[Jpn. J. Parasitol., Vol. 44, No. 2, 112–118, April, 1995]

Larval Digenetic Trematodes from Fresh Water Fish in River Miryang, Korea

HYUN HEE KONG, BYUNG RYOL CHOI, CHU HWAN MOON AND DONG WIK CHOI

(Accepted for publication; March 22, 1995)

Abstract

Fifteen species of fresh water fish collected in the 3 localities, Yuchon, Kagok-ri and Miryang-shi of the river Miryang, Kyungsangnam-do (Kyungnam Province), Korea were examined for encysted larvae of digenetic trematodes from March to October, 1993. Six species of metacercariae, *Clonorchis sinensis*, *Cyathocotyle orientalis*, *Exorchis oviformis*, *Echinochasmus* sp., *Metacercaria hasegawai* and *Metagonimus* sp., and 2 unknown species of larvae were found in the flesh of 13 species of fish. The infestation rate and density with the metacercaria of *C. sinensis* was low on the whole. *Gnathopogon atromaculatus* was most heavily infested, with the average of 13.1 cysts per gram of flesh, while *Pseudorasbora parva* harbored a few cysts only but was most heavily infested with cysts of *C. orientalis*. It is found that the river Miryang is still one of the enzootic areas of *C. sinensis* in Kyungnam Province, Korea.

Key words: Larval digenetic trematodes, *Clonorchis sinensis*, enzootic area, Korea

Epidemiological studies of the liver fluke, *Clonorchis sinensis* in Kyungsangnamdo (Kyungnam Province), Korea have been performed by many investigators. The high prevalence of *C. sinensis* among residents in the vicinity of Chinju was reported for the first time by Kojima and Ko (1919). The following year, Kobayashi (1920) presented the infestation patterns of the larval trematodes in fish host and the infection rate for *C. sinensis* among residents in the Kimhae area. The extraordinarily high prevalence of *C. sinensis* among the residents in the Changyong district was reported by Furuyama (1927a, b) and Kobayashi (1927). The prevalence in the Kimhae plain was reviewed by Sekigutchi *et al.* (1937a, b).

Subsequently, results of further frequent epidemiological studies in Kyungnam Province, Korea have been reported by Kang (1955) and Lee (1956) in Kimhae area; by Walton and Chyu (1959) in Ulsan-shi and Miryang-shi; by Chun (1962, 1964) in the vicinity of the river Naktong; by Ahn *et al.* (1966) in Namhae-gun; by Chang (1967) in Haenam-gun; by Kim and Song (1971) and Song (1971) in several areas of Kyungnam Province; by Choi (1977) in the vicinity of the river Nam; and by Hwang (1978) in the river Hwang basin of Kyungnam Province, Korea. The extensive survey of *C. sinensis* in man, snail and fish hosts in the vicinity of the river Taewha by Joo (1980) indicated that clonorchiasis among the residents was still high. The studies of larval digenetic trematodes from fresh water fish in Kyungnam Province were chiefly concerned with the demonstration of *C. sinensis* metacercaria.

The purpose of this study is to assess the results obtained on the encysted larvae of digenetic trematodes among fresh water fish collected in the river Miryang, Kyungnam Province, Korea.

Department of Parasitology, Kyungpook National University School of Medicine, Taegu 700-422, Korea.

Geography of River Miryang

The river Miryang, about 25 kilometer in length, has its origin in the northern part of Sangdong-myon, Miryang-gun, where the river Chongdo and the river Dongchang join.

The river Miryang runs down to Sanoe-myon, where it is joined by the river Tanjang. It then runs through Miryang-shi and is joined again by small rivulets in the course of running between Sangnam-myon and Samrangjin-up. Finally, it runs into the river Naktong (Fig. 1).

