

Epidemiological Report

Epidemiological Investigation on *Clonorchis sinensis* in Human Population in an Area of South China

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SUMMARY: To detect the prevalence of *Clonorchis sinensis*, one of the important helminthes in the human population of the Guangxi Region where *Schistosoma japonicum* was endemic but eliminated in the late 1980s, the Kato-Katz thick smear technique was used for examining fecal samples from selected townships in Hengxian County. Among 1,552 people examined, 491 (31.6%) were found infected with *C. sinensis*. By counting eggs per gram feces (EPG), it was found that the light, moderate, and heavy intensities of infection occupied 55.4, 33.0, and 11.6%, respectively, with an average EPG of 4,845 in the infected subjects. The survey revealed that the prevalence in the age groups of 0-9 and 10-19 years old was less than 10% but was 45-50% in the groups between 30-39 and 60-69 years old. A much higher prevalence was demonstrated in the male population (41.9%) than in the females (20.5%), and heavier intensity of infection was also found in the males than in the females. These results indicated that the prevalence of this liver fluke is increasing in the past decade in that region, and there is an urgent need to further assess the epidemiological factors in reference to the area's changing socio-economic conditions and human behavior, contamination of the environment and fish ponds, inadequate farming/fishery practices, and the infection of domestic animals.

INTRODUCTION

The Guangxi Zhuang Autonomous Region was one of the provinces in China where *Schistosoma japonicum* was prevalent. Since the mid-1950s, by extensive interventions of mass detection, chemotherapy for both human and cattle population, and intermediate host (*Oncomelania* snail) control, the elimination of schistosomiasis was announced in 1989 (1-3). Meanwhile, other helminthes are highly prevalent in the region due to its relatively poor socioeconomic development, particularly in areas with ethnic populations. In the first nationwide randomly sampled survey on human parasitic infections 10 years ago, the overall infection rate of intestinal parasites in Guangxi was 85%, and the average prevalence of *Ascaris lumbricoides*, hookworms, *Trichuris trichiura*, and *C. sinensis* in the region was 66, 38, 47, and 1.2%, respectively (4). Investigations of *C. sinensis* during 1978-1997 revealed a prevalence ranging from 0.08 to 75% in different areas in which the clonorchis infection had been previously reported (5). *Taenia* spp. was endemic in 30 counties, mostly those with ethnic minorities. An average prevalence was 0.36% with the highest rate of 39% in a township of Rongshui County (6). Natural nidi of *Paragonimus westermani* and other paragonimus species were detected in 15 counties. A positive rate of 42% by skin test and a rate of 7% by sputum or stool examination for eggs were reported in selected villages (7).

In order to understand the prevalence of helminth infec-

tions other than schistosomiasis and to provide evidence for developing control strategies, an epidemiological study in selected areas was proposed. This paper reports the findings from a survey regarding *C. sinensis* infection.

MATERIALS AND METHODS

Areas and subjects: Hengxian County, formerly an area in which schistosomiasis was present, was selected for the investigation of *C. sinensis* infection. Three spots (townships) in the county were identified and the whole population in each place, about 500 people, was included in the study.

Methods: The modified Kato thick smear method (Kato-Katz technique) was used for fecal examination (three slides for each fecal sample) to detect eggs of *C. sinensis* and intestinal nematodes, and an egg count was made to determine the intensity of clonorchis infection (number of eggs per gram feces [EPG]). Following the criteria set by the Chinese Ministry of Health (8), an EPG of <1,000, between 1,000 and 9,999, and >10,000 was identified as light, moderate, and heavy infection, respectively.

By using test kits purchased from Shenzhen Luhan Biotechnic Co. (Shenzhen, Guangdong, China), conventional enzyme-linked immunosorbent assay (ELISA), using crude antigen of clonorchis adult worms, was performed for sera from partial examinees with or without clonorchis eggs. The preparation of the antigen followed a routine procedure: ground powder of lyophilized adult worms was defatted by acetone, normal saline with thiomersalate was added for 72 h extraction, and the supernatant after centrifugation was then used as crude antigen. The stools of those with positive serological test but who were shown to be egg negative by Kato-Katz technique were reexamined using the formalin-ether concentration method.

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Table 1. Fecal examination for the infection of *Clonorchis sinensis* in Hengxian

Township Surveyed	No. examined	Prevalence		Intensity of infection*			
		No. infected	%	Light %	Moderate %	Heavy %	Mean EPG
Fucheng	545	205	37.6	59.5	30.7	9.8	3034.8
Pinglang	502	157	31.3	64.3	30.6	5.1	2451.8
Pingma	505	129	25.2	38.0	39.5	22.5	9049.1
Total	1552	491	31.6	55.4	33.0	11.6	4845.2

* Light infection: eggs per gram feces (EPG) 1-999; moderate infection: EPG 1000-9999; heavy infection: EPG \geq 10000.

