to the distance between houses and the snail habitats (113). Under natural conditions, infected snails have been recorded to release as many as 5840 cercariae per snail per day (8, 40, 181).

The infection rate of the snail varies according to the season. The main peak of transmission in China is in July (113). In the temperate zones of Japan and Korea, cercariae are shed from May to October and earlier in subtropical zones (96, 181). There is no consensus on the viability of infection after estivation. Some researchers have presumed that C. sinensis miracidium which penetrated the snail before estivation, arrested in the redia stage over winter and then shed cercariae around April. Rim (181) suggested that in the area as cold as Korea, the cercariae and rediae are unable to survive estivation and a new infections are initiated each year and shed cercariae in late spring (96, 181).

3.3 The second intermediate hosts

Since 1980, 13 families involving 109 species of freshwater fish and one family involving three species of freshwater shrimp have been documented as the second intermediate hosts (Table 2). Among this total, 76 different species of fish were found to be infected with metacercariae of Clonorchis in China, 42 in Korea, 25 in Japan and nine in Russia. The extent of the change of the geographical distribution of these species is not well documented. In one instance, Abbottina rivularis and Acheilognathus signifer which were known as second intermediate hosts of C. sinensis in Korea, have now disappeared (40). The Family Cyprinidae, involving 95 fish species, plays a major role in the developmental cycle of the fluke. Small sized fish such as, Pseudorasbora parva, genus Acanthorhodeus, Rhodius and Hemiculter, are more frequently and more intensely infected with metacercariae of C. sinensis than large sized fish, e.g., the genus Ctenopharyngodon, Hypophthalmichthys, Carassius and Cyprinus with the exception of the genus Mylopharyngodon (96, 181, 217, 255). The large populations and wide distribution of P. parva, make it the most important fish in the transmission of the disease. In certain provinces in China and Korea, the rate of metacercaria infection in P. parva reached 100% (40, 82, 203, 233, 259, 270). The intensity of C.
sinensis infection has been documented at a maximum of 31,615 metacercariae in a single fish. A small P. parva weighing only 0.2g was found to bear 3429 cercariae in Hunan Province, China. In Guangdong, each gram of the fish tissue can contain as high as 6,548 metacercariae (181).

The most commonly infected fish in China include P. parva, C. idellus, M. aethiops, genus Abbottina, Rhodesus, Hemiculter. In Heilongjiang, Liaoning, Beijing, Shaanxi, Hubei and Guangdong, the infection rate of one or several of these fish was over 60% (52, 126, 218, 233, 237, 255, 259, 270). According to reports summarized by the Korea Association for Parasite Eradication (KAFE), in 1978, the most frequent infection resources in Korea were P. parva (90.3%), followed by Sarcocheilichthys sinensis (78.0%), Hemibarbus laheo (68.2%), Puntungia herzi (50.9%), Pseudogobio esocinus (47.5%), Gnathopogon sp. (44.4%), Cultriculus kneri (38.5%) and A. taenianalis (29.5%) (40, 181, 203). In Japan, the infection of P. parva, C. elongatus, S. variegatus and A. lanceolata were common, whereas the fish which were usually eaten uncooked were limited to C. carpio and C. carassius (96).

Although as early as 1963, it was reported that three species of freshwater shrimps, Caridinia nilotica gracilipes, Macrobrachium superbum and Paleomonetes sinensis, belonging to Class Crustacea, Phylum Arthropoda could be the second intermediate host (181), there is no agreement on this topic. Recently in Hubei Province, Yuan (259) found two infected C. nilotica gracilipes out of 14 examined. Furthermore, in Jiangxi it was also noted that M. superbum had metacercariae of Clonorchis (52). The role of shrimp as the second intermediate host has been doubted by other investigators. Liu et al. (141) examined 703 fresh water shrimps in the ponds of endemic foci in Liaoning, Guangdong and Fujian provinces between 1980 and 1982. None were infected, whereas 60-80% of P. parva in the same ponds were found infected. They concluded that the shrimp might be transport vector carrying metacercariae of C. sinensis released from dead and broken fish. Metacercariae were also not found in 235 freshwater shrimps belonging to six species from an endemic area in Jilin Province (10). Thus, there is no epidemiological evidence of an association between eating raw shrimps and Clonorchis infection.

Wang (217) investigated the infection of freshwater fish with metacercariae in Jilin Province and found that both the rates and intensity of the infection varied according to fish body weight, i.e., the heavier the body weight of the fish, the lower the rate and intensity of the infection. The distribution of metacercaria in fish body was also studied. Most metacercariae were found in the tissue (66.0-99.0%), then in head (6.6-22.6%), skin (0.1-7.8%) and scales (0.3-3.9%) (113).

It has been already known that metacercaria of Clonorchis has a low resistance to high temperature. In a slice of fish tissue, 1 mm in thickness, the metacercariae can be killed in the 90°C hot water after one second. In the water of 75°C, 70°C, 60°C, the killing effect needs three, six, and 15 seconds respectively. Metacercaria can live for two hours in vinegar (with a concentration of 3.4% acetic acid), five hours in soya
sauce (with a concentration of 19.3% sodium chloride). However, while the slices of fish were quick-fried or fried, those harbouring in "thick" slices of fish may still survive after short, inadequate cooking (230). Wang et al. (216) studied the resistance of *Clonorchis* metacercaria to low temperature, chemicals at different concentrations and various sauces in vitro as well as to low temperature in the fish. The extracorporeal metacercariae showed strong resistances in these tests.

3.4 Animal reservoir hosts

In general, host specificity is determined by the extent of the relationship between the host and parasite during evolution. As for *C. sinensis*, many domestic and wild animals have been reported to serve as the reservoir hosts and play important roles in the transmission of clonorchiasis. These definitive natural hosts other than man are dogs, pigs, domestic cats (*Felis domesticus*), *F. bengalensis*, *Vivercula indica*, rabbits (*Lepus cuniculus*), *Rattus rattus*, *R. norvegicus* caraco, *R. flaviventer*, *Ondatra zibethica*, *Mustela sibirica*, buffaloes (*Bos buffalus*), camel and yellow weasel (*Mustela sibirica*) (10, 36, 52, 119, 122, 123, 137, 191, 203, 211, 218, 234, 237, 260, 270, 276, 277). Among them, cats, dogs, pigs and rats are particularly important because of their wide distribution and large populations. In some endemic areas, the prevalence rates and intensity of *Clonorchis* infection of the animals were very high. For example, in Jiangxi and Heilongjiang provinces, China, the infection rates in domestic cats and dogs were as high as 100% (126, 259, 270). 3990 flukes were found in the bile duct of a cat (218). *C. sinensis* ectopic parasitism in the lung of a cat was observed by Yuan (270) at dissection showing 116 adult flukes. Pigs showed infection rates of 11.7% in Sichuan Province and 35.3% in Henan Province, China (127). It is estimated that in the endemic areas the number of pig exceeds the number of cats and dogs, and thus the pig has a major role in the epidemiology of clonorchiasis in some areas (122). The role of the ubiquitous house rats (*R. rattus*) in the epidemiology of clonorchiasis and their potential contribution to the spread of transmission need further investigation (191).

There is no consensus on the role of chickens and ducks as reservoir hosts of *C. sinensis*. Some investigators have confirmed this association (52, 160, 233, 270, 271). However, Zhu (293, 294) and Wang (228) raised questions on this point through two studies in 1965 and 1984. They were unable to detect eggs in the faeces 35 days after feeding orally 100 metacercariae to each chicken or duck, whereas in the control rabbits, a large number of adult worms were seen in the bile ducts. They suggested that domestic fowls are suitable hosts for *Metorchis orientalis* and the eggs of *M. orientalis* are easily confused with *C. sinensis* eggs. Similar conclusions were published by Huang (76).

The importance of reservoir animal in the epidemiology of clonorchiasis is confirmed by their high infection rates in the areas where the prevalence and intensity of infection in people are high and the opposite is observed in low prevalence areas. In Kimhae City, Korea, 59 (18.5%) of 319 pigs, two of four dogs, and 19 (10.9%) of 174 house rats
were infected, whereas in Goyang City with relatively low prevalence, only 2.4% (2/84) of pigs, 21.6% (11/51) of dogs, and 3.8% (14/368) of rats were infected. Therefore, in some endemic areas, the reservoir hosts such as pigs, dogs and rats may play a significant role in spreading the eggs of *C. sinensis* (181). However, the intensity of human infections is usually heavier than the infection of reservoir animals. Faecal examination of animals in some areas showed that 0.14-10.0% of dogs, 7.3% of cats, and 0.33% of house rats passed viable *C. sinensis* eggs. It indicates that infected persons rather than reservoir hosts play the major role in the intensity of transmission of the disease (181, 200) and reservoir animals may serve primarily to maintain the presence of transmission.

Komiyama et al. (96) reported that in the 1950s and 1960s, *Clonorchis* infection among cats was severe in many area of Miyagi Prefecture, Japan where the disease was once prevalent, reaching as high as 87.0%.

Development of *C. sinensis* in laboratory animals such as rabbits, guinea pigs, dogs, cats, rats, hamsters, gerbils (*Meriones unguiculatus*), nutrias, *Macaca mulatta*, *Mus musculus*, *Apodemus agrarius*, *Citellus dauricus*, and *Castor fiber birulai* has been studied to evaluate the suitability of the animals as experimental hosts (10, 43, 58, 68, 69, 89, 102, 172, 254). In these studies of host-parasite relationship, the following are observed or measured: (1) infection rates which refer to the rates of infected individuals among animals challenged with the metacercariae; (2) recovery rates or worm burdens; (3) growth and development expressed as prepatent period and longevity of the infection, and size of the parasite, (4) fecundity or egg-laying capacity. Table 3 presents some of these results.

Kim et al. studied the prepatent period, longevity of the infection and chronological fecundity of *C. sinensis* in six strains of inbred mice, ICR, DDS, GPC, BALB/c, nude and DS. The prepatent period of the fluke ranged from 21 to 25 days and the fertile period (longevity of infection) was 22 to 179 days varying by strains of the mice. In this study the DDS mouse seemed to be the most suitable experimental animal with the shortest prepatent period, the longest fertile period and high (the highest is 28 eggs per gram of mouse faeces) and stable (from 23rd day after infection) egg-laying capacity. However, considering intraspecies differences in host-parasite relation of *C. sinensis*, mice are supposed to be relatively unsuitable as the experimental hosts (89). In experimentally infected gerbils, eggs of *C. sinensis* was detected as early as day 21 after the infection (59).

The recovery rate of *C. sinensis* is influenced by the biological conditions of the metacercaria such as maturity and activity, and anatomical factors of the biliary passage of the host animal. Differences in age of the hosts and in the method of administration of metacercariae may account for some discrepancies in published results (43).

