

## Clonorchiasis in Korea

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**Abstract:** Since the first report on the incidence of egg positiveness of *Clonorchis sinensis* by Matsumoto in 1915, clonorchiasis has been recognized as one of the most important endemic diseases in Korea. Most of the works on clonorchiasis after then had been studied by Japanese workers until 1945. After the Korean War in 1950~1953, health problems of Koreans were extremely aggravated. This gave us attentions for the prevention of contagious diseases as well as parasitic infections in public health point of view. It was greatly recognized that not only ascariasis, hookworm infections, and trichuriasis, but also clonorchiasis and paragonimiasis constitute the important helminthic diseases in Korea.

In the late 1950s, several Korean workers conducted studies of *C. sinensis* on its biology, epidemiology, pathology, clinical symptoms as well as treatment. Thereafter overall epidemiological surveys were carried out actively throughout the country since 1960s. From these surveys, it became clear that endemic areas of clonorchiasis are scattered all over the country along the Nakdong, Kum, Mankyong, Yeongsan and Han rivers. The most extensive and intensive endemic regions were found mainly along the Nakdong river. Since then, clonorchiasis has been one of the most extensively studied subjects during the past 30 years.

The present review mainly deals with current results obtained by Korean investigators on the prevalence of clonorchiasis, as well as its biology, epidemiology, pathogenesis, clinical manifestations, diagnosis, treatment, prevention and control.

**Key words:** *Clonorchis sinensis*, prevalence, biology, epidemiology, pathogenesis, clinical manifestations, diagnosis, treatment, control, review

*Clonorchis sinensis* (Cobbold, 1875) Looss, 1907, "the Chinese or the oriental liver fluke", is an important human parasite and is widely distributed in southern Korea, China, Taiwan, Japan, and northern Vietnam. This parasite was discovered by McConnell on 1875 in Calcutta during an autopsy of a Chinese carpenter.

In Korea, the first human infection with *C. sinensis* was demonstrated from a Korean autopsied liver by a Japanese physician Matsumoto in 1912. A few years later, he reported an

18.6% egg-positive rate of *C. sinensis* after examination of 351 stool samples of Taegu primary school children (Matsumoto, 1915). After this until 1945, numerous reports had been presented by the Japanese workers on the prevalence of clonorchiasis among the Korean population. During this period, there were reports on numerous clinical cases with complications due to clonorchiasis, on the distribution of the first intermediate host *Parafossarulus manchouricus* in the Korean peninsula as well as

on the infestation of metacercariae of *Clonorchis* in the freshwater fish as the second intermediate host. At that time, clonorchiasis was recognized as one of the most important endemic diseases in Korea.

After the liberation from the Japanese reign at the end of World War II, no research activities on parasitic diseases were performed in Korea as in other scientific fields. Furthermore, a few years later, the Korean War broke out in June 1950.

In the early 1950s, health problems in Korea were extremely aggravated by the War. Public attentions were called to the prevention of contagious diseases as well as parasitic infections by public health officials in Korea.

In parasitic infections among Koreans, the first nationwide survey of randomly-selected stool examinations was conducted by the U.S. Army. The result was reported by Hunter *et al.* in 1949. Shortly after the Korean War, several reports were made on the prevalence of helminths among the Korean population in different parts of the country. It was greatly recognized that not only ascariasis, hookworm infections, and trichuriasis, but also clonorchiasis and paragonimiasis constitute the important helminthic diseases in Korea.

In the late 1950s, there had been some reports on human infection of *Clonorchis sinensis* diagnosed by stool examination. The first nationwide survey of clonorchiasis by eggs was conducted by the Ministry of Health and Social Affairs in 1958. According to the results of this survey the total number of inhabitants examined at 7 river basins was 14,519, of which 1,701 (11.7%) were found to be infected. At that time several workers conducted studies of *C. sinensis* on its biology and epidemiology, as well as clinical symptoms and treatment. However, it is noteworthy that Walton and Chyu (1959) carried out for the first time the intradermal skin test using VBS antigen. Out of 9,771 subjects, 2,066 (21.1%) were positive in southern Korea. This had contributed greatly to the epidemiological investigation of clonorchiasis.

During the 1960s, overall epidemiological surveys were actively carried out throughout the country. From the above surveys, it became clear that endemic areas of clonorchiasis were scattered all over the country along the Nakdong, Kum, Mankyong, Yeongsan, and Han rivers and the most extensive and intensive endemic regions were found mainly along the Nakdong river. Since then clonorchiasis has been one of the most extensively studied subjects during the last 30 years in Korea. Recently Rim (1986) made a comprehensive monograph on the current pathobiology and chemotherapy of clonorchiasis, in which he reviewed the recent advances on *C. sinensis* infection based on significant world literature and contributions of Korean workers.

The present review deals mainly with current results obtained by Korean investigators on the well-recognized prevalence of clonorchiasis occurring during last 3 decades, as well as the researches on its biology, epidemiology, pathogenesis, clinical manifestations, diagnosis and treatment.

## THE PARASITE

The adult worm of *Clonorchis sinensis* is flat, elongated, aspinous, and flabby, tapering anteriorly and somewhat rounded posteriorly. The living worm is transparent and slightly pink or brownish in color and may have yellowish pigments of lipofuscin in the body (Cho *et al.*, 1983). The size of the worm varies according to its age, species of the host, number of parasites in one host, and its location in the large or small bile ducts, its minimum and maximum length measuring 4 to 20 mm. Generally speaking, the size of *C. sinensis* ranges from 8 to 15 mm in length by 1.5 to 4.0 mm in breadth and about 1.0 mm in thickness.

