

Collection of *Clonorchis sinensis* adult worms from infected humans after praziquantel treatment

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Abstract: A cohort was established for evaluation of cancer risk factors in Sancheong-gun, Gyeongsangnam-do, Korea. As one of the cohort studies, stools of 947 residents (403 males and 544 females, age range: 29-86 years) were screened for *Clonorchis sinensis* eggs using both Kato-Katz method and formalin-ether sedimentation technique. The overall egg positive rate of *C. sinensis* was 37.7% and individual EPG (eggs per gram of feces) counts ranged from 24 to 28,800. Eight egg positive residents voluntarily joined a process of collection of the passed worms after praziquantel treatment. A total of 158 worms were recovered from 5 of the 8 treated persons, ranged from 3 to 108 in each individual. The worms were 15-20 mm x 2-3 mm in size, and showed brown-pigmented, red, or white body colors. This is the first collection record of *C. sinensis* adult worms from humans through anthelmintic treatment and purgation. The adult worms of *C. sinensis* may be paralyzed by praziquantel and then discharged passively through bile flow in the bile duct and by peristaltic movement of the bowel.

Key words: *Clonorchis sinensis*, praziquantel, human, recovery, adult worms

Clonorchis sinensis Looss, 1907 is one of the major human trematodes in East Asia, such as China, Korea, East Russia, Taiwan, and Vietnam (Hong, 2003; Rim, 2005). In Korea, clonorchiasis is still prevalent and several endemic areas are scattered along major rivers, although other intestinal parasites have been dramatically controlled by the nationwide control program (Hong et al., 2006). The 7th national survey on prevalence of intestinal parasites in 2004 found that egg positive rate of *C. sinensis* was 2.9% and

about 1.3 million people are estimated to be infected in Korea (Korea Association of Health Promotion, 2004).

Most of the infected people of clonorchiasis are asymptomatic, but they usually complain of non-specific symptoms, such as anorexia, nausea, vomiting, loose stool, and abdominal or epigastric discomfort, when they are heavily infected (Hong, 2003). Many complications may occur in chronic clonorchiasis of heavy intensity, and the most serious one is cholangiocarcinoma. Incidence and mortality of cholangiocarcinoma correspond well with clonorchiasis in Korea (Choi et al., 2006; Lim et al., 2006).

Praziquantel (PZQ), a derivative of pyrazinoisoquinoline, is the drug of choice for treatment of trematodes (Greenberg, 2005; Rim, 2005). The recommend-

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Table 1. Summary of worm recovery from 8 volunteers by praziquantel treatment and purgation

Individuals	Age	EPG counts	No. of worms recovered
A	68	28,800	- ^{a)}
B	56	12,240	108
C	47	1,440	6
D	49	1,368	- ^{a)}
E	63	1,344	11
F	46	1,224	30
G	64	1,128	- ^{a)}
H	45	1,080	3

^{a)}No worms were recovered.

ed regimen for clonorchiasis is 25 mg/kg, 3 times at 5-hr intervals in a day. However, the recommended dosage of PZQ is not completely effective, with the cure rate of 83-85% in clonorchiasis (Rim et al., 1981; Seo et al., 1983). Control of clonorchiasis by PZQ treatment was tried in the field, but the result was incomplete in spite of repeated treatments (Hong et al., 1998).

We established a cohort study in Sacheong-gun, Gyeongsangnam-do to evaluate risk factors of cancers in Korea, and examined the prevalence of clonorchiasis as a program of the cohort study in July 2006. The study site is a known endemic area of clonorchiasis. The present study protocol was approved by the institutional review board of the National Cancer Center of Korea, and informed consent was obtained from subjected residents. A total of 947 residents (age range, 29-86 years; median age, 64 years) were subjected to the examination, including 403 males and 544 females. The subjects were examined their stools by both Kato-Katz method and formalin-ether sedimentation technique.

The egg positive rate of *C. sinensis* was 37.7%, and EPG (eggs per gram of feces) counts determined by Kato-Katz method ranged from 24 to 28,800 and most of infected persons had light infection burdens (EPG < 1,000). All of the infected people were treated with 3 doses of 25 mg/kg PZQ in a day.

We tried to recover adult worms of *C. sinensis*, which passed through the intestine after PZQ treatment in 8 volunteers (EPG > 1,000), who fully agreed

to join the worm collection protocol. They were administered with 25 mg/kg PZQ, 3 times a day, and purgation was induced on the next day by drinking of a purgative drug (Colyte[®]-F, Taejoon Pharm Co, Seoul, Korea). During the purgation, they were encouraged to drink as much water as possible to facilitate diarrhea and to prevent dehydration. Whole fecal samples were collected until no worms were detected in stools, and the worms were recovered from diarrheic stools.