The river is about 10 meters above sea level and the soil of the bed is mainly composed of sand and

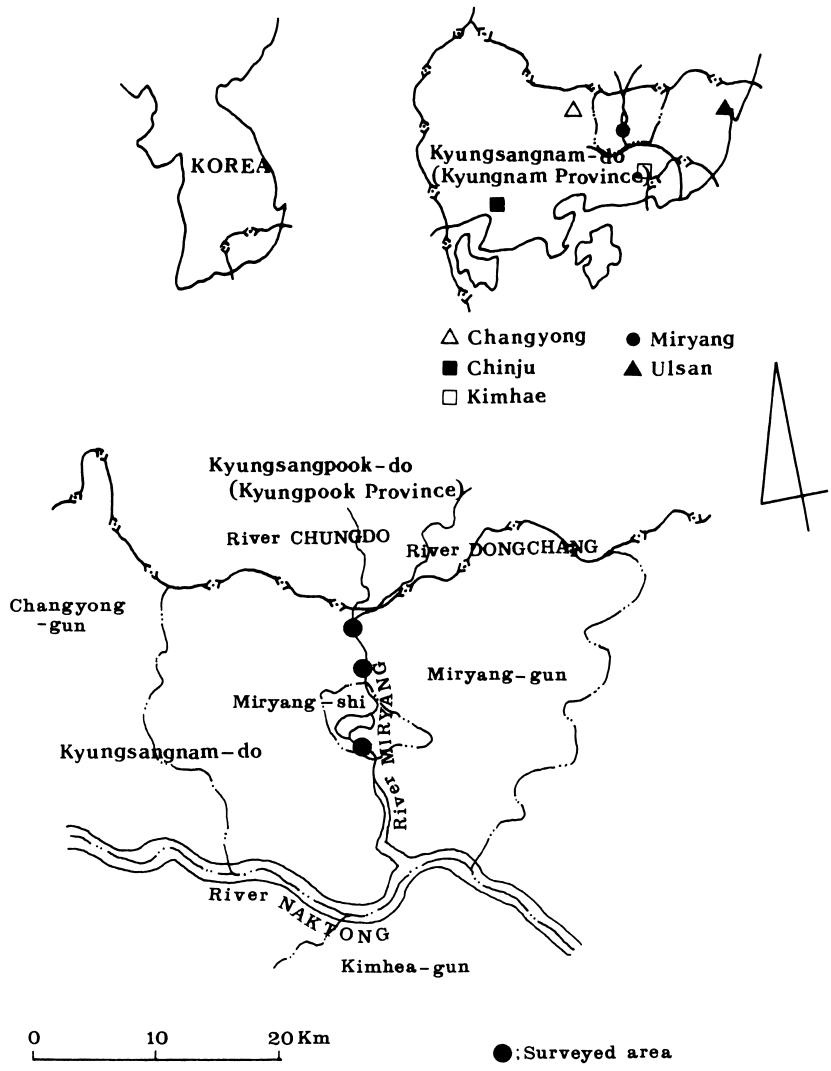

Fig. 1 The map of showing the surveyed areas in the river Miryang, Kyungsangnam-do, Korea.

rock with mud and grasses. All day long, there is abundant flowing water in the river and it holds many fresh water fish.

Materials and Methods

Localities surveyed

Fish were collected at 3 localities, Yuchon, Kagok-ri and Miryang-shi, from the river Miryang, Kyungnam Province, Korea. Unfortunately, it was impossible to collect fish in the lower stream, because of unfavorable fishing and environmental conditions.

Collection of fish

Fresh water fish were collected mostly with a castnet and glass bowls containing crushed oil cake as a bait, and sometimes by fishing with rod and line, from March to October, 1993.

Determination of infestation and density with larval trematodes

The intestinal contents of the fish were removed to prevent autodigestion and transported to the laboratory of the Department of Parasitology, Kyungpook National University School of Medicine, Taegu, Korea.

The species of the fish was identified according to the keys described by Kim and Kang (1993). The weight and length of each fish was measured after species identification.