Usually 160-190 days after infection, the fluke reaches its maximum size in definitive hosts. Both adults and eggs in animal reservoir hosts
are smaller than those in man. Li (123) observed that the average sizes of
adult worms of Clonorchis in cats, R. norvegicus and yellow weasel were
11.1 x 2.3, 8.9 x 2.2, 7.5 x 1.5 mm, respectively and the eggs from cats
and R. norvegicus averaged 27.1 x 15.6 and 27.3 x 16.2 μm. Chung (43)
found that the parasite in the guinea pig is largest with decreasing size
in the hamster, rabbit, rat, dog and mouse, respectively.

The egg-laying capacity of C. sinensis in cats is high. Each of 60-, 90-
and 120-day old adult worms discharged 945 (673-3153) eggs per day on
an average, whereas in rats, only 195 (57-332) eggs per day (123).

Generally, with the increase of the number of metacercaria
administered, the recovery rate decreases, whereas the number of the
parasites recovered increases. As a result of the increase of worm burden,
a decrease of egg-laying capacity was observed (43). The size of the fluke
was also influenced by the intensity of the infection (52, 123). Early
studies on experimental infections showed that 10 to 30 metacercariae were
optimal for the rat and 20 for the hamster and mouse (68).

3.5 Longevity of the parasite

Longevity of a parasite is very dependent on the compatibility of the
host-parasite and the tolerance of the host (7).

In general, C. sinensis can survive 15 to 25 years in human body.
Attwood and Chou (7) reported one case with vital C. sinensis continually
in the bile ducts for at least 26 years. The patient was a Chinese who
migrated to Australia 26 years previously from Hong Kong and never
returned. Flukes can be easily identified at autopsy. Based on
epidemiological data from Shandong Province where the peak infection is in
the 6-15 year old age group, Zhu (293) estimated that most flukes live for
less than 10 years.

4. Epidemiology

4.1 Modes of transmission

4.1.1 Eating habits

The prevalence of clonorchiasis, as well as intensity of human
infection, is related to the custom of eating raw fish. Clonorchiasis is
mainly observed among the peoples of south and northeast parts of China.
On the other hand, in certain provinces of central China where infected
fish and reservoir hosts are present, human infection is rare or absent due
to the habit of eating only cooked fish. Of the racial or social groups,
the Cantonese people in the south and Korean ethnic group in the northeast
have the highest prevalence of Clonorchis infection. The morning congee
(rice gruel) with raw fish enjoyed by the Cantonese contributes to the high
prevalence in southern China and Hong Kong (46). Other modes of
transmission such as, eating half-roasted and undercooked fish were also
found to be associated with high prevalence of clonorchiasis in 54
counties/cities in Guangdong province. Many local residents acquire infections through fish prepared in chafing dishes with short cooking times (29). The eating habits of the Korean ethnic groups have been investigated (10). Several ways of consuming raw fish were observed: ranging from whole raw small fish ingested directly or slices of raw fish marinated with vinegar, soya sauce or chili or prepared as pickled, salted or dried fish. Marinated slices of raw fish is the most common preparation and usually eaten while drinking wine (10). In some areas such as Beijing, infection has been reported through eating fish paste (218). Among the peoples with low intensity of infection, the infection was mainly acquired by eating poorly cooked infected fish (29, 259).

Eating raw shrimps has been claimed to be the only source of infection in the endemic areas of Fujian Province (181). Children living in river basins in Guangdong, Beijing, Hubei, Jiangxi, Shaanxi, Shandong like playing and eating small fish, occasionally raw shrimp, and poorly cooked fish (29, 36, 218, 237, 255, 259, 270, 293). In some of these areas, children have a tradition of holding a live fish in their mouths while fishing and therefore acquire the infection in this manner (218, 259, 270).

Among the Koreans infection with *C. sinensis* is acquired by eating slices of raw freshwater fish coated with hot bean paste. It is the custom in rural social gatherings in Korea to eat this appetizer with the rice wine called, "Maraculee", and is considered to be special health food for males (40). In the Pusan City, Korea, freshwater fish were eaten by both urban and periurban residents after different preparation methods (some people use several methods): boiling by 67.2% of those surveyed, roasting by 18.8%, frying by 5.0%, salting by 3.9%, and drying by 1.9%, as well as raw without preparation by 24.3% (40). Although heavily infected small fish are not generally eaten in raw, they still transmit infection when undercooked (181). Sometimes, children were given raw fish by their mothers who followed traditional ideas that raw fish would make their children strong (40, 181).

As mentioned above, the intensity of metacercaria of *Clonorchis* per weight is greatest in small fish. In Japan, these small whole fish are generally eaten after being boiled with a small amount of soya sauce. Some species of fish are specifically served as slices with vinegar, salt miso (bean paste) or soya sauce. On the other hand, large fish were usually eaten raw without marination (96). Large fish, *C. carpio* and *C. carassius*, which were frequently eaten raw by the Japanese have low rates of infection. As a result, clonorchiasis among the Japanese is not associated with severe clinical manifestations (96). In Vietnam, clonorchiasis is mainly acquired by eating raw freshwater fish in the salads (86).

Utensils used in preparing fish, such as knives and chopping boards, have been reported to be contaminated with metacercariae of *C. sinensis* and can be vehicles of infection (29, 96, 203, 216, 270, 277).

4.1.2 Freshwater contamination
Contamination of freshwater with faeces containing the eggs plays an important role in the transmission of *C. sinensis*. In rural areas with poor sanitation, residents use the water from the ponds or streams which receive drainage from pigpens, cowsheds or even toilets and are contaminated with faeces of man and domestic animals. The faeces are ubiquitous: 1) fresh human night soil is used as fertilizer; 2) roads are contaminated during the transportation of faecal material; 3) fish fry are fed with human and domestic animal faeces (mainly pigs) without treatment; all these promote transmission (22, 29, 126, 259, 270, 293). The infection rates of fish and snails are indirectly proportional to the distance from human settlements (293). The prevalence rate among the residents close to rivers is higher than among those in the areas far from the rivers. In Hubei Province, for example, the prevalence of clonorchiasis was high in the plain areas where houses are always constructed near the streams and rivers and water is easily contaminated, resulting in high infection rates of the snails and fish. In mountainous areas, the population density is low and the sources of freshwater are streams far from the settlements, thus, less contaminated and clonorchiasis is uncommon (259). Some authors have suggested that infection may be acquired through drinking or using freshwater from *C. sinensis* contaminated rivers (96, 203). The epidemiological significance of this possibility is not known.

4.2 Age and sex distribution

The age and sex distribution of clonorchiasis in endemic areas is related to social customs. The mean EPG count of *Clonorchis* infection was higher among the older age groups and such an increase in intensity was considered to be caused by an accumulation of adult worms through repeated infections (5, 181, 202). No evidence of concomitant immunity to reinfection has been found in this review of the literature.

The age and sex distribution of clonorchiasis was reported in Baise District, Guangxi, China (21). The youngest infected person was nine years of age, and the oldest, 70 years of age. The highest rates among males were in the 25-55 year age groups; among females, those over 45 years of age were most frequently infected. The overall rate of infection in men (60.4%) was significantly higher than among women (15.9%) and was directly related to the habit and frequency of eating raw fish. Similar results were reported by Yuan (270) in Jiangxi Province and Bai (10) in Jilin Province. In Guangdong, the prevalence and intensity of infection were highest in the age groups over 40 years of age; among children below four years old these rates were very low. In this same study, the overall prevalence was 21.5% in men and 11.1% in women in 22 counties/cities and the intensity of infection (mean EPG) was 2,648 and 1,234, respectively (36). On the other hand, in the endemic areas of Beijing, clonorchiasis mainly occurred in males less than 30 years of age owing to the fact that the young boys enjoy fishing in the streams, eating poorly cooked fish and their traditional habit of keeping a live fish in their mouths while fishing (218).

In Korea, raw freshwater fish is served as an appetizer when drinking
rice wine at social parties. Since women infrequently participate in such gatherings, they have much less exposure to the infection. Significant differences in age and sex prevalence rates were found in most areas along main rivers (8, 202). The rates among males were significantly higher and increased proportionally with age up to 59 years of age. The tendency to eat raw fish has greatly diminished among children because of effective health education (203). The intensity of infection (mean EPG) averaged 4,963 EPG of faeces in the overall population, but was highest among males, 6057 mean EPG of faeces, compared with females whose mean EPG was 2557. The highest mean EPG (5240-6454) was seen in the 30-59 year old age group (8). The distribution does vary between different localities as reported in highly endemic areas such as, Kimhae County, Korea (162) where the differences in prevalence between men and women was not large, 52.2% vs. 41.0% as well as in other localities (40, 202).

Komiya (96) reported that in Japan the prevalence of Clonorchis infection was higher among the older age groups with the peak between 30-50 years of age. The prevalence tended to decrease over age 50. Unlike other endemic areas, the difference in prevalence between the sexes was small in Japan.

4.3 Seasonal variation

The seasonal variation of both C. sinensis cercaria in the vector snail and metacercaria in fish has been noted by many investigators (96, 126, 217, 293) and related to higher summer water temperatures. In Liaoning Province, China, 4.8% of P. manchouricus, the main snail intermediate host, were infected in June, whereas by July the infection rate had risen to 13.2% and decreased to 2.6% and 1.9% in August and September. After October, no infected snails were found (113). Similar seasonal distribution was observed among populations of P. manchouricus and A. longicorns in Anhui Province. The infection rates of each of these two species during April to June, ranged between 0.58-1.51% and 1.05-15.45%, respectively. None of them were found to be infected after September (113). According to the dynamics of snail populations monitored by Zhu (293), Clonorchis emerged abundantly from July and reached its peak in August. In some areas of Asia the rainy season occurs in July and August. Human and animal excreta are easily washed into ponds and the snail infection rates reach their maximum levels during this period of time. Two or three months after the peak infection in the snail intermediate host, the peak level of cercarial shedding occurs.

In Jilin province (217), the infection rate in more than 10 species of freshwater fish was stable between April and June with an overall mean of 8.83±0.66% (8.33-9.59%). Between July and October the mean overall fish infection rate was 14.60±2.46% (12.05-17.91%), peaking in September (17.91%). Similar seasonal variations were observed among populations of P. parva and Abbottina in Liaoning (113). Further south in Shandong province, Zhu (293) reported that the peak prevalence of Clonorchis infection in fish was October - November. In the most frequently infected species, P. parva and other species as well, the intensity reached the
highest level in November. This suggests that the peak risk period for human infection is between September and November.