A total of 158 adult worms of *C. sinensis* were recovered from 5 of the 8 volunteers (Table 1). Worm recovery in each individual ranged from 3 to 108, and the highest number of worms (n = 108) were collected from a heavily infected person (EPG = 12,240). Although it was difficult to predict the exact worm burden in the infected person due to a small number of subjects in this study, it was expected to recover hundreds of worms from a person with EPG higher than 10,000. Failure of worm recovery in 3 volunteers may be due to evacuation of their stool before the purgation, or by late passing of the worms from the bile duct because of the ductal pathology. Of course there still remained a possibility of lost worms during washing and collection from fecal materials, although the process was carefully carried.

The recovered worms were fully matured and 15-20 mm x 2-3 mm in size, but, interestingly, their body color varied according to individuals; dark brown, red, and white (Fig. 1). On the contrary, the live worms recovered directly from the liver of infected animals are usually red. We considered that the dead worms with damaged tegument by PZQ treatment lost function of their tegument, and therefore, they were stained or destained by bile, digestive fluid, or intestinal contents during the passage through the intestinal tract.

PZQ causes 2 major effects on flatworms by disruption of Ca²⁺ homeostasis; a rapid, sustained muscular contraction and tegumental disruption of flatworms (Greenberg, 2005). Major changes in PZQ-treated trematodes are vacuolization of the tegument and subtegumental parenchymal layers, and narrowing of the intestinal lumens (Seo et al., 1985). The body size

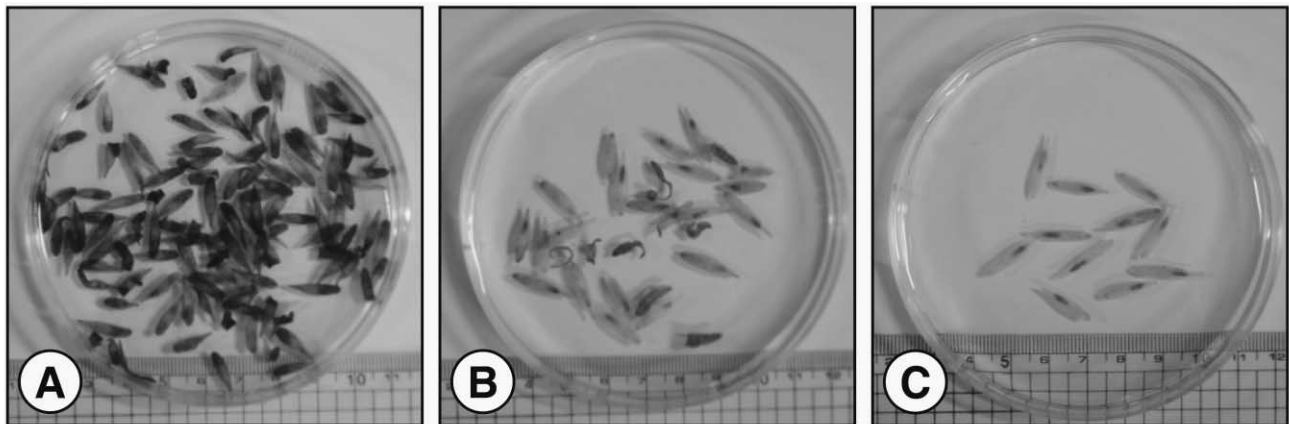


Fig. 1. Adult worms of *Clonorchis sinensis* recovered from infected persons by praziquantel treatment and purgation. The worms were dark brown (A), red (B), or white (C). A, B, and C worms were recovered from the subjects B, F, and E in Table 1, respectively.

decreases by contraction at the early phase, and increases later by extensive vacuolization and relaxation. In the present study, the recovered worms showed no activity and were relaxed, indicating that they were detached from their habitat, intrahepatic bile ducts, and expelled from the infected persons by peristaltic movement of the intestinal tract.

Worm recovery by PZQ treatment and purgation from the infected persons provides crucial information for the definite diagnosis of intestinal infection by trematodes or cestodes (Chai et al., 2000). Especially, in case of intestinal trematode infections, it is very helpful for the diagnosis, because stool examination sometimes can not provide a clue for the species of trematodes due to similar morphology of eggs between heterophyids and *C. sinensis* (Lee et al., 1984). Further human infection by unidentified parasites can be benefited by worm recovery (Hong et al., 1988; Lee et al., 1993). It also provides the information of the worm burden in the infected persons; however, it is not always accurate as shown by this study.

The present study first describes collection of adult worms in *C. sinensis*-infected persons through anthelmintic treatment and purgation. We also believe that the collected worms must be regarded as minimum confirmed numbers recovered from the subject persons.

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