The ultrastructure of the *Clonorchis* tegument has been studied by Kim (1968) and Jeong *et al.* (1978) in Korea. Generally, the fine structure of the tegument is similar to those of other digenean teguments. By scanning electron micro-

scopic observation, the surface showed numerous irregular tegumental ridges encircling the body. Around the oral and ventral suckers, as well as near the excretory pore, were thickly distributed, upraised mammillary papillae which were topped by a short cilium. By transmission electron microscopy, the external surface of the tegumental syncytium showed disc-like granules, and many mitochondria were distributed throughout the body. The tegumental syncytium was bounded on its inner surface by a thin basement membrane which, occasionally, was everted into the tubules penetrating muscle layers. The sensory papillae of *Clonorchis* adult worms are similar in structure to the sensory nerve endings found on other digenetic trematodes. According to Jeong *et al.* (1980b), the inner walls of the oral cavity and esophagus are formed from the tegument, which is of the same basic structure as the body tegument.

The presence of the prostatic gland of *C. sinensis* was also observed by electron microscopy. Jeong *et al.* (1980a) found that the prostatic gland consisted of numerous unicellular glands grouped around the ejaculatory duct. The individual cell was lobulated, tapering in the direction of the ejaculatory duct, and the secreting ducts of the gland penetrated into the ejaculatory duct through the muscular layer, finally opening into the lumen of the duct. Although the prostatic gland was less developed than that of *Fasciola hepatica*, the basic structure was quite similar. There were well-developed lamellae in the epithelia of all ducts concerned with the passage of spermatozoa from the testis to the male genital opening. There were also well-developed flame cells and lamellae along the surfaces of the epithelia of all excretory canals or ducts concerned with evacuation. The excretory pore was 7.5  $\mu\text{m}$  in diameter and the dorsoterminaly opened. The yellow-to-light brown coloured egg measures 26 to 30  $\mu\text{m}$  by 15 to 17  $\mu\text{m}$  and is ovoid in form, narrowing a little posteriorly into the operculum. The operculum takes the shape of a watchglass with a prominent shoulder and is found on the narrow ante-

rior end of the shell. At the thickened posterior end a small median protuberance is seen usually comma-shaped. By scanning electron microscopic observation, the egg surface is covered with an irregularly-shaped, coarse network of delicate membranous exuberance.

The morphology and development of the larval stages of *C. sinensis* were described in detail by earlier investigators such as Faust and Khaw (1927), Komiya and Suzuki (1964) and Komiya (1966).

### DISTRIBUTION AND PREVALENCE

Since the introduction of the cellophane thick smear technique (Kato thick smear method) in 1968, Seo *et al.* (1969) carried out stool examinations by using the Kato method on subjects from all over Korea. The results showed a positive rate of 4.7% out of 40,581 persons (mostly schoolchildren). On the other hand, out of 3,880 adults, 450 (11.6%) positive cases were found by the cellophane thick smear method.

Investigations by the intradermal test showed that the prevalence rates of clonorchiasis were 11.1% to 21.1% among Koreans (Walton and Chyu, 1959; Rim *et al.*, 1973). Surveys using intradermal tests were performed by the Korean Red Cross (1965~1970) on primary school children and school teachers living along the drainage areas of the 5 main rivers in southern Korea (the Han, the Nakdong, the Kum, the Yeongsan and the Mangyong Rivers). Out of 147,811 schoolchildren examined, 18,339 (12.4%) were positive ranging from 5.6% (the Kum River basin) to 25.8% (the Nakdong River basin).

In order to observe the distribution and endemic status of *C. sinensis* infection in Korea, Song *et al.* (1983) surveyed the basins of 6 major rivers, *i.e.*, the Han, the Kum, the Nakdong, the Mangyong, the Yeongsan and the Seomjin Rivers, and a number of nonriver side areas for a 10-year period from 1973 to 1982. During that period, stools from 19,758 people were examined. The infection rate in each area was

observed and then analyzed by age and sex. The intensity of *Clonorchis* infection was determined in the individual by counting the eggs passed per unit of feces. The results of their surveys and the infection rates of *C. sinensis* reported by many other investigators were collated and a map of each river basin prepared by Rim (1986).

Recently Seo *et al.* (1981) examined the stools of 13,373 people living within 6 km of several riversides in order to investigate the actual status of *C. sinensis* infection among riverside inhabitants. The survey results showed an overall infection rate of 21.5%. An infection rate of 40.2% of the Nakdong River basins ranked first, 30.8% of the Yeongsan River basins second, 17.3% of the Seomjin River basins third, 15.9% of the Tamjin River basins fourth, 15.7% of the South Han River basins fifth, 12.0% of the Kum River basins sixth and 8.0% of the Mangyong River basins last. They estimated the number of people infected with *C. sinensis* at approximately 830,000 to 890,000 of the total 4 million inhabitants of the above 7 basins.

Since 1971, prevalence surveys of intestinal parasitic infections have been undertaken every 5 years by the Korean Association of Health under the direction of the Ministry of Health and Social Affairs, Republic of Korea. Nationwide surveys of randomly-selected sample populations in urban and rural areas were conducted in 1971, 1976, 1981 and 1986. According to these survey results, the incidences of *Clonorchis* infection were 4.6%, 1.8%, 2.6%, and 2.7%, respectively. Therefore, the infection status of *C. sinensis* remained virtually unchanged 15 years despite the development of effective anthelmintics such as praziquantel.