4.4 Familial aggregation

Familial aggregation means the cumulative frequency of disease in a family as an epidemiological unit. In most endemic foci it appears that Clonorchis infection is not random and there is a tendency towards familial aggregation. Komiya and Suzuki (96), investigating Clonorchis infection in a village of Miyage Prefecture, found familial aggregation of infection. However, in the highly endemic area like Kimhae, Korea, familial aggregation was not confirmed to be statistically significant until 1983 (162, 202).

5. Pathogenesis

When larvae of C. sinensis reach the biliary tract and mature, the flukes induce pathological changes both by mechanical injury caused by the suckers of the worm and interaction with its metabolic products (157, 207, 208). The eggs of C. sinensis have a lesser role in the pathogenesis of the disease. However, both eggs and disintegrated flukes serve as the nidus of stones in the bile ducts and gallbladder in persons with C. sinensis infection.

Immunohistochemical studies to determine the antigenicity of various organ structure of C. sinensis by Kim et al. (88) suggest that the excretory-secretory substances originating from the digestive and excretory organs, i.e., the intestine and bladder, are the most potent antigens.

6. Pathology

No significant new information on the histopathological response to C. sinensis infection has appeared since the classical study by Hou (Hou, P. C. The pathology of Clonorchis sinensis infestation of the liver. Journal of pathology and bacteriology, 70:53-64, 1955). However, through animal experiments, clinical studies and autopsies, as well as the observation of ultrastructural changes with electron microscope, our understanding on the disease has been improving. The appearances of pathological changes vary with the intensity and duration of the infection, susceptibility of the host, and co-existence of bacterial infections. The pathology has been classified into four different stages by Min based on the characteristics of human and experimental infection. They are: 1) desquamation of epithelial cells, 2) hyperplasia and desquamation of epithelial cells, 3) hyperplasia and desquamation of epithelial cells plus adenomatous tissue formation, and, 4) marked proliferation of the periductal connective tissue, with scattered abortive scini of epithelial cells and fibrosis of the bile duct wall (157).

6.1 Animal experiments

The pathogenesis of C. sinensis in laboratory animals, as assessed by
macro- and microscopic alterations of the hepatobiliary system, suggests that the guinea pig is the most susceptible animal. Light *C. sinensis* infections usually do not cause serious injury whereas heavy worm burdens cause severe damage of the liver, biliary tract and pancreas. The dilatation, thickening and adenomatous hyperplasia in the bile ducts occur to varying degrees in different animals. Macroscopically, the dilated bile ducts are filled with bile and contain various number of worms and eggs. Microscopically, inflammatory cellular infiltration consisting of lymphocytes, plasma cells, histiocytes and fibroblasts, and hyperplasia of epithelial cells are observed (178). Goblet cell metaplasia was found in infected rabbits two months after the infection (107). Small eosinophilic abscess and focal hepatic cell necrosis may be present, but the hepatic lobular structure is intact (131). The flukes ingest blood as a nutrient in the bile ducts of the host and anemia ensues in heavy infections (90). In heavily infected rabbits, duodenal changes including congestion of the mucosa and exudative inflammation with or without the adult flukes in the lumen, and acute interstitial pancreatitis and dilatation and infiltration of the pancreatic ducts were seen (131). In a less susceptible reservoir host of *C. sinensis* such as the rhesus monkey, *Macaca mulatta*, there is extensive eosinophilic infiltration in the portal areas. In the acute stage, i.e., 30 days after infection, plasma cells may be observed in the hepatic sinusoids. The chronic stage is associated with enlargement of intrahepatic bile ducts and adenomatus proliferation of epithelial cells with predominant plasma cell infiltration and fibroblasts and a few eosinophils in the periductal areas (102).

Ultrastructural changes in the bile ducts of *C. sinensis*-infected guinea pigs under transmission electron microscopy include proliferation of epithelial cells of the bile ducts resulting in pseudostratification with proliferation of the surrounding connective tissue. The microvilli of the ducts are irregular, oedematous and fused. The straightening of cell borders, detachment of cytoplasmic processes and fibroblastic processes between adjacent epithelial cells were noted. Changes in the nuclei and the endoplasmic reticulum and mitochondria in the cytoplasm of the epithelial cells are observed (114).

The ultrastructure of the biliary tract in *C. sinensis*-infected rabbits was reported (100). At sixty days after the infection, epithelial changes were noted. At 120 days after the infection, hyperplasia and hypertrophy of the ductal epithelium and deformity, disappearance or irregular arrangement of microvilli with oedema were marked. At 180 days after the infection, apart from the above mentioned changes, abnormal basement membranes, increased cytoplasmic fibrils, and metaplasia of the goblet cells were seen. The intracellular space was dilated and epithelial cells were stratified (100).

6.2 Human pathology

6.2.1 Liver

The pathology of the liver has been described from laparotomy, biopsy
and autopsy materials. The main pathological changes occurring in man are similar to those in experimental infections. Grossly, the liver is usually enlarged. The surface of the liver was altered in most cases and described as uneven, cystic, nodular or fibrotic according to stage and intensity of infection. Cystic areas were seen in proximity to the thickened bile passages. Under Glisson's capsule, dilated branching or clubbed bile ducts were observed (275). On cut section, the biliary tracts were dilated to two or three times their normal diameters and that the bile duct walls were several times thicker than normal. Fibrosis of the periportal and peribiliary spaces was observed (157). In a heavily infected female patient with 154,500 EPG of faeces by Stoll technique, the cut surface of the liver was filled with adherent, bloody mucus with numerous adult flukes at autopsy (275). A pseudo-lobular structure surrounded by fibrous connective tissue with proliferation of the biliary ducts was reported in another case at autopsy (159).

Hepatic cellular injury is not caused by C. sinensis infection per se. The liver structure is basically preserved and liver cirrhosis is rarely seen in C. sinensis infected persons. Superimposed bacterial infections, usually ascending cholangitis due to Escherichia coli, may result in liver abscesses. Thus, a postnecrotic or biliary cirrhosis may develop (128, 208).

It is generally accepted that the pathological changes are usually more significant in the left lobe than in the right lobe of the liver in C. sinensis infection (230). The relationship between hepatomegaly and the anatomical features of right and left hepatic ducts was studied by Yao et al. (264) in 33 autopsies of diseases other than hepatobiliary disease by measuring the 1) length of the hepatic ducts, 2) outer diameters of the ducts, and 3) angle (in degrees) between right and left hepatic ducts with the common bile duct. Compared with the right duct, the left hepatic duct was straight, the diameter was larger, and had a smaller angle with the common duct. These observations may explain why the flukes tend to enter the left hepatic duct rather than the right (278).

In light infections, the flukes tend to locate in the distal intrahepatic ducts, whereas the common bile duct and gallbladder are involved usually only in heavy infections or at a late stage of the infection showing dilatation, proliferation and desquamation, periductal fibrosis, cholangitis and enlargement or oedema of the gallbladder. The gallbladder is not a preferred location due to the concentrated bile. In untreated infections, the pathological changes are progressive and the liver damage depends on the intensity and duration of the infection.

6.2.2 Pancreas

Adult fluke invasion into the pancreatic ducts occurs most frequently in heavy infections, but the pathological changes are usually less extensive than those in the bile ducts. The pancreas is usually not enlarged but firm in consistency (133). The flukes reside in the pancreatic duct, within the main pancreatic duct of the tail portion, and
within its tributary ducts. The changes are similar to the hepatic lesions, namely, adenomatous hyperplasia of ductal epithelium, and sometimes, squamous metaplasia. Dilatation of the pancreatic ducts, cell infiltration in the periblobular areas and fibrosis of the interstitial tissue were seen at autopsy (275). When bacterial infection supervenes, acute inflammation of the pancreas is noted (208).

6.2.3 Spleen

Congestion of the spleen is seen in the early phase of the infection. In chronic infection with hepatic changes, the spleen may be enlarged and shows an increase of fibrous connective tissue especially in the red pulp (159).

7. Clinical presentations

7.1 Incubation period

The prepatent period, the period between ingestion of metacercariae and the presence of C. sinensis eggs in the faeces, varies according to different definitive hosts. In man, about four weeks are necessary for the flukes to attain sexual maturity and the appearance of the eggs in stool (178). Since the onset of clinical symptoms is insidious, and about one-third of the infected persons are asymptomatic (239), the incubation period, the period between ingestion of the metacercariae and the onset of symptoms, cited in the literature varies considerably. According to Xu et al. (Xu, Z. P. et al. Acute clonorchiasis, report of two cases. Chinese medical journal, 92:423-426, 1979), the acute symptoms of the disease occurred 10 and 26 days respectively in two patients after eating raw freshwater fish infected with C. sinensis. More recently acute symptoms - high fever and intestinal complaints - occurred 40 days after eating raw freshwater fish (78).

7.2 Acute phase

The acute disease has been reported by several investigators (27, 78, 196, 231). Chills and fever up to 40°C occur before and associated with pain in the right upper quadrant with loss of appetite, generalized abdominal pains and abdominal distension are common. Occasionally transient urticaria, jaundice, even ascites can be observed. On physical examination, the liver is usually enlarged and tender, and in some patients, a slightly enlarged spleen can be palpated (178, 196, 230). Two instances of a leukemoid haematological reaction in the acute stage have been reported. In one case the peripheral leukocyte count was 4.2x10⁹/L with 20% immature leukocytes (231), and in the other, the peripheral leukocyte count was 15.8x10⁹/L with 86% eosinophils (78). A bone marrow biopsy in the latter showed a proliferation in the granulocytic series. After treatment with praziquantel, clinical symptoms disappeared and the peripheral blood counts returned to normal.
7.3 Chronic phase

About one third of the chronic *C. sinensis* infections are asymptomatic whereas eggs are present in stool or duodenal fluid (17, 239). Generally the severity of chronic disease is related to intensity and duration of the infection. However, nutritional status and intercurrent infection have an important role in its natural history.

Usually lightly infected persons are asymptomatic in the early stage of the infection. In moderate to long duration of the infection, the symptoms are evident only by careful clinical questioning. Gradual onset of discomfort in the right upper quadrant, anorexia, indigestion, abdominal pain or distension and irregular bowel movements are the usual complaints. In heavily infected persons, symptoms are significant including weakness, dizziness, weight loss, epigastric discomfort, abdominal fullness, diarrhoea, anaemia and oedema. The clinical features in some cases are progressive in severity and may persist for years. In severe cases or in the late stages of heavy infection, jaundice, portal hypertension, ascites and upper gastrointestinal bleeding may occur (48, 181, 230). However, the role of intercurrent infection such as hepatitis B was not assessed.

It is noteworthy that some persons have predominantly neuropsychiatric symptoms, such as, insomnia, vertigo, paresthesia, hypomnesia, mental depression, palpitation of the heart, etc. These symptoms in association with the infection were proven by their disappearance after effective treatment with praziquantel (25).