Table 2. Sex distribution of *Clonorchis sinensis* infection in Hengxian

Township Surveyed	No. examined	Male			Female		
		No. examined	No. egg positive	%	No. examined	No. egg positive	%
Fucheng	545	283	147	51.9	262	58	22.1
Pinglang	502	266	100	37.4	236	57	24.2
Pingma	505	260	92	35.4	245	37	15.1
Total	1552	809	339	41.9	743	152	20.5

$\chi^2 = 25.2367, P < 0.001$.

The $R \times C$ chi square (χ^2) test was used for statistical analysis.

RESULTS

Prevalence of the infections of *C. sinensis* and intestinal nematodes: A population of 1,552 from the townships of Fucheng, Pinglang, and Pingma in Hengxian were sampled for the survey. The overall prevalence of helminth infections including intestinal nematodes and *C. sinensis* was 48.9% ranging from 44.2 to 53.8%. The average prevalence of *A. lumbricoides*, *T. trichiura*, and hookworm infections was 15.9, 12.8, and 7.5%, respectively. The prevalence of clonorchis infection was 37.6, 31.3, and 25.2% in the three townships, respectively, with an average of 31.6%.

The intensity of clonorchis infection: According to the Manual of Intestinal Parasite Control, the number of EPG is used to measure the intensity of clonorchis infection. Among the positive fecal specimens, light infection occupied 55.4% (ranging from 38 to 64.3% in the townships), moderate infection 33.0% (30.6-39.5%), and heavy infection 11.6% (5.1-22.5%). The average EPG was 5159.5 (Table 1). The highest EPG was 105,840 in one case in Pingma.

Age distribution of clonorchis infection: The prevalence was 4.3 and 8.7% in the 0-9 and 10-19-year-old age groups, respectively. It reached a plateau from the 30-39-year-old age group (50.2%) to the 60-69-year-old age group (45.4%) (Fig. 1). Statistical analysis showed an extremely significant difference in the prevalence among the age groups from 0-9 to 30-39 years old ($P < 0.0001$). In regard to the intensity of infection, all the cases in the 0-9-year-old group showed light infection; heavy infection occupied 6-7% in the 10-19 and 20-29-year-old groups, and from 11.3 to 16.7% in the other groups. Evidently, the adult population was more affected.

Sex distribution of clonorchis infection: As revealed in Table 2, the prevalence was much higher in males (41.9%) than in females (20.5%) ($P < 0.001$). The intensity of infection was also heavier in the male population than in the female population: light, moderate, and heavy infection

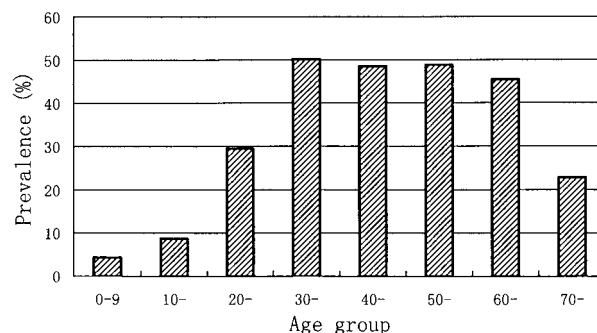


Fig. 1. Age distribution of *Clonorchis sinensis* infection. $\chi^2 = 184.7649, P < 0.0001$ (among age groups from 0-9 to 30-39).

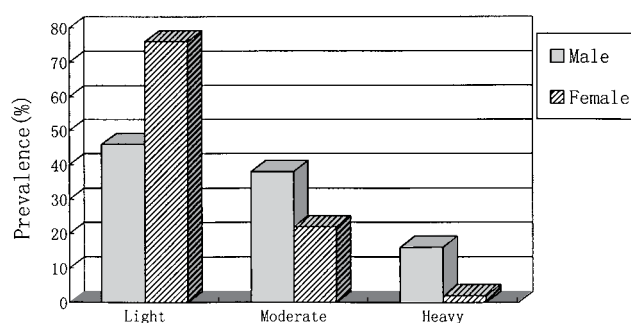


Fig. 2. Intensity of infection in male and female population groups.

occupied 46, 38, and 16%, respectively, in the males, while in the females, 76, 22, and 2%, respectively (Fig. 2) ($P < 0.0001$).