## EPIDEMIOLOGY

The geographical distribution of clonorchiasis closely parallels the distribution of the intermediate host, particularly the snail host, known as *Parafossarulus manchouricus*. This snail is the only known molluscan host for *Clonorchis*

*sinensis* in Korea. The incidences of *C. sinensis* cercariae in the snail, *Parafossarulus manchouricus*, collected from endemic areas in Korea, were reported from as low as 0.08% to 3.1% of the snails examined (Rim, 1986). In endemic regions in the basins of the Nakdong River, the population density of snails usually ranged from 200 to 500/m<sup>2</sup> in the areas of snail habitat during the summer (Report of Korea Association for Parasite Eradication, 1978). Considering the large population of snails found in many bodies of water and the large numbers of shedding cercariae from an infected snail, such a low infection rate in the snails is still highly significant in terms of propagation of the infection. In Korea, the shedding of cercariae from snails was generally observed during the period from May to October (Chun, 1963; Kim, 1974).

Approximately 36 species of freshwater fish were found to serve as second intermediate hosts in Korea. The majority of these fish belong to the family Cyprinidae. Among these fish, the infection of *Clonorchis* metacercariae is found most frequently in *Pseudorasbora parva*, *Sarcocheilichthys sinensis*, *Hemibarbus labeo*, *Acanthorhodeus gracilis*, *A. taenianalis*, *Puntungia herzi*, *Pseudogobio esocinus*, *Gnathopogon* species, and *Acheilognathus limbata*. *P. parva* shows an extremely high degree of infection, usually hundreds to thousands of the metacercariae in a fish, with a maximum of 31,516 (Kim, 1974). In Goyang Gun, Kyonggi Do, the maximum number of metacercariae per gram of flesh of this fish was 6,090. In the lower basins of the Nakdong River (in Kimhae Gun), the mean number of metacercariae per gram of flesh of *P. parva* ranged from 46.7 to 3,189.4 in different localities (Kim, 1974). According to reports summarized by the Korea Association for Parasites Eradication (KAPE) in 1978, the most frequently infested fish was *Pseudorasbora parva* 90.3%, followed by *Sarcocheilichthys sinensis* 78.0%, *Hemibarbus labeo* 68.2%, *Puntungia herzi* 50.9%, *Pseudogobio esocinus* 47.5%, *Gnathopogon* species 44.4%, *Cultricolus kneri* 38.5% and *Acanthorhodeus taenianalis* 29.5%.

Choi *et al.* (1976) examined the intensity of infestation with larvae in various freshwater fish from the Kumho River, one of the tributaries of the Nakdong River. In their results, *Pseudorasbora parva* was the most heavily infested, and the mean number of metacercariae per gram of flesh was 51.4. *Puntungia herzi*, *Gnathopogon* species, *Pseudogobio esocinus*, *Sarcocheilichthys sinensis*, and *Hemibarbus labeo* were infested moderately (10.4 to 19.5%), whereas *Acheilognathus rhombea*, *Acheilognathus limbata*, and *Acanthorhodeus taenianalis* were infected with only a few cysts (1.5 to 3.3%). Park *et al.* (1984) compared the metacercarial density of *Pseudorasbora parva* in a period of 10 years in the Kimhae area which is one of the high endemic areas. Although the metacercarial infestation rate of *P. parva* did not change, the metacercarial density decreased from 1,312.4 ea/fish in 1972 and 1,233.4 ea/fish in 1973 to 160.8 ea/fish in 1983.

According to Rhee *et al.* (1982), a large number of clavate cells were found in the epidermis of *Misgurnus anguillicaudatus*, *Parasilurus asotus*, *Cyprinus carpio*, and *Carassius carassius* which are not suitable as the second intermediate hosts for *C. sinensis*, while clavate cell were not found in *Pseudorasbora parva*, *Zacco platypus*, *Microphysogobio koreensis*, *etc.*, which are known as second intermediate hosts. From these results, he suggested that the degree of infection of *C. sinensis* metacercaria would be closely related to the occurrence of clavate cells in the epidermis of freshwater fish. Rhee *et al.* (1983b) also conducted an experiment with *Cyprinus carpio nudus* on the suitability as a second intermediate host of *C. sinensis*. When *Cyprinus carpio nudus* were exposed to a number of cercariae in the beaker, only a few cercariae could invade through the epidermis. Most of the invaded cercariae were killed before forming the cyst. Even rarely encysted cercariae were found to be dead within 48 hours, whereas in the control fish, *Pseudorasbora parva*, numerous cercariae penetrated the epidermis and encysted in its muscle.

A survey on reservoir hosts was conducted in Korea by Kim (1974) who examined the prevalence of *Clonorchis* infection in animals (hogs, dogs, and house rats) in high- and low-endemic localities. In Kimhae Gun, the highest endemic area in Korea (68.8% of the population infected), he found that 59 (18.5%) out of 319 hogs, 2 out of 4 dogs, and 19 (10.9%) out of 174 house rats were infected. In Goyang Gun a relatively low endemic area (15.2% of the population infected), 2.4% (2/84) of the hogs, 21.6% (11/51) of the dogs, and 3.8% (14/368) of the house rats were infected. The prevalence rate of infection among the animals in the high endemic locality was significantly higher than in the low endemic locality. Therefore, in many endemic areas, the reservoir hosts such as hogs, and rats may play a significant role in transmitting the eggs of *C. sinensis*. However, in the endemic areas, more humans are infected than reservoir animals. This indicates that infected humans rather than reservoir hosts play a major role in the epidemiology of the disease.