Repeated or heavy infection during childhood has been reported to cause dwarfism. Physical growth and sexual development are retarded. Zhu et al. (291) reported that 34 (40.6%) out of 85 children with clonorchiasis below 15 years of age among a total of 2214 patients who were hospitalized for treatment of clonorchiasis showed growth retardation. As compared with normal children, their weight and/or height was lightly (a decrease of <10%), moderately (a decrease of 10-20%) and heavily affected (a decrease of >20%) in 6.7%, 36.7% and 56.6% of them, respectively. Proportionally short and thin stature, lack of secondary sexual characteristics, and amenorrhoea in women were recorded (239). All these growth retard persons are mentally alert and it has been proposed (263), but not proved, that their clinical state is related to a specific pituitary disfunction with inadequate secretion of gonadotropin in response to *C. sinensis* infection, similar to that documented in dwarfism due to schistosomiasis.

On physical examination, the entire liver is usually enlarged with predominance of left lobe enlargement. The liver is smooth and firm, with or without tenderness, during the chronic stage. The hardness of the liver increases as the disease advances. In severe cases, the surface may be nodular or irregular due to fibrosis of the liver or complications. A slightly enlarged spleen is palpated in a small proportion of infected persons and is probably due to portal hypertension. Ascites may be seen in the late stage of clonorchiasis.
In a clinical study of 3769 patients hospitalized due to clonorchiasis from several counties of Guangdong Province, China by Weng et al. (239), there were 2374 males and 1395 females. Of these, 1289 (34.2%) were asymptomatic. Among those that were symptomatic, main symptoms were tiredness (26.3%), upper abdominal discomfort (24.1%), abdominal pain (23.1%) and dizziness (19.3%), whereas loss of appetite, insomnia, headache, diarrhoea, constipation, hypomnesia, etc. were less frequent complaints. Hepatomegaly was palpated in 1961 (52.0%) persons and splenomegaly in only 28 (1.2%) persons on physical examination. The clinical manifestations were classified in seven groups including the asymptomatic group: hepatitis type in 1514 cases (40.2%), indigestion in 631 cases (16.7%), cholangiocystitis in 234 cases (6.2%), neurosis in 78 cases (2.1%), hepatic cirrhosis in 21 cases (0.6%), dwarfism in two cases. Those classified as "hepatitis" did not have confirmatory laboratory tests.

In another series of 752 hospital patients reported by Liu et al. (146) also from Guangdong Province, 24.6% (185 cases) were asymptomatic and 59.6% (448 cases) had hepatomegaly.

7.4 Laboratory findings

7.4.1 Haematology

An increase in the concentration of blood leukocytes and eosinophils is seen in acute infections. In chronically infected persons, the mean eosinophil counts increased in parallel with the intensity of infection. The eosinophil counts were 197.8±229.5, 658.7±570.4, 951.0±808.0 and 2881.0±1626.0 per mm³ in four groups of 50 patients with the light (1-999 EPG), moderate (1000-9999 EPG), heavy (10,000-29,999 EPG) and very heavy (over 30,000 EPG) infections respectively (83). In another series of 369 children with clonorchiasis in Pingdu County, Shandong Province, China, 86.3% had a high eosinophil count, and in 60.6% the leucocyte count was above 10×10⁹/L (67). Red blood cell count was normal in 77.6% of 406 patients examined as reported by Liu et al. (146) in China. Kim et al. (91) examined 287 clonorchiasis patients in several endemic areas in South Korea. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were all within normal limits. By using chromium radioisotope, ⁵¹Cr, Kim et al. (90) showed that the adult flukes ingested blood in the bile duct of rabbit hosts and utilized it as a nutrient. This explains that in heavy infection, anaemia may be one of the effects of C. sinensis infection. In acute infection, anaemia and increased erythrocyte sedimentation rate (151 mm/hr) were reported in a single patient (231).

7.4.2 Liver function tests

In acute clonorchiasis, serum alanine transaminase (ALT) is usually significantly elevated, and a decrease of serum albumin and increase of globulin concentration are observed. During the chronic stage, liver function usually remains within normal limits since liver cell damage is not significant. Blood biochemistry variables were analyzed systematically.
among 287 hospitalized Koreans with clonorchiasis according to the intensity of infection by Kim et al. (91). ALT and aspartate aminotransferase (AST) were relatively normal except in the heavily infected group, of whom, 22.2% had slightly elevated ALT and 33% had slightly elevated AST. Alkaline phosphatase was occasionally slightly above normal levels. Other liver function tests, such as 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 the infection. On electrophoresis, gamma globulin levels were increased in 66.7% of 21 cases (146). In clonorchiasis in children, an slight increase in ALT activity was reported in 66.2% of 151 hospitalized children and a slightly increased icterus index was recorded in 27.1% (67). In another hospital series, 17 (81.0%) out of 21 children with clonorchiasis had increased ALT, and after chemotherapy, the enzyme activity in 15 of 17 children (88.2%) returned to normal (291).

7.4.3 Immunoglobulins

Antibody response to infection with C. sinensis was studied in cats and rabbits (20, 265). Elevated serum IgM antibody was shown in cats seven days after the infection, followed by elevation of IgA and IgG (20). In patients with chronic infection, serum IgA was normal, whereas IgG and IgM were still elevated (139). Serum total IgE in clonorchiasis patients was several times higher than normal persons, and Clonorchis-specific IgE was detected in 44.8% of 38 clonorchiasis patients, but none was detected in normal controls (289). A close correlation between the serum IgE level and specific IgE to C. sinensis in individuals with the infection (coefficient of correlation r=0.945) and a little correlation between serum IgE and EPG (r=0.606) and between specific IgE and EPG (r=0.569) were observed by Min et al. (156).

7.5 Radiography, ultrasonography and computed tomography

7.5.1 Radiography

Plain abdominal radiography may show gallstones in the biliary tract. Retrograde endoscopic cholangiopancreatography (ERCP) was claimed to be a useful means to find pathological changes in the biliary passage, although other types of cholangiography are also used, such as drip infusion cholangiography, percutaneous transhepatic cholangiography and postoperative cholangiography. However, the role of cholangiography has decreased after ultrasonography and computed tomography (CT) have been introduced in the diagnosis of hepatobiliary diseases.

Multiple uniform small irregular filling defects of several mm in diameter, a size compatible to the size of the fluke, are observed (42, 48). The defects are described as linear, round, filamentous, rice-like, oval or elliptic in shape, and bile duct wall appears scalloped. The defects occur mostly within small intrahepatic bile ducts and their peripheral branches, whereas the extrahepatic bile ducts are normal or only slightly dilated. Short segmental stenosis of bile duct may also be
observed (132). Significant dilation of the second and third distal branches of the bile ducts and dilation and blunting of the terminal branches of the biliary tree are common. Clusters of mature flukes may produce radiolucent defects in the common hepatic duct (205).

ERCP in 52 persons with _C. sinensis_ infection in Hong Kong showed filamentous or oval filling defects in 36 (69.2%), blunting of the terminal branches or interruptions in the bile ducts in 24 (46.2%), irregularity of the lumen of the bile ducts, or pancreatic ducts in 29 (55.8%), and dilated small ducts in 11 (21.1%) (242). An _in vitro_ observation on radiographic appearances of the flukes was reported by Leung et al. (112). In the study, fresh adult _C. sinensis_ harvested from a patient through nasobiliary catheter drainage and the flukes were put in silastic tubes filled with contrast used for routine ERCP. The _in vitro_ appearances of the fluke with radiography were: filamentous, wavy, curled up, elliptical wavy and elliptical shaped filling defects. Filamentous wavy and/or elliptical appearance of the fluke is considered to be a pathognomonic finding on ERCP examination (112). Three patients with proven clonorchiasis of the pancreas were examined with ERCP. Diffuse irregular dilatation of tributaries and main duct in the tail of the pancreas was shown, but the main pancreatic duct and tributaries in the body and head were normal (133).

7.5.2 Ultrasonography

Ultrasonography is a useful and simple technique to assess disease of the hepatobiliary tract, to identify the flukes in the bile ducts, and to evaluate the effect of specific treatment. It is now considered a first line clinical diagnostic procedure for clonorchiasis.

Ultrasound features of biliary clonorchiasis was reported by Morikawa et al. (158). In an asymptomatic woman with _C. sinensis_ infection, ultrasonography revealed a slight dilatation of the common bile duct and gross dilatation of intrahepatic bile ducts with numerous spotty echoes and thin homogeneous linear echoes ranging from several mm to 1 cm. The walls of the dilated bile ducts showed thickening and highly echogenic, whereas the common bile duct showed only mild dilatation and slightly thickened walls. The movement of the flukes in bile ducts was clearly apparent by simultaneous use of an M-mode sonogram. The adult flukes in the gallbladder are demonstrated by the finding of floating or dependent, discrete, echogenic foci in the lumen (134). The effectiveness of treatment was assessed by the cessation of movement of the flukes and a reduction in the number of small echogenic areas in the bile ducts four days after treatment with praziquantel, although the thickness or dilatation of the bile ducts remained unchanged. Dilatation of the peripheral intrahepatic bile ducts with increased echogenicity of the duct walls in the absence of dilatation of the extrahepatic bile ducts by ultrasonography, is highly suggestive of a diagnosis of clonorchiasis (132, 134).

Biliary stones can be identified by ultrasound. Clonorchiasis with
only flukes in the intrahepatic bile ducts can be misdiagnosed as intrahepatic biliary calculi by ultrasound (288). An ultrasound survey for gallbladder disease among 947 Hakkanese people with C. sinensis infections in Taiwan was reported by Hou et al. (74). 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%. The difference between C. sinensis-infected and non-infected groups was not statistically significant and the authors considered that there was no causal relationship between C. sinensis infection and gallstone among the subjects investigated. These observations are a rare exception to the weight of the literature which concludes that there is a definite association between clonorchiasis and biliary stones, particularly common duct and intrahepatic stones (181).

7.5.3 Computed tomography (CT)

CT detects the same pathologic processes as cholangiography and ultrasonography, i.e., the flukes within the dilated bile ducts and periductal changes. However, the flukes per se within the bile ducts are usually difficult to identify by CT scan (38), although innumerable tiny dense foci within dilated ducts are suggestive of aggregates of the flukes (132). Choi et al. (38) analyzed CT images in 42 persons with clonorchiasis; 17 had clonorchiasis alone and 25 had clonorchiasis and hepatobiliary malignancies. Among the 17 persons with clonorchiasis alone, 16 (88.4%) showed diffuse, minimal, or mild dilatation of the intrahepatic bile duct whereas in three, the CT scans were normal. None had thickening of bile duct wall on CT scan. All 25 persons with clonorchiasis and hepatobiliary cancer had diffuse dilatation of the extrahepatic bile ducts on CT. The authors suggested that uniform dilatation of the biliary tree from the common bile ducts in the porta hepatis to the ducts in the periphery of the liver is typical of clonorchiasis. Furthermore, the diagnosis of clonorchiasis can be suspected when CT shows diffuse, uniform, and minimal or mild dilatation of the intrahepatic bile ducts, particularly distally, without evidence of extrahepatic biliary dilatation (38, 39).