One gram of flesh was removed from the trunk of each fish and examined for the presence of the encysted larvae of digenetic trematodes by compressing it between 2 large slide glasses (70×90mm) under a dissecting microscope.

In order to isolate the encysted larvae from the flesh and to count the number of the larvae, the artificial digestion technique was employed. One gram of the flesh was mixed with artificial gastric juices, which consisted of 3.0 ml of diluted hydrochloric acid and 0.3 g of pepsin per 100 ml of distilled water. The mixture was incubated in a water-bath at 37°C for 3 hours, stirred with a glass rod and allowed to stand for a minute to gather the isolated cysts on the central portion of the beaker.

In order to differentiate the larval trematodes, the cysts obtained were observed morphologically for the determination of species following Komiya and Tajimi (1941), and Yamaguti (1958).

Results

Table 1 shows the species and number of fresh water fish collected in the river Miryang, a large tributary of the river Naktong in Kyungnam Province, Korea. Fifteen species of fish were collected in the river. The species of fish were more abundant in the flow near the Miryang-shi than in that of Yuchon and Kagok-ri. Seven species of fish, the oily bitterlings, *Acheilognathus limbata*, the Korean shiners, *Gnathopogon atromaculatus*, the steed barbels, *Hemibarbus labeo*, the dark sleepers, *Odontobutis obscura*, the flat bitterlings, *Paracheilognathus rhombea*, the pale chubs, *Zacco platypus*, and the dark chubs, *Zacco temmincki* were common inhabitants of the river. In the present study, the crucian carps, *Carassius auratus*, the bride bitterlings, *Rhodeus uyekii* and the southern top-mouthed minnows, *Pseudorasbora parva*, which are common pond inhabitants were collected in the river.

Table 2 lists the infestation rates and densities of the encysted larvae of digenetic trematodes from fresh water fish in the river Miryang.

Six species of metacercariae, *C. sinensis*, *Cyathocotyle orientalis*, *Exorchis oviformis*, *Echinochasmus* sp., *Metacercaria hasegawai* and *Metagonimus* sp. and 2 unknown species of larvae were found in the flesh of 13 species of fish. The encysted larvae of *C. orientalis* were demonstrated from 11 species of fish, with the recovery rate from 25.0 to 100 %. The larvae of *E. oviformis* were demonstrated from 10 species of fish, followed by *C. sinensis* and *M. hasegawai* from 7 species, *Echinochasmus* sp. from 2 species and *Metagonimus* sp. from 1 species of fish.

Of the 7 species of fish with *C. sinensis*, the most frequently infested fish were *P. parva* and the sandy fish, *Microphysogobio koreensis*, followed by *G. atromaculatus*, the striped shiners, *Puntungia herzi*, the slender bitterlings, *Acheilognathus intermedia* and *P. rhombea*. The least frequently infested was *A. limbata*.

Except for the cysts of *C. orientalis* in *P. parva*, the density of larval trematodes in the fish was somewhat low. The average number of metacercariae per gram of flesh ranged from 0.1 to 16.6

In the density of infestation with *C. sinensis* metacercaria, *G. atromaculatus* was the most heavily infested and the average number of the metacercariae per gram of flesh was 13.1. *P. parva* and *P. herzi* were lightly infested, the average number of the former being 3.0 and the latter 2.3. The remaining 4 species of fish were infested with 1 or less than 1 cyst.

The metacercarial density of *C. orientalis* in the flesh of *A. limbata*, *M. koreensis*, *P. rhombea* and *P. parva* were heavier than those of *C. sinensis*. *P. parva* was most heavily infested, with an average of 27.0 metacercariae, *P. rhombea* and *A. limbata* were next with 16.6 and 11.8, respectively. *M. koreensis* was lightly infested with 5.0 cysts. The remaining 7 species of fish harbored few, ranging from 0.4 to 3.2.

The density of *E. oviformis* in the flesh of fish was similar to that of *C. orientalis*, but somewhat low in all species of fish.