In the mode of human infection, *Clonorchis* infection is acquired by eating uncooked fish containing infectious metacercariae. The intensity of human infection is dependent upon the eating habits of the population (Rim, 1982). It is well known that the Korean people have a custom of eating raw fish at drinking parties. Therefore, in endemic areas, more men are infected than women. The incidence in children is low, but from 20 years of age onwards the incidence increases, being the highest at 40 to 50 years of age. The large fish, *Cyprinus carpio* and *Carassius carassius*, which are frequently eaten by the inhabitants in endemic areas, have a low rate of infestation. However, repeated consumption of the raw fish may lead to heavy infections and high incidence. In most endemic areas, people consider raw fish a health food. Sometimes, ignorant Korean mothers feed raw fish to their children believing that it will help them grow strong. On the other hand, heavily-infected small fish are not generally eaten raw, but they may be undercooked and still transmit infection (Rim,

1986).

In the 1970s, epidemiological research on clonorchiasis was carried out in most actively in Korea. In the correlation of *Clonorchis* infection with epidemiological and clinical significance, a quantitative examination of stool was greatly helpful. Stoll's egg counting method is especially suitable for field works. The egg count in terms of number per gram of feces (EPG) is important in estimating the worm load and is applicable in quantitative analysis of *Clonorchis* infection as well as in assessing the efficacy of treatment. According to the egg counts, patients can be divided into 4 groups, light infection EPG between 1 and 999; moderate infection EPG between 1,000 and 9,999; heavy infection EPG between 10,000 and 29,000; and very heavy infection EPG 30,000 or over.

In order to observe the endemicity of *C. sinensis* infection, Rim(1984) attempted a quantitative epidemiological analysis on the levels of intensity or endemicity, age and sex distribution, the features of transmitting *C. sinensis* infection, as well as the epidemiological changes of clonorchiasis including familial aggregations in highly endemic areas. The degrees of intensity or endemicity of *C. sinensis* infection in endemic areas were compared by the regression lines compiled with regression equations, which were calculated by the cumulative percentage of EPG number of *C. sinensis* infection in the surveyed areas. Quantitative analysis on the age and sex distribution and the features of transmitting *C. sinensis* infection as well as epidemiological changes in the endemic areas, were applied by the Muench. catalytic model. It was noted that the theoretically obtained simple and two-stage catalytic curves fitted so well with the observed data and that the catalytic model was found useful in understanding the epidemiological features of the transmission of *C. sinensis* infection (Park *et al.*, 1984; Rim, 1986).

### PATHOGENESIS

The main cause of pathogenesis in clonorchiasis

is a mechanical obstruction of the biliary tract by the worms, congestion of bile, and the effects of soluble substances (metabolites) released from the flukes into the ducts and surrounding tissues. The severity of the dysfunction depends upon the number of worms present and the period of infection. It is generally accepted that more than 1,000 flukes caused immediate dysfunction of the liver from the parasitism of *C. sinensis*. Chung(1959) recovered 27,600 flukes at an autopsy of a person who died from an obstructive jaundice with hepatomegaly and enlargement of the gallbladder resulting from transient obstruction of the common bile duct.

The histopathological response to infection with *C. sinensis* in man was described classically by Hou(1955). The degree of pathological change depends on the intensity as well as duration of the infection. In the early stages of infection or in a mild infection, there is no detectable change in the liver. With heavy infection the most prominent changes are in the bile ducts. On the surface of the liver, pale cystic areas can be seen where the fibrosed bile ducts approach the surface, and in section, the dilated bile ducts are seen to be filled with bile and contain a various numbers of worms. The main pathological changes occurring in humans are similar to those seen in infected animals. In experiments on animals, early infection led to the proliferation of bile ducts and pseudostratification of the biliary epithelium. Later metaplastic squamous cells appeared in conjunction with glandular proliferation, giving an appearance suggestive of adenomatous hyperplasia (Lee *et al.*, 1978).

As in the schistosomes and *Fasciola hepatica*, the intestinal ceca of *C. sinensis* contain a black pigment which was proved by benzidine test to be the degenerated products of hemoglobin (Chu *et al.*, 1982). Many blood cells were observed by electron microscope in the lumen of the ceca of adult worms, and their morphology was confirmed to be the same as that of the host. Chu *et al.* (1982) examined 648 specimens of *C. sinensis* collected from the liver of rats infected experimentally with metacercariae of *C. sinensis*,

They observed weekly the appearance and amount of black material in the cecal lumen of the worms during the course of development of the fluke in the body of the host. The results showed that *C. sinensis* starts to intake blood in the bile duct 2 weeks after infection, gradually increasing the amount, and in the 5th week, most of the intestine of *C. sinensis* has become filled with intestinal contents made up of blood components.

The above results suggest that anemia could be one of the consequences of heavy infection of *C. sinensis*. On the assumption that *C. sinensis* in the bile duct ingests the blood of the host, considerable changes should then occur in the blood picture indicating anemia. However, although the mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) values in accordance with the degree of infection with *C. sinensis* do not show any change at all. As the degree of infection becomes higher, the mean values of the hemoglobin, hematocrit and red blood cell counts also become higher (Kim *et al.*, 1982b). Kim *et al.* (1982b) measured the blood loss caused by *C. sinensis* infection with  $^{51}\text{Cr}$  and compared it according to intensity of infection. The results showed that the blood volume, red blood corpuscular volume and plasma volume of the heavy infection group were lower when compared with those of the uninfected normal control. From these results, we can understand the reason why the blood picture in patients with heavy infection seldomly shows anemia and why the volumes of red blood corpuscles, hemoglobin, and hematocrit are higher than those of the light infection group.

As for the complications of clonorchiasis, formation of calculi in the intrahepatic biliary passages is one of the most characteristic pathological features. Sometimes it is accompanied by suppurative cholangitis, cholecystitis, and biliary abscess or so-called cholangitis and, ultimately, can cause primary liver cancer, specifically cholangiocarcinoma.