7.6 Complications

7.6.1 Secondary bacterial Infections of the hepatobiliary system

The flukes in the biliary ducts can cause obstruction and bile retention with consequent histopathological damage of the biliary tract. This creates optimal conditions for secondary bacterial infection, most frequently due to E. coli. Pyogenic cholangitis and cholecystitis and their association are common complications of C. sinensis infection, and their geographical distribution is similar to that of clonorchiasis (132). In a clinical study of 2214 patients with C. sinensis infection in Guangzhou, China during 1960-1979, Zhu et al. (290) reported that there were 381 (17.2%) with cholecystitis of which 168 (7.6%) were acute cases and 213 (9.6%) were chronic cases. Although this hospital data is biased towards severely ill persons, the rates of cholecystitis in the control group from the same hospitals were statistically significantly lower, i.e.,
only 0.82% of 15,389 persons without clonorchiasis, confirming the association with clonorchiasis.

The association between *C. sinensis* infection and cholangitis, cholecystitis has frequently been reported (15, 41, 42, 67, 70, 84, 85, 136, 167, 205, 207, 239, 275, 290) from Chinese, Korean, and Asian immigrants outside to the endemic areas. Animal experiments have confirmed this association (114, 131). However, when bacteria are induced into the bile ducts of infected experimental animals, the flukes either died or their growth was retarded. Bacterial cholangitis in persons infected with *C. sinensis* is associated with a significant reduction of faecal egg count. This may be due to death and reduced fertility of *C. sinensis* (181). In patients with both clonorchiasis and biliary tract infection, treatment of *C. sinensis* alone sometimes gives complete cure. Generally, concomitant antibiotic treatment is required. For those with biliary obstruction caused by the flukes who do not respond to medical treatment, surgical intervention is required.

7.6.2 Cholelithiasis

Biliary stones in clonorchiasis are formed due to bile stagnation caused by mechanical obstruction and changes in bile constituents. There is a consensus in favour of an association between *C. sinensis* infection and cholelithiasis (64, 136, 178, 181, 290). Gao et al. studied the changes in the concentration of bilirubin, cholesterol, phospholipid, bile acid and the activity of bacterial β-glucuronidase in bile of rabbits infected with *C. sinensis* metacercariae. They suggested that an increase in bacterial β-glucuronidase activity and glycoprotein in bile is favorable to the formation of pigment stones, and this may explain the higher rates of cholelithiasis in clonorchiasis patients (64). In South Korea, a series of studies reviewed by Rim (181) showed that *C. sinensis* infection was a main cause of cholelithiasis, choledocholithiasis and intrahepatic stones. In Guangzhou, China, 93 (4.2%) of 2214 hospital patients had gallstones whereas among 15,389 cases with diseases other than clonorchiasis only 0.3% showed stones in the biliary tract (290). However, a population-based survey in southern Taiwan showed that the prevalences of gallstones in persons with and without clonorchiasis were 9.4% of 947 and 5.6% of 144, respectively (74).

Bilirubin stones are the most common, in contrast to the cholesterol stones in gallbladder disease without clonorchiasis. In patients with recurrent pyogenic cholangitis, the stones are usually muddy and clay-like, and are often multiple, packing the intra- and extrahepatic bile ducts with casts (132). Gallstones in eight out of 11 autopsy cases with *C. sinensis* infection had eggs inside as nidus of the stones (290). When multiple intrahepatic stones are found, few or no flukes (181) or many flukes (79) have been reported.

7.6.3 Pancreatitis

An association between clonorchiasis and pancreatitis has long been
suggested. Although symptoms of pancreatitis are not frequently seen in *C. sinensis* infections, pancreatic involvement in clonorchiasis is not rare, especially in those with heavy infections. The flukes are usually found in the main and branch ducts of the pancreatic tail, and the ducts may be dilated and packed with the flukes (132). Remarkable adenomatous hyperplasia in the mucosa of the ducts and inflammatory cellular infiltration in periportal area of the pancreas with interlobular fibrosis were reported in a severely infected cases at autopsy with the diagnosis of pyogenic cholangitis, cholecystitis, pancreatitis and malabsorption syndrome of the small intestine (275). The main pancreatic duct and tributaries draining into the body and head portion may not be dilated (133). When pancreas is parasitized by the flukes, concomitant hepatobiliary disease is always present. In *C. sinensis* endemic areas, patients with acute pancreatitis are usually screened for this infection (41). Only one case of adenocarcinoma of the pancreas associated with *C. sinensis* infection has been reported recently (45).

7.6.4 Liver cirrhosis

There is no consensus in the literature on the possible association between clonorchiasis and cirrhosis. In some instances the diagnosis is clinical or not supported by a clear description of the pathology. In Zhu et al.‘s series (290), 2214 hospitalized persons with clonorchiasis, 128 (5.8%) had a clinical diagnosis of liver cirrhosis compared to 96 (0.6%) of 15,389 in the control group. However, in another series from the same area of 3769 infected persons, only 21 (0.6%) had liver cirrhosis (239). In some infected persons, malnutrition as a sequela to heavy worm burden may be a factor in the development of cirrhosis (290). In experimentally infected guinea pigs with an average worm burden of 240 (56-690) per animal, acute damage of the hepatic lobules was seen with disintegration of the lobular structure, fibrotic proliferation of perilobular interstitium and ascites, in addition to adenomatous proliferation of bile duct epithelia (290). In a few case reports of chronic human clonorchiasis, a hard palpable liver with an enlarged spleen, oesophagogastric varices, abdominal collateral vein dilatation, jaundice, or ascites are seen in the cirrhotic patients with haematemesis and melaena (159, 207). The cirrhosis is postnecrotic or biliary in nature, and unlike schistosomiasis, "clay pipe stem" fibrosis is never seen in uncomplicated cases (208).

7.6.5 Cholangiocarcinoma

*C. sinensis* infection and cholangiocarcinoma are causally related. Apart from earlier literature, many reviews and case reports have been published during the past 13 years since 1980 (57, 93, 94, 132, 136, 178, 181, 189, 190, 208, 246, 247, 290). However, hepatocellular carcinoma, has been found only occasionally associated with *C. sinensis* infection (189). The extensive epidemiological data confirming the association between clonorchiasis and cholangiocarcinoma has been almost unanimous. However, the mechanism of carcinogenesis is still controversial.

The data in favour of the association between cholangiocarcinoma and
clonorchiasis are as follows:

(a) Geographical correlation: Cholangiocarcinoma is more prevalent in the Far East, particularly in China, where clonorchiasis is endemic than in the non-endemic areas such as Europe and North America (189). In Guangdong, the most heavily endemic province for *C. sinensis* in the mainland of China, a high correlation between the prevalence of clonorchiasis and incidence of primary liver cancer (which kind of liver cancer was not mentioned by the authors) (246). The prevalence rate of clonorchiasis is significantly higher in carcinomatous liver disease than in the non-cancerous group (93). Furthermore, prevalence rate of cholangiocarcinoma is significantly higher in population of *C. sinensis* endemic areas than in that of non-endemic areas (136, 208). In the future it will be important to determine in which countries clonorchiasis overlaps the distribution of opisthorchiasis, another cause of cholangiocarcinoma.

(b) Original tumour site: In *C. sinensis* infection, cholangiocarcinoma arises from metaplastic change of epithelial cells (57) and usually occurs in the secondary intrahepatic bile ducts where the flukes are preferentially situated (136). On the other hand, in Asia, the majority of liver cancers are hepatocellular in origin.

(c) Histopathology: Most of the investigators have endorsed a causal relationship between clonorchiasis and cholangiocarcinoma, whereas the correlation with hepatocellular carcinoma is absent. In South Korea, the ratio of hepatocellular carcinoma to cholangiocarcinoma in a heavily endemic area (Pusan) (10:1) was significantly different from that in a lightly endemic area (Seoul) (4:1), and a higher association of clonorchiasis with cholangiocarcinoma than with hepatocellular cancer was indicated (181). Similar data could be traced from Hong Kong in the early literature. Cholangiocarcinoma is an adenocarcinoma, and extensive mucinogen infiltration and squamous metaplasia are often found at the tumour sites which is considered to be sequelae of *C. sinensis* parasitism (189). A multicentric origin of cholangiocarcinoma in clonorchiasis coincides with the multiple sites of parasitism of *C. sinensis* in the bile ducts (93).

(d) Animal experiments: Descriptions of experimentally induced cholangiocarcinoma in *C. sinensis* infected animals were not found in the recent literature. Experimental infection inducing cholangiocarcinoma in dogs and cats was described by Hou in the 1960s (Hou, P. C. Primary carcinoma of bile duct of the liver of the cat infected with Clonorchis sinensis. *Journal of pathology and bacteriology*, 1964; 87:239-244. and Hou, P. C. Hepatic clonorchiasis and carcinoma of the bile duct in a dog. *Journal of pathology and bacteriology*, 1965; 89:365-367.). The structure and development of these carcinomas were identical with clonorchiasis related tumours in man.

Although most published studies have endorsed a positive correlation between *C. sinensis* and cholangiocarcinoma, the pathogenesis of cholangiocarcinoma is still inconclusive, and the explanations of its
mechanisms are partial, at best. Adult worm extract of \textit{C. sinensis} showed no mutagenicity in an Ames bacterial assay (77). The flukes \textit{per se} do not provide the sole carcinogenic stimulus leading to malignancy as millions of persons harbouring \textit{C. sinensis} in their bile ducts never develop bile duct tumour, and cholangiocarcinoma arises in only a relatively small percentage. The peak age for cholangiocarcinoma in long-standing cases of \textit{C. sinensis} occurs between fifth and seventh decades (57). A severe, chronic infection may produce adenomatous hyperplasia and dysplasia of epithelial cells and goblet cell metaplasia in the bile duct which is considered to be the predecessor of cholangiocarcinoma (93). It is most probable that the aetiology of cholangiocarcinoma is multifactorial in origin (57, 157, 181, 208). The fluke itself may be only weakly carcinogenic and acts synergistically with other factors in the induction of the carcinoma (189). The contributing factors promoting cholangiocarcinoma listed by some investigators are: ingestion of exogenous carcinogens or co-carcinogens, and endogenous influences such as malnutrition, immune defects and genetic factors (57, 93, 181, 189).