The density of infestation with *M. hasegawai* in the 7 species of fish was very low, except for *G.*

Table 1 Fresh water fish collected in the river Miryang, Miryang-gun, Kyungsangnam-do in 1993

Species	Common name	Length‡ (mm)	No. of fish collected			
			Yuchon	Kagok-ri	Miryang-shi	Total
Acheilognathus intermedia (T et S*)	Slender bitterling	56.5	–	2	2	4
Acheilognathus limbata (T et S)	Oily bitterling	56.9	23	10	17	50
Carassius auratus (Linnaeus)	Crucian carp	77.8	–	–	8	8
Coreoperca herzi Herzenstein	Korean brook perch	46.2	–	–	3	3
Gnathopogon atromaculatus (N et P†)	Korean shiner	56.7	175	45	14	234
Hemibarbus labeo (Pallas)	Steed barbel	140.6	2	3	5	10
Lepomis macrochirus Rafinesque	Bluegill	47.5	–	–	4	4
Microphysogobio koreensis Mori	Sandy fish	82.5	–	2	–	2
Odontobutis obscura (T et S)	Dark sleeper	83.5	1	–	9	10
Paracheilognathus rhombea (T et S)	Flat bitterling	5.52	15	6	8	29
Pseudorasbora parva T et S	Southern top-mouthed minnow	73.4	–	–	3	3
Pungtungia herzi Herzenstein	Striped shiner	68.7	4	–	–	4
Rhodeus uyekii (Mori)	Bride bitterling	42.6	3	2	3	8
Zacco platypus T et S	Pale chub	66.2	17	11	10	38
Zacco temmincki T et S	Dark chub	67.3	21	18	14	53

*Temminck et Schlegel. †Nichols et Pope. ‡average body length.

atromaculatus and *P. rhombea*. The average numbers of metacercaria in *G. atromaculatus* and *P. rhombea* were 7.4 and 1.5, respectively. However, the average number in the remaining 5 species of fish was less than 1.

As shown in Table 2, the density of infestation with *Echinochasmus* sp. in 2 species of fish, *A. limbata* and *G. atromaculatus*, *Metagonimus* sp. in *G. atromaculatus*, and the unknown species of larvae in 5 species of fish was also very low.

Discussion

The present authors have been carrying out surveys to determine the sources of trematodal infection from fresh water fish collected in rivers and ponds in Kyungsangnam and pook-do (Kyungsang Provinces), Korea. It is obvious that the infestation of fresh water fish with metacercariae of digenetic trematodes in both Provinces has been decreasing from the results of recent surveys. Coincidentally, infection rates of the residents in the Provinces have also been decreasing (Kojima and Ko, 1919; Kobayashi, 1920, 1927; Furuyama, 1927a, b; Sekigutchi *et al.*, 1937a, b; Nishimura, 1943; Kang, 1955; Walton and Chyu, 1959; Shin, 1964; Ahn *et al.*, 1966; Chang, 1967; Kim and Song, 1971; Song, 1971; Seo and Lee, 1975; Choi, 1977; Hwang, 1978;

Table 2 Infestation rates and densities (in parenthesis) of larval digenetic trematodes from fresh water fish collected in the river Miryang, Miryang-gun, Kyungsangnam-do in 1993