Chang and Rim(1981) undertook experiments

with white rats to find out the influences of bacterial infection in the bile ducts on the existence and growth of *C. sinensis* already living in the bile ducts. When the bile ducts were experimentally challenged with bacteria, the flukes either died or were retarded in their growth.

Extensive studies on the frequency of disease caused by *C. sinensis* have not been carried out in Korea. However, Pae(1968) showed that in Taegu City, one of the heavily infected areas, 7(20.6%) out of 34 cases of cholelithiasis had *C. sinensis* eggs in the gallstone, and he said that *C. sinensis* was a main cause of cholelithiasis. On the other hand, in Seoul, 2(1.5%) out of 134 cases of cholelithiasis (Song, 1978) and 2 (0.9%) out of 225 cholelithiasis cases were associated with *C. sinensis* infections (Park *et al.*, 1979). In addition, Kim(1969) found out through clinical experiences that 6(5.0%) out of 120 cases of acute cholecystitis in the Pusan area were caused by *C. sinensis*, but Kim *et al.* (1976) reported only 2(1.7%) out of 120 cases of acute cholecystitis in Seoul were associated with *C. sinensis* infection. Hur *et al.*(1969) stated that 12(11.7%) out of 103 cases of biliary surgical disease without calculi in Seoul were caused by *C. sinensis*. However, Kim(1976) reported that 43(21.8%) out of 197 cases of acalculous cholecystitis in the Nakdong River basin were associated with *C. sinensis* infection. According to Cho *et al.* (1971), among 345 cases of bile duct disease in the Seoul area, 15(4.3%) cases also suffered from *C. sinensis* infection. In the endemic areas, however, 7.7% of 587 cases of hepatobiliary surgery in the Pusan area (Kim, 1976) and 13.3% of 180 cases of hepatobiliary surgery in the Taegu area (Lee and Kang, 1976) were associated with the infection of *C. sinensis*. On the other hand, Chung and Lee(1976) investigated the relationship of primary liver carcinoma and clonorchiasis in the Pusan area, one of the areas most heavily infested with *C. sinensis* in the southern part of Korea. A series of 206 consecutive cases of primary liver carcinoma were subjected to path-

ological evaluation during the year 1963 to 1974. Out of 206 cases, 47 cases (22.8%) were associated with *Clonorchis* infection. Among them, out of 36 cases of cholangiocarcinoma, 19 cases (52.8%) were found to be infected with *C. sinensis*, but of 170 cases of hepatocellular carcinoma, 28 (16.5%) were associated with *C. sinensis*. Lee *et al.* (1979) reported that 3 (8.1%) out of 37 cases of primary carcinoma of the gallbladder were also associated with *C. sinensis*. It is generally believed that the majority of liver cancers associated with *Clonorchis* infection are cholangiocarcinoma. Recently Kim (1984) made a hypothesis on cholangiocarcinoma in clonorchiasis. According to him, at the stage of adenomatous hyperplasia of the bile duct due to *Clonorchis* infection, exogenous carcinogens or co-carcinogens as promoters and endogenous influences, such as nutritional, immunological and/or genetic factors, are assumed to participate in provoking goblet cell metaplasia, as well as dysplasia of the bile duct epithelial cells, and consequently, cholangiocarcinoma.

Choi and Yang (1974) studied 699 cases for surgical intervention on the hepatobiliary tract during a 2<sup>1</sup>/<sub>2</sub>-year period from January 1970 at Pusan Gospel Hospital, finding that 65 cases (9.3%) were infected with *C. sinensis*. They confirmed that clonorchiasis cases were associated with the following hepatobiliary diseases: cholecystitis (26.2%), cholelithiasis (18.5%), choledocholithiasis (12.3%), intrahepatic stone (4.6%), cholangiohepatitis (15.4%), pancreatitis (7.7%), cholangiocarcinoma (13.8%), and hepatocellular carcinoma (3.1%). Likewise, Lee and Kang (1976) found adult worms of the liver fluke in 24 cases (13.3%) out of 180 cases of hepatobiliary surgery during 3-year and 7-month period on January 1972 at Taegu and also reported similar results of associated hepatobiliary diseases.

### SYMPTOMATOLOGY AND CLINICAL FEATURES

Clinical manifestations result from the number of worms, infection period, condition of the

worms and complications. In the early stage of infection or when the number of worms is small, both subjective and objective symptoms are almost nil. However, the symptoms are aggravated in accordance with the intensity and duration of the infection.

To understand the significance of the clinical features of clonorchiasis, Kim *et al.* (1982a) carried out examinations for hematology, blood biochemistry, and clinical symptoms on patients in several endemic areas whose stools were positive for eggs of *Clonorchis sinensis*. Stools of a total of 287 clonorchiasis patients were examined by the Stoll's egg-counting method for measuring the intensity of infection. Twenty-two (40.0%) out of 55 patients in the light infection group and 27 (18.8%) out of 144 patients in the moderate infection group had no complaints of any symptoms at all. Furthermore, 4 (6.9%) out of 58 patients and 3 (10.0%) out of 30 patients in the heavy and the very heavy infection groups, respectively, also had no complaints. Accordingly the number of patients complaining of subjective symptoms increased as the intensity of the infection became higher. But even in the light infection group, 60% of the patients complained of subjective symptoms. Among the subjective symptoms, lassitude, asthenia, mental depression, and dizziness were the most common symptoms. The frequency of such symptoms increased as the degree of infection increased. Besides these, in unusual cases, tachycardia, sweating, sleeplessness, fever, edema on the face, itching and dyspnea were complained of. As gastrointestinal symptoms, abdominal discomfort, epigastric pain, nausea, indigestion, anorexia, and cramps were common; sometimes diarrhea and vomiting were also complained of. In general, these symptoms occurred more frequently as the intensity of the infection become higher, but the patients in the light infection group complained of those symptoms in considerable number. Neurological symptoms included headache, back pain, and neuralgia (joint pain), regardless of the intensity of infection. In some cases the patients complained of shoulder pain



and tremor.