Up till now, all the epidemiological or clinico-pathological data showing the association of these two diseases have been from retrospective surveys in a limited population. Further large scale, population-based field surveys and prospective epidemiological investigations in areas endemic for the disease in association with laboratory studies will help to explain the aetiology of the cancer in man. With the available effective treatment, praziquantel, control programmes can be successfully implemented in some areas. As the incidence and prevalence of clonorchiasis decreases, the incidence of cholangiocarcinoma in endemic areas should regress, but the decrease may not be observed until many years later.

Three patients with another type of cholangiocarcinoma, mucoepidermoid carcinoma, of the extrahepatic bile duct were reported in association with \textit{C. sinensis} infection from Hong Kong. The clinical and pathological appearances of this rare type of tumour were similar to the usual biliary adenocarcinoma. The co-existence of the fluke infection suggested the possibility of an aetiological association (98).

8. Diagnosis

8.1 Parasitological examinations

Definitive diagnosis of \textit{C. sinensis} infection is based on the finding of its eggs either in faeces or duodenal fluid, or the adult flukes during laparotomy and parasitological examination remains the "golden standard". Examination of duodenal drainage fluid for the eggs is very sensitive but inconvenient and not easily to be accepted by patients.

No significant progress has been made in the past decade on parasitological diagnosis and the major faecal examination techniques currently being used in endemic areas are the formaldehyde ether concentration technique (Ritchie technique), Kato faecal thick smear
technique and Stoll dilution technique. The egg positive rates obtained from different faecal examination techniques vary considerably (125, 142, 227). Single examinations by current techniques have a high false negative rate, especially in persons with the light infections or those with a history of treatment. Repeated stool examinations are necessary to improve the sensitivity of the examination. Ma (153) evaluated the sensitivity of a precipitation technique. The study group was 661 villagers of whom 210 persons had eggs in the stool at least once in 12 stool samples from six specimens (2 samples from each specimen). Two examinations from one specimen could only detect 45.7%; and four, six, eight or ten examinations from two, three, four or five specimens detected 69.0%, 79.0%, 87.6% and 94.8%, respectively. While repeated examinations increase sensitivity, repeated stool collection is difficult in practice.

8.1.1 Formaldehyde ether concentration technique

Though the sediment is small in quantity, the eggs are easily observed microscopically. This technique is considered to be quite sensitive (155), and intensity of the infection can also be estimated. A single examination has been claimed to detect about 90% of persons who are actually excreting eggs (230). This technique is 10 times more sensitive than the direct fecal smear technique (142) and twice as sensitive as the simple sedimentation technique (54). Using this technique with three faecal specimens to evaluate cure four months after praziquantel treatment, 52 persons were found to have the low levels of egg excretion. Among them, 55.8%, 84.6% and 100% were positive according to one, two or three examinations, respectively (25). This sensitive technique is a technically complicated procedure and requires volatile materials.

8.1.2 Kato faecal thick smear technique

This quantitative technique has been widely used in field surveys in China and Korea (13, 181, 193, 249, 286) because of its simplicity, low cost, reproducibility of results between investigators. Its sensitivity to detect C. sinensis eggs is not significantly lower than the formaldehyde ether technique (142, 249). A modified Kato technique was developed by Cai et al. (13) in which a fine screen of 260 meshes per inch was used. Only small eggs like C. sinensis eggs and fine debris could pass through the mesh, making it easier to detect the eggs. The calculated numbers of EPG from the same stool sample were much higher than those obtained by the formaldehyde ether technique (13) and the merthiolate-iodine-formaldehyde concentration (MIFC) technique (286). However, the identification of C. sinensis eggs in the Kato thick smear requires training to distinguish transparent or altered eggs.

8.1.3 Stoll's dilution egg counting technique

This technique uses sodium hydroxide which facilitates identification of the eggs. The detection rate is higher than the direct smear technique and the zinc sulfate floating technique (125, 131), but not as high as formaldehyde-ether concentration and modified Kato technique (209). Since
it is simple, cheap and reproducible, it is considered to be suitable for field work (29, 182, 259). It is also used to evaluate the intensity of C. sinensis infection (155).

8.1.4 Duodenal drainage

Examination of duodenal drainage for C. sinensis eggs has a sensitivity approaching 100% (230). Since the procedure is not as simple as other techniques, it is less frequently used and mainly in the hospital. The Entero test (1) using a cotton string fixed to a very light weight enclosed in a capsule, has been used to detect C. sinensis eggs after swallowing the capsule and examination of the material sticking to the string after three hours. The sensitivity of this techniques was significantly higher than the Kato technique (P<0.05) (251). The main limitation of this simple technique is that it can only be used for hospitalized patients.

8.1.5 Egg identification

There are several species of trematodes that can parasitize man and the morphology of their eggs are similar to the eggs of C. sinensis. These trematodes belong to Opisthorchidae and Heterophyidae. Xu (248) described the identification of these eggs. By measuring the sizes of a number of the eggs, it is comparatively easier to differentiate C. sinensis eggs from those of Centrocestus formosanus (eggs with larger size) and Procerovum and Stellantchasmus falcatus (eggs with smaller sizes). However, as for Metorchis orientalis, Haplorchis spp., Heterophyes heterophyes and Metagonimus yokogawai, the differentiation is difficult based only on the measurement of the size of the eggs. According to the width and height of operculum, significance of shoulder-like rim of the narrow anterior end of the shell, he divided the eggs into nine types. Most of the C. sinensis eggs belong to types 1-3, most of the M. orientalis and M. yokogawai eggs belong to types 2 and 3, and most eggs of H. heterophyes and Haplorchis belong to types 3-5 and 4 and 5, respectively. When the eggs under microscope are mostly types 1-3, C. sinensis should be considered. When they are mainly types 4 and 5, most probably these eggs are produced by Haplorchis. The differentiation of C. sinensis from Opisthorchis viverrini and O. felineus was described (135). Since under normal conditions C. sinensis eggs do not enter blood vessels, any eggs similar to C. sinensis observed in tissues and organs other than those in the hepatobiliary system, should be first considered to be Heterophyidae eggs. A comparative morphological study between eggs of Heterophyidae and C. sinensis in Korea was conducted by Lee et al. (108) who concluded that in spite of some similar morphological characteristics of Heterophyidae and C. sinensis 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 remember that immature Clonorchis eggs may not be distinguishable from Metagonimus eggs (Kammerer, W.S., Van Der Decker, J.D., Keith, T.B. & Mott, K.E. Clonorchiasis in New York City Chinese. Tropical Doctor 7: 105-106, 1977).
8.2 Immunological tests

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

8.2.1 Intradermal tests

Adult *C. sinensis* crude antigen used intradermally is highly sensitive among persons known to be excreting *C. sinensis* eggs (4, 181). The coincidence rate between fecal egg examination and intradermal test was as high as 91.8% and no cross reaction was observed with nematode infections, i.e., *Ascaris*, hookworm and whipworm infections (4). However, persons with paragonimiasis and schistosomiasis were reactive (21, 181, 211, 218, 259). Cao *et al.* (14) used the same skin test antigen at three different sites, i.e., the flexor and the extensor surface of the forearm, and the interscapular region on the back. There was no significant difference between the reactivity rates at the different sites. However, the faecal egg positive rate was much higher in persons with positive intradermal reactions in all three sites than in those with a positive reaction in only one or two sites. No eggs were detected in those who were negative at the three skin sites. In other studies (124), the reactivity rate among those excreting eggs was significantly higher with purified adult worm antigen than with a crude antigen. The false positive rate was lower with the purified antigen (precipitated with ammonium sulfate); the molecular weight of the main band was 27.8 kDa (124). Seven years after treatment, among 34 persons without detectable eggs in the stool, the intradermal test became negative in 79.4% (14).

8.2.2 Serological tests

8.2.2.1 Antibody detection

i. Enzyme-linked immunosorbent assay (ELISA)

ELISA has been widely used since its introduction to China and Korea in the late 1970s. This technique has the advantages of higher sensitivity and specificity, ease of use and reading and convenience for field work. It may be the immunological test of choice in the field surveys for *C. sinensis*. It has recently been endorsed that ELISA is the most appropriate immunodiagnostic test for the disease (50).

Among those with *C. sinensis* eggs in their stool, the positive rates by ELISA ranged between 78.7-95.5% in various studies (18, 19, 61, 63, 138, 151, 182, 219, 226, 256, 282). The sensitivity of ELISA was reported to be higher than that of indirect haemagglutination test (IHA), counter-immunoelectrophoresis (CIEP) (282), complement fixation test (CFT) (65, 282), and indirect fluorescent antibody test (IFAT) (65). The antibody titer detectable by ELISA was higher than CFT and CIEP (23, 24). The seroreactivity rate was higher using fresh serum samples compared with
serum samples prepared more than six months previously (258, 282). The false positive rate in healthy controls ranged between 1.0-4.4% (63, 151, 282). Cross reactions were reported among one third of persons with paragonimiasis, and 5-25% with schistosomiasis due to *S. japonicum*, cysticercosis, hepatitis, liver cancer and tuberculosis (168, 282). The cross reactions with other trematode infections was reduced by purification of the crude antigen (105). In experimentally infected rabbits, the positive rate and average extinction value (optical density) of the test increased in parallel with the worm burden and the seroreactivity was induced earlier in heavily infected animals (12). The average reciprocal titer also increased with intensity of *C. sinensis* infection in 25 persons (63).

An ELISA-inhibition test using a specific monoclonal antibody to detect antibodies against *C. sinensis* in human sera was reported by Yong et al. (267, 268). The sensitivity of this test was 77.1% and no false positives were observed among normal controls and persons with paragonimiasis. In their experience, the sensitivity of the ELISA-inhibition test was similar to the conventional ELISA (75%), but its specificity was higher.

A series of other modified ELISA techniques have been reported, such as dot-ELISA (158, 272), dig-ELISA (diffusion in gel-ELISA) (273), ABC-ELISA (avidin biotin-horseradish peroxidase complex ELISA) (147, 194, 269), K-ELISA (kinetic-dependent ELISA) (33), PFA-ELISA (horseradish peroxidase cross-linked *Staphylococcus aureus* protein A-ELISA) (12), SPA-ELISA (staphylococcal protein A-ELISA) (194, 281) and SPA-dot-ELISA (197). Among these, the simple and rapid dot-ELISA can be easily used at room temperature and has been recommended by some authors for field use (158, 272). A recently developed diagnostic ELISA kit for clonorchiasis was evaluated among 141 infected persons of whom 90.7% were positive (280).