Result of serological test: ELISA was performed for serum samples taken from clonorchis egg-positives, with 191 out of 205 sera (93.2%) showing positive reaction. The serological test was also used in 207 subjects in which no eggs had been found by the Kato-Katz method; 12 (5.6%) were positive for the antibody. Fecal samples from these 12 cases were examined again using the formalin-ether concentration

method, in which approximately 2 g of fecal material was used, and clonorchis eggs were found in eight cases (66.6%).

DISCUSSION

In 1989, Hengxian County was identified as a pilot area for the national survey on human parasites (4), and the prevalence of clonorchiasis was 18% and those of ascaris, hookworm, and trichuris infection was 71, 34, and 55%, respectively. In more than a decade, the prevalence of ascaris, hookworms, and trichuris infection significantly decreased to 15.9, 7.5, and 12.8%, respectively. This was partly due to the local control activities in the 1990s, including deworming of students of primary and secondary schools. The improvement of living standards, environmental hygiene, and health literacy among local people also helped reduce the transmission of the soil-transmitted helminthes. The investigation in Hengxian County revealed again that clonorchis infection is apparently an important problem, with an increase of its prevalence from 18 to 31.5%. There may be epidemiological and socioeconomic factors that positively affected the prevalence of clonorchis infection. For instance, people in that area received higher incomes due to the socioeconomic reforms of the past decade and they preferred and could afford raw freshwater fish – a dish which was believed to be highly delicious, nutritious, and expensive. In addition, no specific control program was established for liver fluke, and the routine single-dose anthelmintics are not effective for the fluke.

People at any age can be infected by *C. sinensis*. Depending on the modes of fish-eating, the most affected population groups in China can be either children who became infected by eating small roast fish while playing in the field such as in some central provinces, or adults who contracted infection by consuming fish dishes which are raw or undercooked (9). This study showed a much higher prevalence in the adult population than in children and adolescents, a phenomenon often seen in the southern provinces.

As to the relationship between prevalence and sex, the investigation demonstrated that the infection rate was double in males in comparison to females. The intensity of infection also showed great degrees of difference with more heavy and moderate infections and less light infections in males than in females. It is not clear why the males contract more and heavier clonorchis infections. One possible reason might be the more frequent social activities and dining opportunities at restaurants for male adults than for females. It is interesting to note that the age and sex distribution of clonorchis infection in some areas of South Korea was quite coincident with what we found in Guangxi (10).

In some areas, serological tests have been used for epidemiological screening of clonorchis infection. In this study, ELISA was used in a portion of the subjects with or without a positive stool examination. It was interesting that by applying the formalin-ether concentration technique, eggs were found in fecal samples of two-thirds of the anti-clonorchis antibody positives. Apparently, the concentration method is more sensitive for detecting clonorchis eggs than is the Kato-Katz technique because the former technique requires a much larger quantity of fecal material and provides a clearer microscopical field for identifying the small eggs. This is virtually consistent with the previous studies which recommended that Kato-Katz method, with its practicality and feasibility, be used for mass survey in the field while the formalin-ether concen-

tration technique be conducted for clinical diagnosis due to its higher sensitivity, though it is more time-consuming (9).

It seems necessary to indicate that it has been difficult to confirm an infection of *C. sinensis* only by eggs in cellophane-covered (Kato-Katz) thick smears in areas where intestinal trematodes in the family Heterophyidae may exist, which excrete similar small-type eggs in feces. In the southeastern provinces such as Guangdong and Fujian, by identification of adult worms expelled, trematodes other than *C. sinensis* were reported including *Hyterophyes hyterophyes*, *Metagonimus yokogawai*, *Haplochis pumilio*, and *Centrocestus formosanus*, and one case of the latter (*C. formosanus*) was recorded in Guangxi (4). Although these intestinal trematodes were not widely prevalent, mixed infection with *C. sinensis* is possible in some areas. Though intestinal trematodes other than *C. sinensis* were not found in the area surveyed previously and this time, it would be of interest to determine in further examination if these flukes are present in Hengxian County.

While the endemicity of soil-transmitted helminthes is decreasing, *C. sinensis* has become more prevalent than it was a decade ago in the region surveyed. It is not clear what important epidemiological factors may have contributed to the increase of the clonorchis prevalence. Further study is of great importance, especially in reference to the area's changing socioeconomic conditions and human behavior, contamination of the environment and fish ponds by the unhygienic latrines and human/animal excreta, inadequate farming/fishery practices, and the infection of domestic animals such as cats, dogs, and pigs. Epidemiological clarification of these factors will be essential for developing an effective control strategy for clonorchiasis.

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