Species	No. of fish examined	Infestation rate and density						
		*C. sinensis**	*C. orientalis*†	*E. oviformis*‡	*Echinochasmus* sp.	*M. hasegawai*§	*Metagonimus* sp.	Unknown species
A. intermedia	4	50.0 (0.5)	50.0 (1.8)	25.0 (0.8)	–	–	–	–
A. limbata	50	20.0 (0.3)	90.0 (11.8)	80.0 (6.1)	6.0 (0.2)	16.0 (0.3)	–	–
C. auratus	8	–	–	–	–	–	–	12.5 (0.2)
C. herzi	3	–	–	–	–	–	–	–
G. atromaculatus	234	96.2 (13.1)	64.5 (3.2)	1.7 (0.1)	7.5 (1.2)	94.9 (7.4)	14.5 (0.2)	15.0 (0.3)
H. labeo	10	–	40.0 (2.0)	–	–	–	–	–
L. macrochirus	4	–	–	–	–	–	–	–
M. koreensis	2	100 (1.0)	100 (5.0)	100 (3.5)	–	–	–	–
O. obscura	10	–	–	50.0 (0.4)	–	30.0 (0.1)	–	–
P. rhombea	29	41.4 (1.1)	96.6 (16.6)	75.8 (11.1)	–	31.0 (1.5)	–	10.3 (0.4)
P. parva	3	100 (3.0)	100 (27.0)	–	–	–	–	33.3 (1.0)
P. herzi	4	75.0 (2.3)	50.0 (0.5)	75.0 (6.3)	–	–	–	–
R. uyekii	8	–	25.0 (0.4)	62.5 (2.0)	–	12.5 (0.1)	–	–
Z. platypus	38	–	84.2 (2.8)	50.0 (5.3)	–	15.8 (0.5)	–	13.0 (0.3)
Z. temmincki	53	–	47.2 (1.7)	7.5 (0.1)	–	13.2 (0.1)	–	–

**Clonorchis sinensis.* †*Cyathocotyle orientalis.* ‡*Exorchis oviformis.* §*Metacercaria hasegawai.*

Chung *et al.*, 1991).

In the present study, thirteen out of 15 species of fish harbored 6 species of encysted larvae, *C. sinensis, C. orientalis, E. oviformis, Echinochasmus* species, *M. hasegawai* and *Metagonimus* species and 2 kinds of unknown larvae were found. Except for the cyst of *E. oviformis*, the density of larval trematodes in the fish were somewhat low. The average number of the larvae per gram of flesh ranged from 0.1 to 16.6 cysts. In spite of relatively high infestation rates ranging from 20.0 to 100 % for *C. sinensis* in 7 kinds of fish, density of infestation was also low, ranging from 0.3 to 13.1 cysts.

The results in the present survey are much lower in the infestation rates for the larval trematodes in fish than those reported by Chun (1962, 1964). However, the results are in accordance with those of recent surveys in the river Naktong and its tributaries (Kim and Choi, 1986; Choi and Koo, 1988; Moon and Choi, 1988; Choi *et al.*, 1990a; 1990b; 1990c; Cho *et al.*, 1991a; Choi *et al.*, 1991b).

The decreasing infection rates appear to result from the mass survey and treatment of infected residents, the decreasing infestation of fresh water fish with metacercariae of digenetic trematodes, and public health education not to eat fresh water fish in raw or undercooked.

There may be several reasons for decreasing infestation of fish intermediate hosts of digenetic trematodes. First of all, the inflow of flukes eggs into rivers and ponds has been decreasing because of lowered prevalence of flukes infections in residents, the decreased numbers of wild reservoir hosts, sanitary treatment of human excreta, and use of chemical fertilizers instead of night soil in agriculture. Secondly, the population density of first intermediate hosts has been decreasing probably owing to water pollution. In Kyungsang Provinces, Korea, almost all ponds are used as piscinae, and large amount of antibiotics and feeding stuffs are put into the ponds. Water in those ponds is too polluted to be a suitable aquatic environment for snail intermediate hosts of trematodes. Water in rivers in the Provinces are also polluted by inflow of house waste and agrichemicals.

Khan and Thulin (1991) speculated that ecto- and endoparasites of fish might be affected in a number of different ways by pollutants. Thus, pollutants might influence, directly and indirectly, the prevalence and intensity of metacercariae in fish

although there is little information which relates the intensity of the parasitic infections to pollutants (Skinner, 1982).