**ii. Indirect fluorescent antibody test (IFAT)**

Using serum IgG titer 1:16 or over against frozen section antigen of adult *C. sinensis* as the positive cut-off, the positive rates ranged between 61.5-80.5% among clonorchiasis patients (37, 65, 101). In different studies, Yan (257) and Zhou and Zhuo (287) showed that at serum dilutions of 1:10 and 1:20, the positive rates among infected persons were 88.7-89.1% and 58.5-60.9% respectively, and the false positive rates were 9.3-10.0% and 1.7-1.9% respectively. Cross reactions were reported in 3.6% of schistosomiasis patients. Kwon et al. (101) reported that seroreactivity rates paralleled the intensity of the infection. In light, moderate and heavy infections, the positive rates were 28.1%, 68.9% and 77.8-84.6% respectively. False positive reactions occurred in one out of nine normal controls, but none were reported among 16 persons with other parasitic infections.
iii. Indirect haemagglutination test (IHA)

Higher sensitivity and specificity were recorded with IHA in the diagnosis of clonorchiasis. In egg-positive persons, IHA positive rates were 75.8-82.6% (130, 152, 253) and the false positive rate was 2.0% among normal persons (150). No cross reactions were observed in individuals with *Ascaris*, hookworm, whipworm and filarial infections and hepatitis. However, cross reactions were seen in 10.2-20.0% of persons with other trematode infections (schistosomiasis, paragonimiasis, fasciolopsiasis) (130, 150, 152, 253). Specific antibodies against *C. sinensis* appeared earlier in heavier infection with higher titers compared with light infections in rabbit experiments (30).

iv. Complement fixation test (CFT)

CFT is not frequently used nowadays. Hahm et al. (65) reported positive reactions at a dilution of 1:8 or below in 56.4% (31 of 55) of clonorchiasis sera. The false positive rate was 12.0% (3 of 25). In another report, only 13 (26.0%) out of 50 infected persons were positive by CFT (282). Compared with ELISA and IPA, CFT seems to be less sensitive and specific in the diagnosis of clonorchiasis (65, 282).

v. Counter-immunoelectrophoresis (CIEP)

Zhang and Ye (274) reported that the CIEP was positive in all 37 rabbits experimentally infected with 50-600 metacercariae of *C. sinensis*, 40 days after the infection. Among infected persons, 67.1% were found to have positive reactions related to the intensity of infection. However, Yen et al. (266) reported all of the 70 *C. sinensis* infected persons were positive with CIEP. Cross reaction was observed in toxocariasis and angiostrongyliasis. Other report also showed a high sensitivity of this technique (283).

vi. Other serological tests

Using dot-immunogold-silver staining (dot-IGSS) to detect antibody against *C. sinensis*, Wu et al. (243, 244) found that all of 35 persons with the infection were positive when the serum samples were diluted at 1:20, 1:40 and 1:80. No cross reactions were with schistosomiasis. However, four out of 10 cases with paragonimiasis were positive. Radioimmuno-precipitation polyethylene glycol assay (RIPEGA) was used to detect antibodies against *C. sinensis* in 128 patients, and a 90.3% positive rate was recorded. The false positive rates were 2.0% and 3.3%, and cross reactions in schistosomiasis and fasciolopsiasis were 10.2% and 6.1%, respectively. All 27 experimentally infected rabbits except one showed positive RIPEGA reaction. The negative rabbit revealed only four adult flukes upon dissection. A rectilinear correlation (r=0.62) was observed between the stool EPG (log+1) in man or worm burden in the infected rabbits and the precipitating rate of the RIPEGA (140).

8.2.2.2 Circulating antigen detection
Reports on monoclonal antibodies produced against C. sinensis have been rare in recent literature (62). Detecting circulating antigens (CAGs) in sera of C. sinensis-infected persons by ELISA double sandwich method was reported by Chen et al. (32, 34, 35). This technique can detect the CAGs at 30 ng/ml concentration. Among 117 infected persons, 94.9% were positive. Three months after treatment with praziquantel, 70 out of 86 of those followed-up were Cag negative and all were egg negative; 16 were Cag positive, of whom 12 were egg positive and four were negative. The concentration of CAG declined more rapidly than that of circulating antibody after treatment. A good correlation between CAG detection and stool egg examination was found and the authors suggested that this technique could be used in the evaluation of treatment.

8.2.3 Stool antigens detection

A CIEP technique was developed by Wen et al. (235) to detect C. sinensis antigens in the stool with anti-C. sinensis rabbit IgG. Among 75 persons with the eggs in their stool, 52 (69.3%) showed a precipitating line in the CIEP. However, seven (15.6%) out of 45 normal controls were also positive.

8.2.4 Urinary antibody detection

Urinary antibody detection by ELISA with a veronal buffered saline antigen of C. sinensis was reported by Kim et al. (95) in human clonorchiasis. The sensitivity was 87.0% among 470 urine samples and 88.4% among serum samples from the same patients. Among 201 control urine samples, 14 (7.0%) showed false positive reactions. Using a P. westermani antigen, 34.7% of the urinary specimens from C. sinensis infected persons also were reactive. The authors suggested that ELISA applied to urine samples may have application in the large scale screening for clonorchiasis.

9. Effects of treatment on disease

9.1 Parasitological effects

Praziquantel has been the drug of choice since the end of 1970s after its excellent efficacy against C. sinensis was shown, although several other drugs have also been quite effective. Prior its availability, the drugs used had a significantly lower efficacy and greater side effects. These drugs were antimonials, gentian violet, emetine, chloroquine, dithiazanine iodide, hexachlorophene, hexachloroparaxylol (herbol), dehydroemetine, niclofolan, etc. (179, 230), as well as Chinese herbs (26, 173, 174, 176).

9.1.1 Praziquantel

9.1.1.1 Animal experiments

The effect of praziquantel on experimental C. sinensis infection in
rats, guinea pigs, rabbits and dogs has been reported (3, 120, 199). When infected rats were treated with a single dose of praziquantel of 500 mg/kg, or 3x25 mg/kg for two days, all were cured (120, 199). Infected guinea pigs were also cured by a 2-day treatment of 3x25 mg/kg (3). The flukes recovered from the bile ducts of experimentally treated rats in different developmental stages were seriously damaged (184).

9.1.1.2 Human treatment

The standard dose of praziquantel is 25 mg/kg three times a day for two consecutive days. When lower dose or doses of praziquantel were used, the egg negative conversion rate was lower. The cure rate of a single dose of 40 mg/kg praziquantel was between 22.7% - 33.3% according to the intensity of infection, but egg reduction rate was high in those who were not cured (177). When a total daily dose of 75 mg/kg divided into three doses for one and two days was given, the cure rates were 67.4-85.7% and 97.7-100% respectively (169, 177). However, when a total of 150 mg/kg of praziquantel was given in 15 divided doses over five consecutive days, the cure rate (72.9%) was significantly lower than the same dosage over two days (169). The cure rate of praziquantel against C. sinensis is related to the intensity of infection. When the same dose, 75 mg/kg in one day, was given to both groups, Chen et al. (25) found that the egg negative conversion rate was 84.1% among persons with light infections (<500 EPG) and 58.1% in moderate (500-4990 EPG) and heavy infections (>5000 EPG). In large-scale treatment programmes, a single dose of 40 mg/kg for light infections (<1000 EPG), 2x30 mg/kg for moderate infections (1000-10,000 EPG), and 3x25 for heavy infections (>10,000 EPG) was recommended by Rim (177) and Rim et al. (186). However, others have suggested that for large scale treatment, a single dose of 60 mg/kg, or 60 mg/kg divided into two doses in one day appears to have higher cure rates (106, 284) than 40 mg/kg either in single dose or in two divided doses. Treatment of individuals, especially hospital patients, with moderate or heavy infections, a total of 150 mg/kg (6x25 mg/kg) over two days has been recommended by several investigators (169, 171, 182, 250). Many reports on clinical trials on praziquantel have been published from Korea, China and other countries since 1978 (2, 3, 25, 73, 75, 81, 99, 106, 111, 129, 143, 144, 149, 169, 171, 177, 181, 182, 186, 187, 192, 199, 204, 212, 213, 214, 215, 220, 223, 224, 229, 232, 238, 241, 245, 250, 252, 262, 284, 292). A part of them are summarized in Table 4.

The dosage of praziquantel can be several times higher than dosages used in the treatment of schistosomiasis, yet the side effects are mild and temporary. The tolerance of clonorchiasis patients to the drug is good, and most of them can complete the treatment courses as scheduled. The main side effects are dizziness, headache, fatigue, nausea, abdominal pain and diarrhoea (25, 81, 187, 192, 212, 252, 292). Allergic shock (129) and grand mal seizure (204) appearing after the drug administration have been reported. Intracranial hypertension with coma was observed in a clonorchiasis patient complicated with cerebral cysticercosis after treatment with praziquantel (198). No deterioration in liver function tests was observed after praziquantel treatment in patients with
clonorchiasis complicated with hepatitis B virus infection. As praziquantel possesses the merits of a high efficacy, low toxicity and short treatment course, as well as no mutagenicity, teratogenicity and carcinogenicity, it has been recommended as the drug of first choice in the treatment of clonorchiasis.

9.1.2 Albendazole

Good therapeutic efficacy of albendazole have been shown both in animal experiments and human studies against C. sinensis infections. Bae et al. (9) reported that when experimentally infected rats with 50 metacercariae were treated with albendazole of 2x25 mg/kg for two days, its anthelmintic effect was confirmed. Similar results were also shown in experimentally infected dogs (145). Zhong et al. (285) treated 50 clonorchiasis cases with a daily dose of 400 mg for seven days and a 90% cure rate was observed. Other reports showed that when albendazole was used in 2x10 mg/kg for seven consecutive days, the stool egg negative conversion rates were 93.1% (279) and 100% (145), whereas the cure rates of the dose of 2x10 mg/kg for three days were only 20.7% and 35.5% (145, 225). The side effects are mild and transient. Comparative studies between albendazole and praziquantel were carried out by Wen et al. (236) and Zhang et al. (279). They concluded that although albendazole is less effective than praziquantel and requires a longer treatment course, its side effects during treatment seem to be milder and it is also effective against nematodes.

9.1.3 Hexachloroparaxylol

In the 1970s hexachloroparaxylol was used to treat 7773 persons with clonorchiasis with an acceptable therapeutic effect and low cost, as reported by Wan et al. (214). A total of 17.5 g of emulsified powdered drug was given over 5-7 days, with cure rates at 4-6 months after treatment ranging between 82.8% and 97.6% according to different investigators, and no significant differences were seen between the drug and praziquantel (25, 214, 223, 224). However, the drug is no longer being used due to its important side effects, especially neuropsychiatric effects and haemolysis.

9.1.4 Amoscanate

Amoscanate was also used in treating C. sinensis infections. When a daily dose of 2 mg/kg covering 3-5 days was used, egg negative conversion rate was 38.5-75% (206, 223, 240). Its hepatotoxic side effects now preclude its use to control clonorchiasis.