Physically obvious signs such as hepatomegaly, tenderness of the liver and jaundice were frequent when the degree of infection was higher, *i.e.* hepatomegaly was detected in 12.7% of the patients in the light infection group compared to 63.3% in the very heavy infection group. Tenderness of the liver was detected in 12.7% of patients in the light infection group, while the proportion in the very heavy infection group was 43.3%. Jaundice was found in 3.6% of the patients in the light infection group but 26.7% in the very heavy infection group. Only 1 case of cirrhosis of the liver was found in each of the moderate and heavy infection groups.

Kim *et al.* (1982a) also conducted a hematological observation in 224 cases of *Clonorchis* infection. The data were analyzed also by the intensity of infection. The mean hematological values were within the normal range except for the differential eosinophil count in all infection groups. However, as the degree of infection became higher, the mean values of hemoglobin, hematocrit and red blood cell counts also became higher. On the other hand, the mean values of MCH, MCV and MCHC in accordance with the degree of infection of clonorchiasis did not show any difference. The increase of eosinophils was the most obvious among the changes of the blood picture in *C. sinensis* infection. As the degree of infection became higher, the eosinophil rate also became higher.

Joo and Rim (1982) experimentally infected rabbits once or several times with various doses of *Clonorchis* metacercariae and observed the eosinophil changes in the blood every week. The results confirmed that usually at the 5th week after infection the eosinophil value began to increase, and during the 7th to 9th week the value was either maintained or further increased. In the repeated infection, the increase of eosinophils was more obvious. Even though the EPG was low or the number of experimentally infesting larvae of *C. sinensis* was small, the value of eosinophils was found to be high. Accordingly, the changes in eosinophils have some relation

to repeated infections except for the degree and duration of infection.

In the blood biochemical tests in *Clonorchis* infection in Korea, many workers observed the values of GPT, GOT, alkaline phosphatase, total bilirubin, indirect bilirubin, BUN, total protein, albumin, A/G ratio, total cholesterol, cholesterol ester, TTT, *etc.* In their results, the mean values of all of the above tests were within the normal range, but the mean values of GPT, GOT, bilirubin, and cholesterol rose in accordance with infection intensity, while the total protein and albumin and the A/G ratio showed a tendency to be lowered as the degree of infection increased (Rim *et al.*, 1973; Hyun and Rim, 1977; Kim *et al.*, 1982a).

## DIAGNOSIS

The parasitological diagnosis is based on finding the characteristic eggs in the feces or duodenal bile drainage. The eggs require differentiation from those of other *Opisthorchis* spp. and heterophid flukes (Lee *et al.*, 1984). The direct smear method of recovering eggs from the feces is possible in cases of heavy infection, but in light infections the eggs may escape from recognition by this method. The method of formalin-ether or other sedimentation techniques are more reliable for detecting the eggs in feces (Rim, 1975a & 1981b). Since the introduction of the cellophane thick smear technique (Kato thick smear method) by Kato and Miura in 1954, it has become one of the most effective methods used in mass stool examination (Cho *et al.*, 1969; Lee *et al.*, 1979). It was evaluated, standardized, and adopted to large-scale routine annual fecal examination of schoolchildren in Japan by Komiya and Kobayashi (1966). Since 1969, the Korea Association of Parasite Eradication (KAPE) has conducted mass stool examinations annually of over 15 million specimens by cellophane thick smear technique for students and inhabitants throughout the country. It was recognized that the cellophane thick smear technique is very simple, economical and highly

effective for detecting the eggs of helminths. In order to correlate *Clonorchis* infection with epidemiological and clinical significance, a quantitative examination may be helpful. The quantitative methods of egg-counting in the fecal samples are of 3 types; direct smear (Beaver's direct egg-count technique), dilution (Stoll's egg-counting technique), and concentration. Among them, Stoll's dilution egg-counting method is more accurate than the others and suitable for field work. According to the level of egg counts, the patients can be divided into four groups, light infection (EPG 1~999), moderate infection (EPG 1,000~9,999), heavy infection (EPG 10,000~29,999) and very heavy infection (EPG 30,000 or over). This classification was applied first by Seo *et al.* (1969) in their studies on the status of helminthic infections in Koreans. Later Rim *et al.* (1973) analyzed clinical symptoms with this classification.

There are some other techniques for detecting eggs or adult worms in diagnosis of clonorchiasis. One is an examination of duodenal fluid collected by duodenoscopy, in which the aim is to collect the eggs as near as possible to where they were laid and before they become diluted in the feces. The other method is the Entero test<sup>®</sup>, which uses a cotton thread fixed to a very light weight, both of which are enclosed in a capsule, except for the free end of the thread. The patient swallows the capsule and the end of the thread is held outside the mouth. After digestion of the capsule, the weight unrolls the encapsulated part of the thread and descends to the terminal part of the duodenum. The whole thread and weight are drawn back after about 3 hours, and all the material sticking to the thread is collected by scraping (Ambroise-Thomas and Goullier, 1984). Recently radiologic findings including cholangiography, sonography, and CT were attempted for the diagnosis of clonorchiasis (Lim *et al.*, 1989; Choi *et al.*, 1989; Lim, 1990). However, all these techniques present certain inconveniences to the patient and examiner alike. Nevertheless, the most reliable diagnostic technique for this infection is still the detection of

the eggs by stool examination. However, practical problems such as difficulties in stool collection and mass stool examinations require more suitable methods (a kind of immunodiagnosis) for epidemiological survey in the field.

A number of attempts have been made to use immune reactions for the diagnosis of infection with *C. sinensis*. However, immunodiagnosis is still regarded as a supplementary diagnostic method. Since the 1930s, various immunological tests; the intradermal (ID), complement fixation (CF), and indirect hemagglutination (IHA) tests, have been applied in the diagnosis of *Clonorchis* infection. Recently a number of techniques such as gel-double diffusion (DD), immunoelectrophoresis (IE), indirect fluorescent antibody (IFA) tests and enzyme-linked immunosorbent assay (ELISA) have been evaluated. The intradermal test has been widely used in Korea for the last 3 decades for the purpose of epidemiological study of clonorchiasis. This test is a rapid, sensitive and useful epidemiologic tool for studies on the prevalence of *Clonorchis* infection. However, the cross reactions with other helminth infections and moderate false reactions are the main disadvantages in its practical application for diagnosis. The CF test and IHA test are rather specific, but their clinical application is difficult because of a low sensitivity and complicated procedures.

Im (1974), and Cho and Soh (1974) attempted to evaluate the indirect fluorescent antibody test using adult worm particle antigen. Their results revealed 100% sensitivity and about 15% cross reactions with paragonimiasis, but cross reactions disappeared after the absorption of test serum with particle antigen of *Paragonimus westermani*. Recently Kwon *et al.* (1984) evaluated IFA in the diagnosis of human clonorchiasis using frozen sectioned adult worms of *C. sinensis* as an antigen. Positive results were revealed only in 72 (61.5%) out of 117 sera from clonorchiasis patients. According to the intensity of the *Clonorchis* infection, the positive reactions of IFA titers were observed in 28.1% of light infection, in 68.9% of moderate infection, in 77.8% and

84.6% of the heavy and very heavy infection groups, respectively. All serum samples from 16 cases infected with parasites other than *Clonorchis* were negative. Only 1 out of 9 sera from non-infected healthy cases showed a false positive reaction. The IFA test has a great practical advantage since the antigen is easy to prepare and reveals consistent results. Sectioned worms gave superior results to particle preparation of adult *C. sinensis*. Moreover, this test, which uses the sectioned parasite as antigen, makes it possible to localize the main antigenic sites in the parasite. In the case of *C. sinensis*, the adult worm sections displaying intense green fluorescence were located mostly in the tegumental region of the surface, as well as the intestinal epithelium of the worms. Recently ELISA has been proved as the most convenient serological method for mass screening of many parasitic diseases. Lee *et al.* (1981) stated that ELISA can be applied satisfactorily in the serologic diagnosis of clonorchiasis. Jin *et al.* (1983) compared ELISA with the Ouchterlony immunodiffusion test using immune animal sera of clonorchiasis and paragonimiasis. Yang *et al.* (1983), using veronal-buffered saline extract of *Clonorchis* as an antigen, applied ELISA in the diagnosis of human clonorchiasis. They found that ELISA is applicable in the diagnosis of human clonorchiasis as a reproducible test in compared with other immunological tests. Hahm *et al.* (1984) made a comparison of the sensitivity of 3 diagnostic tests such as ELISA, IFA and CF tests on 55 human clonorchiasis cases. Their findings indicated that ELISA was the most sensitive and specific in the diagnosis of human clonorchiasis among the 3 serological tests. To observe the usefulness of collected blood on filter paper in serodiagnosis of *C. sinensis*, Han *et al.* (1986) undertook ELISA in 142 *Clonorchis* egg-positive cases and 70 negative controls. Samples on filter paper were positive in 221 out of 140 egg-positive cases (sensitivity: 86.4%) and 69 out of 70 controls were negative (specificity: 98.6%) by ELISA, whereas serum samples showed that 132 out of 140 egg positive cases were positive

(sensitivity: 93.0%) and 67 out of 70 controls were negative (specificity: 95.7%). Therefore, blood collection on filter paper revealed a similar specificity and lower sensitivity when compared with serum samples. The mean absorbance in both samples increased with the intensity of the infection. Therefore, the blood collected on the filter paper could be used in epidemiological surveys of clonorchiasis by ELISA.

## TREATMENT

Since the late 1950s, several investigators have studied the treatment of clonorchiasis with various drugs. However, none had been proved to be effective as well as nontoxic to the host. In 1965 the excellent therapeutic effect of Hetol (1,4 bis-trichloromethylbenzol) on clonorchiasis was observed by investigators in Japan and Korea (Yokogawa *et al.*, 1965 a & b; Seo *et al.*, 1965; Cho *et al.*, 1966a & b). However, the use of Hetol was halted for clinical trials because of its toxicity. There were no drugs of choice for the treatment of clonorchiasis. Rim (1972 & 1975b) carried out animal experiments and clinical trials by using dehydroemetine-late-release tablets (a synthetic compound with emetine-like properties but less toxic than emetine) against *C. sinensis*. The results showed a high effectiveness with a dose of 2.5 mg/kg every other day for 25 to 30 days. Although dehydroemetine is less toxic than emetine, it should not be given to patients with cardiac insufficiency. Meantime, Rim (1972 & 1975b), and Rim and Lee (1979) treated 49 adult patients with *C. sinensis* ova in their stools with niclofolan (Bayer 9015; Bilevon®). The result showed that the drug in doses of 1.0 or 2.0 mg/kg body weight for 2 or 3 days was highly effective against clonorchiasis. The same results were confirmed by Soh and Im (1977). Therefore, niclofolan showed highly curative effects with only 2 medications, but adverse effects were so severe and frequent that the drug was not adopted in clinical cases.

Praziquantel, an acetylated isoquinolino-pyrazine, is a new anthelmintic agent with a high

rate of activity against various cestode and schistosome infection in man and animals. Rim (1978 & 1983) reported for the first time that praziquantel is the drug of choice for the treatment of clonorchiasis, paragonimiasis and metagonimiasis. Rim *et al.* (1979 & 1981) described that for the complete treatment of clonorchiasis 25 mg praziquantel per kg of body weight should be administered orally 3 times a day (at interval of 5 to 6 hours) for 2 days. With a dosage of  $3 \times 25$  mg/kg, even in a single day, more than 85.0% were cured and an egg reduction rate of 99.5% was obtained. Praziquantel is very well tolerated, and side effects consist of mild and transient headache and dizziness. An electron microscopic study observed the morphological changes of *C. sinensis* obtained from experimentally infected rats treated with praziquantel (Rim *et al.*, 1980). By scanning electron microscopic observation, the regular pattern of the tegumental ridges was significantly changed on the outer surfaces of the parasite. On the other hand, by transmission electron microscopic observation, the numerous mitochondriae in the syncytial tegumental layer of the treated parasites appeared to be degenerated and to have formed small vacuoles, of which different sizes were interspersed in the distal part of the syncytial tegument and also in the subepithelial region of the urinary bladder. These vacuoles were fused with each other and lead to the disruption of the apical region of the syncytial tegument along the basement layer, so that the tegumental layer appeared as a large balloon. Kim *et al.* (1982) observed that degeneration of the vesicles occurred in the nerve bulb of the sensory papillae around the suckers and excretory pore at 6 hours after treatment with praziquantel. At 12 hours after treatment, degenerated changes on the tegument of *C. sinensis* occurred by considerable vacuolization of the tegumental syncytium near the sensory papillae. At 24 hours after treatment, vacuolization had increased markedly in the tegumental syncytium, and vacuoles appeared in the tegumental ridge. The vacuoles finally ruptured on the tegumental surface and

formed bleb-like structures. In the course of time, the bleb-like structures increased in number all over the surface of the worm. The balloon-shaped structure that formed at the anterior part of the worm 12 hours after treatment is supposed to be caused by accumulated excretory material due to the loss of excretory function. Therefore, the bleb-like structures and a balloon-shaped structure on the surface of the worms led to its death by the bursting of the tegument in this region.

Recently, albendazole (Zentel®) has been found to be a broad-spectrum anthelmintic with high activity against intestinal nematodes, as well as trematodes and cestodes infections. Rim *et al.* (1984) reported that several clonorchiasis patients were treated successfully with 3 oral doses of 600 mg of albendazole daily for 2 or 3 consecutive days. Considering the ease of administration and its good acceptance as well as excellent efficacy, however, it seems praziquantel can play a great role in the control of clonorchiasis.

In 1982, the Ministry of Health and Social Affairs and the Korea Association for Parasite Eradication conducted a pilot project of treatment for clonorchiasis using praziquantel in order to find the most convenient and effective dosage schedule of the drug when used in mass chemotherapeutic control. As a result, a single dose of 40 mg/kg of praziquantel was recommended for large-scale treatment of *C. sinensis* infection under field conditions (Lee, 1984).

## PREVENTION AND CONTROL

According to Rim (1986), the control of clonorchiasis is fundamentally an enterprise directed at reducing or eliminating the transmission of the disease, so that no more new infections, reinfections or superinfections occur. There are several methods to control clonorchiasis in endemic areas: (1) Reduction of the sources of infection by treatment, (2) Sterilization of the feces, (3) Protection of fish ponds from contamination with night-soil, (4) Control of snail hosts, and (5) Health education.

The control of clonorchiasis is theoretically very simple, because the infection can only be contracted by ingestion of encysted metacercariae when the raw intermediate host (freshwater fish) is eaten. Therefore, the most practical method of preventing human infection is to avoid eating raw, freshly pickled, or imperfectly cooked freshwater fish (1982). However, it is exceedingly difficult to carry out such simple measures in the face of centuries-old traditions, to which the population clings with great tenacity. In districts where it is customary to eat raw fish, educational programs and materials stressing the importance of thoroughly cooking all freshwater fish appear to be the most effective means of preventing clonorchiasis (Rim, 1976 & 1977).

Educational efforts should be directed primarily toward schoolchildren because they are less entrenched in their food habits. Consequently, the educational process should be comprised of various levels to successively change knowledge, attitudes, behaviour, habits and customs of their lives. The choice of methods must be dictated by the nature of the environment, the habits and customs of the people, the pattern of transmission and the resources of the country (Rim, 1984). However, chemotherapy has proved to be a rapid and effective method of control. Therefore, a combination of efforts with major emphasis on health education and mass treatment with praziquantel coupled with governmental aid could reduce the disease.

According to Choi *et al.* (1976), who compared the prevalence rate of clonorchiasis in certain areas in Korea between 1964 and 1976, a marked reduction in prevalence was encountered in the younger age group, but there was no significant difference in the older age groups. The overall prevalence rate for clonorchiasis was reduced from 27.7% to 19.6% during a 10-years period. They concluded that health education was influential in the general decrease in infection. However, traditional ways of living are changing in Korea. The mechanization of farms, the use of chemical fertilizers, pesticides, and insecticides may have affected the parasite or its

intermediate hosts. At the same time, cultural, dietary and economic changes may have had their effect (Rim, 1979).

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