References

1) Ahn, Y. K., Suh, J. K. and Lim, H. C. (1966): Survey of intestinal parasites in Namhai island (Namhai Kun, Kyung-Nam province, Korea). Korean J. Parasitol., 4, 21.
2) Chang, C. H. (1967): A study on the infection with *Clonorchis* and *Paragonimus* among school children and inhabitations in Haman gun, Kyungsang Nam do, Korea. Thesis of school of Public Health. Seoul Natl. Univ., 1–30 (in Korean, English summary).
3) Cho, J. H., Chung, D. I. and Choi, D. W. (1991a): Infestation status of digenetic larval trematodes in fresh water fish caught in the river Dongchang. Kyungpook Univ. Med. J., 32, 331–338.
4) Choi, D. W., Chung, D. I. and Kee, S. K. (1990a): Infestation of larval trematodes from fresh water fish collected in the river Chongdo. Kyungpook Univ. Med. J., 31, 32–38 (in Korean, English summary).
5) Choi, D. W., Chung, D. I. and Kim, J. S. (1990b): Infestation status of digenetic larval trematodes from fresh water fish collected in the river Yang, Kyungpook Province, Korea. Kyungpook Univ. Med. J., 31, 17–25.
6) Choi, D. W., Chung, D. I. and Kim, Y. H. (1990c): Monthly incidence and intensity of infestation by *Clonorchis sinensis* metacercariae from fresh water fish collected in the river Chongdo. Kyungpook Univ. Med. J., 31, 26–31.
7) Choi, D. W., Chung, D. I. and Song, S. H. (1991b): Further study of infestation with digenetic larval trematodes in fresh water fish collected in river Bongwhang. Kyungpook Univ. Med. J., 32, 462–469.
8) Choi, D. W. and Koo, H. M. (1988): Infestation of *Clonorchis sinensis* metacercaria from fresh water fish collected in Yongcheon area, upper stream of river Kumho. Kyungpook Univ. Med. J., 29, 127–133 (in Korean, English summary).
9) Choi, J. J. (1977): Epidemiological studies of *Clonorchis sinensis* in vicinity of Chinju, Kyungnam Province, Korea. J. Pusan Med. Coll., 17, 79–89 (in Korean, English summary).
10) Chun, S. K. (1962): Epidemiological studies of *Clonorchis sinensis* in vicinity of Chinju, Kyungnam Province, Korea. J. Pusan Med. Coll., 17, 79–89 (in Korean, English summary).
11) Chun, S. K. (1964): Studies on the experiment of mode of infection of *Clonorchis sinensis*; 1. studies on the metacercaria of some trematodes parasitic in fresh water fishes. Bull. Pusan Fish. Coll., 6, 1–11 (in Korean, English summary).
12) Chung, D. I., Kim, Y. I., Lee, K. R. and Choi, D. W. (1991): Epidemiological studies of digenetic trematodes in Yongyang county, Kyungpook Province. Korean J. Parasitol., 29, 325–338.
13) Furuyama, T. (1927a): Distribution of intestinal helminths in the Changyeong area, Kyungnam Province, with special reference to prevalence of Clonorchiasis. Mansen No. Ikai., 80, 41–48 (in Japanese).
14) Furuyama, T. (1927b): Distribution of intestinal helminths in the Changyeong area, with special reference to prevalence of Clonorchiasis. J. Chosen Med. Assoc., 82, 881 (in Japanese).
15) Hwang, W. K. (1978): Epidemiological studies of *Clonorchis sinensis* in vicinity of river Hwang, Kyungnam Province, Korea. J. Pusan Med. Coll., 18 (Suppl.), 17–24 (in Korean, English summary).
16) Joo, C. Y. (1980): Epidemiological studies of *Clonorchis sinensis* in vicinity of river Taewha, Kyungnam Province, Korea. Korean J. Parasitol., 18, 199–214.
17) Kang, H. Y. (1955): Studies on the intestinal parasites of the children in Kimhae area (the irrigated area of Nakdong River), especially for the *Clonorchis sinensis*. Seoul J. Med., 1, 84–89 (in Korean, English summary).
18) Khan, R. A. and Thulin, J. (1991): Influence of pollution on parasites of aquatic animals. Advances in Parasitol., 30, 201–238.
19) Kim, D. K. and Song, S. B. (1971): The observations on the rate of parasite infection of school children in Pusan and Kyungnam Province. J. Pusan Med. Assoc., 1, 9–12 (in Korean, English summary).
20) Kim, I. S. and Kang, E. J. (1993): Coloured fishes of Korea. Academy Publishing Company, Seoul (in Korean).
21) Kim, S. H. and Choi, D. W. (1986): Infestation of *Clonorchis sinensis* metacercaria in fresh water fish collected in river Dongchang. Keimyung Univ. Med. J., 5, 15–21 (in Korean, English summary).
22) Kobayashi, H. (1920): Intestinal parasites of Koreans. Nippon-no-ikai, 10, 889–892 (in Japanese).
23) Kobayashi, H. (1927): Endemic foci of *Clonorchis sinensis* in the vicinity of Naktong River, Korea. J. Chosen. Med. Assoc., 82, 881–882 (in Japanese).
24) Kojima, R. and Ko, T. S. (1919): A survey of intestinal parasites among Koreans in the vicinity of Chinju, Kyungnam Province, Korea, with special reference to distribution of *Clonorchis sinensis*. J. Chosen Med. Assoc., 26, 42–86 (in Japanese).
25) Komiya, Y. and Tajimi, T. (1941): Metacercaria from Chinese *Pseudorasbora parva* TEMMINCK and SCHLEGEL with special reference to their excretory system I. J. Shanghai Sci. Inst., 5, 69–106.
26) Lee, I. S. (1956): Biological studies about *Clonorchis sinensis*. Seoul Univ. J. Biol. Agricult. Series, 4, 91–154 (in Korean).
27) Moon, H. K. and Choi, D. W. (1988): Infestation of *Clonorchis sinensis* metacercaria in fresh water fish in river Yang. Kyungpook Univ. Med. J., 29, 127–133 (in Korean, English summary).
28) Nishimura, S. (1943): Incidence of intestinal parasites in Taegu and Yongchon areas. J. Taegu Med. College, 4, 40–50 (in Japanese).

29) Sekigutchi, I., Nakade, K. and Usaka, T. (1937a): Intestinal helminths survey for inhabitants of Daejeo Myun, Kimhae gun, Kyungsang nam do 1. Mansen No Ikai, 195, 31–46 (in Japanese).
30) Sekigutchi, I., Nakade, K. and Usaka, T. (1937b): Intestinal helminths survey for inhabitants of Daejeo Myun, Kimhae gun, Kyungsang nam do 2. Mansen No Ikai, 196, 1–5 (in Japanese).
31) Seo, B. S. and Lee, S. Y. (1975): Epidemiological surveys of clonorchiasis in two areas of South Kyungsang do. Korean J. Parasitol., 13, 173 (Korean abstract).
32) Shin, D. S. (1964): Epidemiological studies of *Clonorchis sinensis* prevailed in the peoples of Kyungpook Province. Korean J. Parasitol., 2, 1–13 (in Korean, English summary).
33) Skinner, R. H. (1982): The interrelation of water quality, gill parasites, and gill pathology of some fishes from South Biscayne Bay, Florida. Fishery Bulletin, 80, 269–280.
34) Song, S. B. (1971): Parasitological examination of primary students in Dongnai and Yangsan. Theses collection in commemoration of Pusan National University's 25th Anniversary, 515–530 (in Korean, English summary).
35) Walton, B. C. and Chyu, I. (1959): Clonorchiasis and paragonimiasis in the Republic of Korea. Bull. WHO., 21, 721–726.
36) Yamaguti, S. (1958): Systema Helminthum. volume 1 – The Digenetic trematodes of Vertebrates, part 1 and 2. Interscience publishers, INC., N.Y.