9.1.5 Microfolan

In small doses, i.e., 1-2 mg/kg per day, niclofolan was given for two to three days and was considered to be effective against C. sinensis. However, due to a serious side effect affecting the optic nerve with ensuing blindness (55, 72, 177, 182), it cannot be recommended for treatment of human clonorchiasis.
9.2 Structure changes of the flukes

9.2.1 In vitro studies

When adult worms of *G. sinensis* were put into culture with praziquantel at 0.1 μg/ml and 0.01 μg/ml, the average survival time was 2.4±1.1 and 11.8±4.5 days respectively, whereas in the control culture without praziquantel, it was 15.4±2.1 days; these differences are statistically significant (195). Exposure of the adult flukes to 0.1 μg/ml praziquantel resulted in many bleb-like structures all over the external surface and some subsequently ruptured (221). In a medium with a praziquantel concentration of 1.0 μg/ml, the mortality was 100% (185, 199).

9.2.2 In vivo studies

Praziquantel acts on all organs of *G. sinensis* inducing degenerative changes. After treatment with praziquantel, the flukes in rats were contracted, deformed and blebs of various sizes appeared on the surface. The internal structure of testes became indistinct and deformed (165, 166). By electron microscopy, swelling and vacuolization of integument, deterioration of basement membrane, degeneration or vacuolization of mitochondria and degenerative changes of tubular membrane of protoplasmic microtubules, spermatocytes and oocysts were noted (188, 199, 210). By scanning electron microscopy, bleb-like structures were observed on the sensory papillae around the two suckers of the flukes (104, 117). Upon reaching the surface, vacuoles burst causing erosion of tegumental tissue (92, 154, 210). Swelling, disarrangement and lysis of the circular and longitudinal muscles were increasingly observed as the time interval after treatment increased (115). Praziquantel causes degenerative damage on microvilli of the gut of *G. sinensis* in infected rats. Partial lysis and degeneration of the microvilli occurred (116). The separation of the intestinal epithelium and basement membrane was seen by transmission electron microscopy (104). Thus, the effects of praziquantel are mainly on tegument, intestinal tract and reproductive organs.

Histochemical changes within the flukes after contact in vitro with praziquantel in a concentration of 0.1 μg/ml for six hours included a marked reduction of glycogen as compared with that of the controls (222). Glycogen content in flukes from infected rats decreased significantly 24 hours after praziquantel treatment, and disappeared at 48 hours post-medication. Protein in fluke tissues increased significantly and RNA content decreased gradually, but no changes in DNA were recorded (161). The metabolic pathways of glucose, nucleic acids and of protein synthesis of the fluke were altered by praziquantel treatment.

Albendazole also has a destructive effect on the tegument and gut of *G. sinensis* as observed in experimentally infected rats and cats by scanning and transmission electron microscopy. Swelling, protrusion formation and erosion of the tegument and degeneration and lysis of the gut microvilli were shown in the ultrastructural observations (121, 210).
9.3 Decrease in morbidity

9.3.1 Symptomatic improvement

After treatment of acute clonorchiasis with praziquantel, fever subsided simultaneously with the disappearance of abdominal pain and distension and other symptoms (78, 231).

Symptomatic improvement after treatment was also seen in chronic clonorchiasis, especially in those with heavy infections. Children with *Cl. sinensis* infections usually show an increase of height and body weight as well as improvement in their general nutritional status (25, 169, 212). Zhu et al. (292) analyzed the changes in symptoms in 180 persons with clonorchiasis one month after praziquantel treatment. The rates of disappearance of the symptoms were: fatigue - 92.7%, anorexia - 86.7%, abdominal distension - 82.3%, pain in the right hypochondrium - 80.9% and irregular bowel movements - 78.4%.

9.3.2 Hepatosplenomegaly

Post treatment regression of the enlarged liver and spleen was reported (25, 292). 40.8% of 129 persons with hepatomegaly and four of six persons with splenomegaly before treatment did not have palpable organs at 4-month follow-up (25). Zhu et al. (292) reported that among the original cohort of 180 cases, physical examination one month after praziquantel treatment revealed hepatomegaly reduced from 80.6% to 65.0% below the right costal margin, and from 58.9% to 37.8% below the xiphoid, whereas splenomegaly reduced from 7.8% to 4.4%. Ascites and jaundice in one patient each disappeared upon follow-ups.

9.3.3 Changes in antibody levels

In experimentally infected rabbits, treatment cure was confirmed by repeated stool examinations after administration of hexachloroparaxylol. The titer of antibody in IHA decreased one month after medication. It reduced to low levels (1:16-1:32) at six months, and almost disappeared at 15 months after treatment (60).

In some follow-up studies, serological tests were performed to determine the changes of antibody levels as an indication of cure. Antibody levels were decreased significantly three months after treatment with praziquantel and at 6-month follow-up, 51.8% (170) and 60.8% (28) of them showed a negative conversion by ELISA. Wang et al. (219) reported that 36.4-40.4% of the infected subjects became negative by ELISA four months after treatment with praziquantel. Serum specific IgG level was decreased at six months after treatment and the decrease was more significant in the group with highest pretreatment intensity of infection, as shown by Lee et al. (103). Hong (71) reported that the period for serological negative conversion after praziquantel treatment was between nine weeks and seven months in human clonorchiasis. In general, the heavier the intensity of infection, the longer the interval before specific
antibodies disappeared. Negative sero-conversions in ELISA after treatment may be considered as cures (31).

Absorbance values of the ELISA in urine decreased gradually, and 18 months after medication and parasitological cure, the values were only reduced about one-half compared with pre-treatment levels (87).

10. Prevention and control

No endemic country has yet launched a large scale national control programme for the prevention and control of clonorchiasis. Its prevention and control will involve many different sectors including the health sector. The disease is fundamentally related to the food habits based on cultural and societal traditions. Prevention therefore will require new approaches in the public sector to communicate innovation in food selection, food preparation and food processing. Diagnosis and treatment is only one component of control and in itself can only have short term effects without a well-planned prevention programme. There are several approaches which can be used for the control of _C. sinensis_ infection:

(a) improvement of sanitation,
(b) health education,
(c) diagnosis and chemotherapy, and
(d) control of snail intermediate hosts.

The first approach can be implemented only when the economic development is such that the people and government in these areas can afford to construct enough hygienic toilets. In most endemic areas, however, this approach must be linked to general development of infrastructure and as part of progressive hygiene education - as long term investments.

Education is a useful means to change the traditional ways of preparing and processing raw fish. The food habits of the peoples of endemic areas are not amenable to change in the short term. Promotion of proper food preparation seems to be the most effective available measure. However, public health authorities need to use better communication techniques for this message. An insistent and persistent educational propaganda stressing the importance of thoroughly cooking fresh water fish and shrimps seems to be an effective means to prevent the disease (66, 180).

Irradiation of fresh fish, while effective, does not seem currently feasible on a large scale. Recently, radiation of fish flesh infected with metacercariae of liver flukes including _C. sinensis_ with Co-60 gamma irradiation has been demonstrated as an effective method of control (109, 148, 201). A minimum dose of 0.1 kGy is effective against metacercariae of another liver fluke, _i.e.,_ _O. viverrini_, without changing physicochemical properties of the fish flesh, as reported by Loaharanu and Sornmani (148). The LD₅₀ of the Co-60 gamma irradiation dose for metacercariae of _C. sinensis_ in fish was 0.05 kGy, and the minimum effective dose was 0.15 kGy.
This technique shows promise as an effective method to ensure the quality of freshwater fish.

Now chemotherapy plays a major role in both morbidity control and transmission control by reducing prevalence of *C. sinensis* and intensity of the infection if it is properly used. The support of health education associated with chemotherapy might accelerate the control effects.

Until now the efficacy and feasibility of snail control for control of food-borne trematodes, including clonorchiasis has not been demonstrated. In the long term this option for control through environmental modification and/or molluscciding must be carefully evaluated before inclusion in any national programme.

11. Summary

*C. sinensis* infection is a public health problem in the four Asian countries and the Asian part of Russia. Up till now, nine different species of snails belonging to four families and 109 species of 13 families of freshwater fish have been confirmed to be the first and second intermediate hosts of *C. sinensis* respectively. Although three species of shrimp have been claimed as the second intermediate hosts, there are different opinions on this topic. Many domestic and wild animals have been reported as the reservoir hosts for *C. sinensis*, but their roles in the intensity of transmission of the disease seem to be not as important as the roles played by humans. The tradition of eating raw or poorly cooked freshwater fish is linked to the distribution of the disease. Pathological changes are mainly seen in the hepatobiliary system and pancreas and are induced by mechanical injury of the fluke and chemical stimulation of its metabolic products. Immunity of an infected person to re-infection is low. Morbidity of the disease is related to the intensity and duration of the infection, susceptibility of the hosts, the coexistence of bacterial infection and its complications. Clinical manifestations vary from person to person. Acute infection is rarely seen by physicians. Chronic infection manifestations range from subclinical cases without symptoms to severe hepatobiliary disease. In some cases, the severe disease can develop if treatment is not given. Hepatomegaly is the most common sequel a but splenomegaly is not common. In heavily infected persons or in the late stages of the disease, jaundice, portal hypertension, ascites and upper gastrointestinal bleeding, as well as complications, such as secondary bacterial infections of the hepatobiliary system, cholelithiasis, pancreatitis and cholangiocarcinoma appear and may cause death. The causal relationship between *C. sinensis* infection and cholangiocarcinoma is no longer a subject of controversy. In endemic areas the incidence and mortality of this carcinoma is definitely higher than in non-endemic areas and there is a consensus regarding the correlation between these two diseases based on epidemiological data, histopathological studies and animal experiments. On the other hand, our understanding of the pathogenesis of cholangiocarcinoma is not conclusive. The role of *C. sinensis* as a carcinogen or cocarcinogen needs to be explored by population-based, prospective epidemiological studies in endemic areas as
well as with laboratory investigations are needed to elucidate the mechanisms of carcinogenesis. Diagnosis of the infection is based on parasitological techniques. Different immunological tests can provide useful information in the management of individual patients and, with improvements, contribute to epidemiological surveys. Praziquantel is now the drug of choice and has specific role in control. Prevention and control of clonorchiasis includes involvement of many sectors to improve sanitation, health education targeted at improved understanding of the need for proper selection, preparation and processing of freshwater fish. Repeated chemotherapy with praziquantel coordinated with intensive education are contribute to control. In the long term, innovation and public awareness will achieve control.
REFERENCES


parasitology, 26:1-8.


231. Wei, S. F. (1989) Leukemoid appearance in Clonorchis sinensis infection:


