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Larval Trematodes from Fresh-water Snails in Cheongdo county, Kyongbuk, Korea*

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=Abstract=

In order to determine the incidence of cercarial larvae of trematodes, the snail intermediate hosts collected in Cheongdo River, an endemic area of *Clonorchis sinensis* in Kyongbuk Province, Korea, were studied from June, 1997, to October, 1998. Four kinds of larval trematodes, *Cyathocotyle orientalis, C. sinensis, Exorchis oviformis*, and *Loxogenes liberum* were found from *Parafossarulus* snails examined.

Of these, *L. liberum* was found frequently liberated cercaria with 1.75%, followed by *C. orientalis* with 0.91% and *E. oviformis* with 0.3%. The least frequently liberated was *C. sinensis* with 0.12%. The infection rates showed variation with the shell length of *Parafossarulus* snail.

Among the *Semisulcospira* snails examined, seven species of larval trematodes and 3 kinds of undetermined cercariae were found in 70 snails, which becomes an overall infection rate of 3.8%.

Of these, the most frequently emerged cercaria was *C. orientalis* with 1.14%, followed by *Cercaria nipponensis* with 0.6%, *Cercaria incerta* with 0.49%, and cercaria of *Metagonimus* species with 0.43%. In general, the larval trematodes began to emerge from May, and peak in their emergency were found in July.

The present study indicates that cercarial larvae of trematodes in the snail intermediate hosts are less infective than those of several decades ago.

Key words: ***Parafossarulus manchouricus, Semisulcospira libertina,*** **Cercarial larvae,** ***Cyathocotyle orientalis, Exorchis oviformis, Loxogenes liberum, Clonorchis sinensis, Cercaria nipponensis, Cercaria incerta,*** **Cheongdo river, Kyongbuk Province**

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Introduction

Clonorchiasis is one of the popularly known human parasitic diseases among Koreans.

It is distributed widely all over Korea, especially along the Naktong River and its tributaries. Considerable data relating to the ecology of fresh-water snails such as, *P. manchouricus* and *S. libertina*, and infection patterns of the snail with the larval trematodes in Korea were reported by some investigators after Hirose (1913) first recorded.

Investigations dealing with the infection of *Parafossarulus* snails with *Clonorchis* cercaria and other larval trematodes were made by Lee & Kim (1958), Chun (1963), Choi *et al* (1975), Chung *et al* (1980), Joo (1980), Bae *et al* (1983), Rim (1986), and Chung & Soh (1991).

The surveys on *Semisulcospira* snails in Korea have been done by Kobayashi (1917, 1918, & 1922), Shiba (1933), Miyanaga (1938), Han & Chun (1963), and Choi *et al*(1982).

In the early 1970s, the Korean Government instituted plans for control of human parasitic diseases by mass treatment, extensive public health education and sanitary agricultural practice.

The control measure resulted in a gradual decrease in human helminthic infections and the number of larval trematodes as well as a decrease in the distribution of the intermediate hosts.

Recently Kwak *et al* (1999) reported that the endemic foci of *C. sinensis* exist in the vicinity of the river Cheongdo and that infection rates with *Clonorchis* metacercaria in fresh-water fishes varied greatly according to the species of fish and their habitats.

However, the ecology of snail hosts and infection patterns of snail with the cercariae of digenetic trematodes were not undertaken at that time.

This paper deals with the infection patterns of the larval trematodes in snail hosts in one of the endemic areas of clonorchiasis in the vicinity of the river Cheongdo.

Materials and Methods

The snail habitat selected in the study is river-bed of Cheongdo located near the Seowon village, Cheongdo county, Kyongbuk Province, Korea (Fig. 1.) where the fresh-water fishes, such as *G. atromaculatus, P. parva, A. intermedia, P. herzi, P. rhombea* and *P. esocinus*, infected with the metacercaria of *C. sinensis* was found (Kwak *et al*, 1999). The snail habitat is about 30 meters above sea level and the soil is mainly composed of sand, mud and rock. The water in the river is relatively permanent, slow-flowing and usually contains abundant organic materials and sewage. Many types of marsh plants and grasses covers the entire area. There are many kinds of freshwater fish, *Parafossarulus* and *Semisulcospira* snails in the water. The collection of snails were made once or twice monthly in the habitat from June, 1997, to October, 1998. The snails were collected by hand and put into dry plastic buckets with marsh plants and grasses and forwarded to the Parasitology laboratory. Snails were measured and placed individually in petri-dishes containing about 50 *ml* of tap water and observations made on the cercarial emergence for one week.

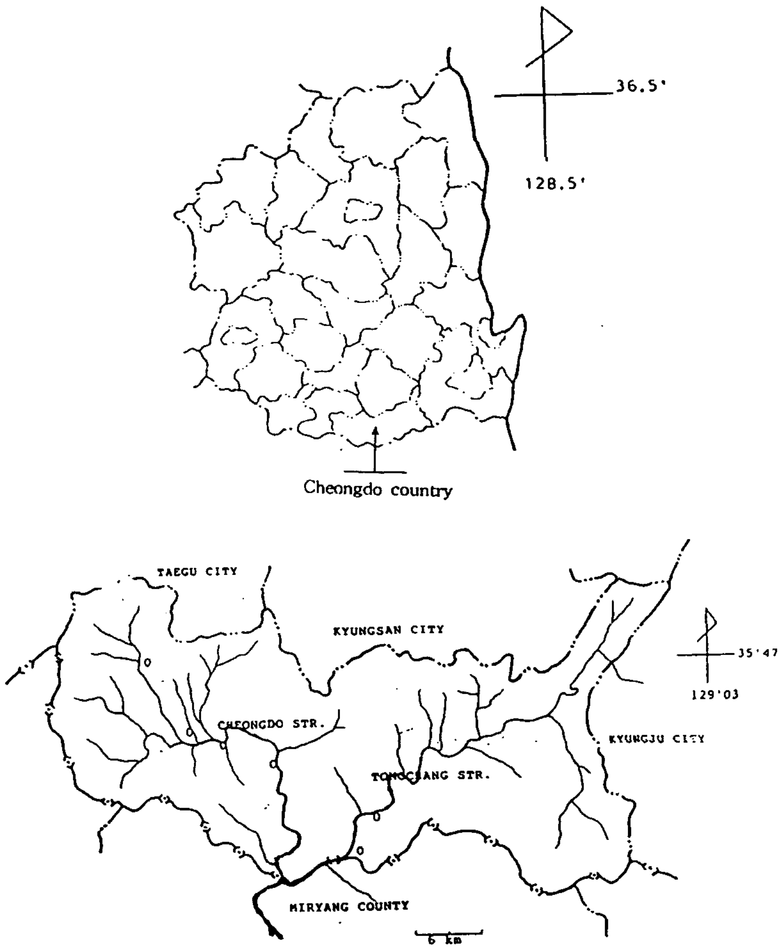

Fig. 1. Surveyed areas(0) in Chenogdo country in Kyongbuk Province, Korea

In order to determine the infection rate in relation to the size of the hosts, the *Parafossarulus* snails were divided into the three size groups: small (7.0-8.9 ㎜), medium (9.0-10.9 ㎜) and large (11.0-12.9 ㎜) shell length.

The *Semisulcospira* snails were also divided into the five groups: Ⅰ (10.0-14.9 ㎜), Ⅱ (15.0-19.9 ㎜), Ⅲ (20.0-24.9 ㎜), Ⅳ (25.0-29.9 ㎜) & Ⅴ (30.0 ㎜ or more) groups.

Uninfected snails (not shedding cercariae) were crushed to confirm absence of any larval stage.

In order to determine the specific name of cercariae, the morphological features of cercariae liberated from the snails were first studied in the living specimens.

Some of the cercariae shed from the snails were stained with Semichon' s acetocarmin and the observations made supplemented those made on living specimens. In order to determine the arrangement and the total number of flame cell in a cercaria, the living specimens stored in a refrigerator were put on the slide glass and the cover glass slipped on. All the edges of the cover glass were then sealed with paraffin-vaseline mixture. Observations were made under the oil immersion lens. Usually, flame cells and tubules could be seen under considerable cover glass pressure, but the excretory system of *Clonorchis* cercaria is very difficult to trace because of the existence of pigmented masses in the entire body and the fine structure of canals. The cercaria obtained were studied morphologically for the determination of the species following "A monograph on cercariae in Japan and adjacent territories" by Ito (1964).

The keys of morphological characteristics of cercariae obtained from *P. manchouricus* and *S. libertina* snails are as follows:

Cercaria of *Cyathocotyle orientalis* Faust, 1921

The body (130～230 x 70～130 ㎛ in size)is ellipsoidal in shape. The oral sucker (35～45 x 25～30 ㎛), prepharynx (5 ㎛), pharynx (15～18 x 15～20 ㎛) and esophagus (10～15 ㎛) are distinct. The forked-tail attaches to dorsal end of body, the tail stem is 230～350 x 30～50 ㎛ in size and that of the furcal is 200～250 x 20～30 ㎛. Minute spines on the furca are present and the excretory canal of the tail opens at the terminal of the furca. Minute spines and 10 pairs of sensory hairs are present on the surface of the body. The excretory bladder is small and the main excretory canals consists of 2 pairs anastomoses together. The flame cell formula is 2[(3+3)+(3+3)]=24. The details of morphological features were reported by Yamaguti (1940), Inatomi(1953), Ito(1964), Chun (1963), and Chung *et al* (1980).

Cercaria of *C. sinensis* (Cobbold, 1875)

The cercaria is parapleuro-lophocercous form and its body length is 200～240 x 60～80 ㎛. The tail is 360～480 x 40～45 ㎛. The oral sucker (30～40 x 25～30 ㎛) and pharynx (10㎛ in size) are distinct, but the ventral sucker is oval in shape and one third of the oral one in size, is not fully developed. Minute spines and 7 pairs of slender sensory hairs are present on the body surface.

The penetrating glands consisting of 7 pairs are fine granular and the esophagus and intestinal ceca are faintly outlined. The excretory bladder is inverted triangular or oval in shape and occupies the posterior part of the body.

The genital anlage is faintly outlined as a cell mass dorsal to the ventral sucker. The main excretory canal originates at the upper corner of the bladder and runs upward to mid-body, where the canal divides into anterior and posterior collecting tubes. The anterior collecting tube divides again into two tertiary collecting tubes and the posterior tube into three, each connecting with three flame cells by their collecting capillaries. Thus the flame cell formula is 2[(3+3)+(3+3+3)]= 30. The details of morphological and biological features of this cercaria were presented by Muto (1918 a & b), Ito (1926), Komiya & Tazimi (1940a), Komiya (1966), Ito (1964), Chun (1963), Chung *et al* (1980), and Joo (1980).

Cercaria of *Exorchis oviformis* Kobayashi, 1915

The cercarial body (150-200 x 80～100 ㎛) is elongated pyriform and it has a pair of pigmented eye spots. The oral sucker is round

(22 μm) and has a small brush-like arrangement at the dorsal side of the mouth.

The esophagus and intestine are not outlined.

The ventral sucker is not yet developed.

Seven pairs of fine granular penetrating glands are large cells situated in the middle of the body. The tail attached to the dorsal part of the body is about twice the body length and has a thin broad membrane on the ventral and dorsal sides.

The excretory bladder is V-shaped.

The flame cell pattern is 2[(2+2)+(2+2)]=16.

The details of morphological features were reported by Okabe (1936), Komiya & Tazimi (1940b), Chun(1963), and Chung *et al* (1980).

Cercaria of *Loxogenes liberum* Seno, 1907

The Oval shaped body has minute spines and 13 pairs of sensory hairs on the surface. The body measures 150~200 x 60~80 μm, the tail 90~180 x 20~30 μm and the oral sucker 40~45 μm. The ventral sucker is 28~30 μm in size, situated a little behind the middle of the body. The stylet(20~30 x5 μm in size) is present in the oral sucker. Four pairs of penetrating cells are present, of which the anterior two pairs are of coarse granular and the posteriors of fine granular materials. The flame cell formula is 2[(2+2)+(2)]=12. The details were reported by Kobayashi (1922), Faust (1924), Okabe (1937), Yamaguti (1938a), Chun (1963), and Chung *et al* (1980).

Cercaria of *Notocotylus magniovatus* Yamaguti, 1934

The larva is a sort of monostomous cercaria. The body (270-330 x130-145 μm) is ellipsoidal in shape and has three eye spots. The tail is 250~450 x60-80 μm in size, and the excretory canal opens in the middle of the tail.

The oral sucker (28~35 x 30~37μm) is oval. The body surface has five pairs of sensory hairs, but no spines. The posterolateral margin of body is transformed to a so-called adhesive organ. The body is opaque due to the coarse granules in the body. The excretory bladder is small and occupies the posterior part of the body. The flame cell formula is 2[(2+2)+(2+2)]= 16. The details were reported by Kobayashi (1922), Ueno *et al* (1930), Yamaguti (1938b), Ito (1959), and Choi *et al* (1982).

Cercaria of *Metagonimus* species

The cercarial body (180~230 x 60~75 μm) is elongated pyriform and it has a pair of pigmented eye spots. The body is light yellow in color and has a relatively thin cuticle. The oral sucker (43 x 35 μm) is oval and has about thirty specialized spines around the anterioventral mouth. The ventral sucker is small (19 x 20 μm) situated in the middle of the body. The esophagus and intestinal ceca are not fully developed. Seven pairs of penetrating glands have a pair of clustered openings dorsal to the mouth. The excretory bladder is clover-shaped and occupies the posterior part of the body.

The tail is 350~400 x 33~35 μm in size and has a dorsoventral fin, which joins at the end of tail. The flame cell formula of the cercaria of *M. yokogawai* is represented as 2[(3+3+3)+(3+3+3)]= 36 by Komiya (1966).

However, the excretory pattern of the cercaria of *M. takahashii* reported by Takahashi (1929) is 2[(2+2+2)+(2+2+2) =24. The differentiation of the excretory pattern of *M.*

yokogawai from that of *M. takahashii* is very difficult.

In this study *Metagonimus* species includes these two species. The details of morphological and biological features of this cercaria were presented by Muto (1917), Kobayashi (1818), Takahashi (1929), Yokogawa & Wakeshima (1934), Shimizu (1958), Ito *et al* (1959), Han & Chun (1963), and Choi *et al* (1982).

Cercaria of *Centrocestus armatus* (Tanabe, 1922) Yamaguti, 1933

The cercaria is relatively small and oval in shape, tapering anteriorly and rounded posteriorly. The body (100-120 x 50-65 μm) is somewhat transparent and has a pair of minute eye spots which lie in the lateral anterior third of the body.

It measures the tail 80～120 x 12～17 μm, and the oral sucker 30 ×35 μm .

The ventral sucker (13 ×13 μm) is small and not fully developed. Four pairs of penetration glands contain fine granular materials. The body surface is covered with spines and eight pairs of sensory hairs. The oral spines consisting of 9 spines are arranged in 2 rows on the mouth. The number of sensory hairs and oral spines varies from cercaria to cercaria. The oral sucker and prepharynx is faintly outlined and no intestinal ceca is found. The excretory bladder is large and thick walled. The tail (120～180 x 12～17 μm) has no spine and fin.

The flame cell pattern is 2[(2+2)+(2+2)]=16. The details were reported by Kobayashi (1922), Takahashi (1929), Yamaguti (1933 & 1938b), Ito & Watanabe (1958), and Choi *et al* (1982).

***Cercaria yoshidae* (Osafune, 1898) Cortet Nichols, 1920**

The cercaria, belonging to cystophorous type, has peculiar organs, the bulbus and the excretory projection. The body (160～240 x 50～55 μm) is ellipsoidal in shape. The slender tail (300～350 x 18～20 μm) is flat laterally at the posterior one third to form a fin. The oral sucker (30～32 x 35～36 μm), the ventral sucker (30～35 x 34～36 μm), and the intestinal ceca are fully developed, but no penetrating and encysting glands are present. The bulbus, situating between the body and the tail, is a large globular cavity 85×90 μm in size. The excretory projection is a slender tube with a canal in it, and 150～360 x 15～20 μm in size.

It originates from the anterior corner of the bulbus and protrudes anteri- obliquely. The distal end of the tube is thickened. No minute spines and sensory hairs are present on the body surface. The body and the excretory projection of the mature cercaria are frequently invaginated in the cavity of the bulbus.

The flame cell formula is 2(2+2)= 8. The details were reported by Yoshida (1917), Cort & Nichols (1920), Faust (1924), Ito (1952), Shimizu (1958), Han & Chun (1963), and Choi *et al* (1982).

***Cercaria nipponensis* (Ando,1915) Faust, 1924**

The oval body is 140～145 x 50～65 μm in size. The oral sucker (40～50 x 40～50 μm) has a large virgula organ. The stylet (14 ×4 μm) and pharynx (10× 12 μm) are distinct, but the ventral sucker (15-20 x 20-25 μm) and intestine are not fully developed. Minute spines and sensory hairs are present on the surface. The penetration glands consisting of 3 pairs are fine granular. The small tail is one half of the body length. The excretory bladder is cup-shaped, and the flame cell formula is 2[(2+2+2)+(2+2+2)]=24. The details were reported by Kobayashi (1917), Faust (1924), Yokogawa & Wakeshima (1934), Ito (1952), Shimizu (1958), and Choi *et al* (1982).

Cercaria incerta **(Kobayashi, 1922) Faust, 1924**

The body (260~280 x 80~90 ㎛) is cylindrical in shape and light yellow in color.

The tail (60~70 X 40~50 ㎛) is short. There is a stylet (13×8 ㎛) on the dorsal side of the oral sucker (45~50 x 40~43 ㎛). The anterior one third of the stylet is pointed and the posterior two thirds thickened. three pairs of penetrating glands lie on the lateral side of the pharynx (19×20 ㎛). The body surface has 7 pairs of sensory hairs but no spine.

The genital primordia, consisting of two compact masses, occupy both the anterior and posterior borders of the ventral sucker (50~55 x 40~45 ㎛).

The excretory bladder (80~90 x 50~55 ㎛) is I-shaped. The flame cell formula is 2[(2+2)+(2+2)]=16. The details of morphological features were reported by Kobayashi (1918 & 1922), Faust (1924), Yokogawa & Wakeshima (1934), Ito (1953), Han & Chun (1963), and Choi *et al* (1982).

Results

The relationship between infection rates for larval trematodes and the length of *Parafossarulus* snails are summarized in Table 1. Among the 1,654 snails of various sizes examined, one or more species of larval trematodes were found in 51, which becomes an overall infection rate of 3.08%. Four species of larval trematodes, *C. orientalis, C. sinensis, E. oviformis*, and *L. liberum*, were found.

Of these, *L. liberum* was found most frequently liberated cercaria, the rate being 1.75%, followed by *C. orientalis* with the infection rate of 0.91% and *E. oviformis* with 0.30%. The least frequently liberated was *C. sinensis* with 0.12%. However, The percent-

Table 1. Relationships between Infection rates for larval trematodes and length of *Parafossarulus* snails (1997-1998)

Size group	No.	*C.orientalis*		*C.sinensis*		*E.oviformis*		*L.liberum*		Total	
(㎜)	examined	No.*	%	No.	%	No.	%	No.	%	No.	%
Small (5.0- 7.9)	57	0	0	0	0	0	0	1	1.75	1	1.75
Medium (8.0-10.9)	1,227	13	1.06	1	0.08	3	0.24	21	1.71	38	3.10
Large (11.0-13.9)	370	2	0.54	1	0.27	2	0.54	7	1.89	12	3.24
Total	1,654	15	0.91	2	0.12	5	0.30	29	1.75	51	3.08

* No. means the number of snail infected.

age of infection showed variation with the shell length of snail. There was an increase in the infection rate with the increase in the length of the snail. The infection rate was 1.75%, 3.10%, and 3.24% in the small, medium, and large size group, respectively.

Table 2 shows the monthly liberation rates for larval trematodes from *Parafossarulus* snails. In general, the liberation rates in three species of larval trematodes, *C. orientalis, E. oviformis*, and *L. liberum*, were higher in July than other months of the years studied.

The liberation rates for *L. liberum* was high, 4.35% in May, continuing at fairly high rates from June to July, then dropping abruptly in August. It then increased to 1.16% in September and to 1.68% in October. The cercarial infection rate for *E. oviformis* was low, being between 0.14% in August and 2.13% in July. Similar rate was obtained in the cercaria of *C. orientalis*.

Table 2. Monthly liberation rates for larval trematodes from *Parafossarulus* snails(1997-1998)

Month	No examined	*C.orientalis*		*C.sinensis*		*E.oviformis*		*L.liberum*		Total	
		No.*	%	No.	%	No.	%	No.	%	No.	%
May	23	0	0	0	0	0	0	1	4.35	1	4.35
June	28	0	0	0	0	0	0	2	7.14	2	7.14
July	47	2	4.26	0	0	1	2.13	8	17.02	11	23.40
Aug	708	5	0.71	1	0.14	1	0.14	6	0.86	13	1.84
Sept	432	8	1.85	1	0.23	2	0.46	5	1.16	16	3.70
Oct	416	0	0	0	0	1	0.24	7	1.68	8	1.92
Total	1,654	15	0.91	2	0.12	5	0.30	29	1.75	51	3.08

* No. means the number of snail infected with larval trematodes.

The cercarial liberation rate for *C. sinensis* was quite low. The average rate was 0.12%, with the range from zero to 0.23%.

In Table 3, The infection rates of larval trematodes from *Semisulcospira* snails, and the relationship between the liberation rates for cercariae and shell length of snails are tabulated. In 1,840 *Semisulcospira* snails examined, seven species of larval trematodes and 3 kinds of undetermined cercariae were found in 70, which becomes an overall infection rate of 3.8%.

Of these, the most frequently emerged cercaria was *C. orientalis* with 1.14%, followed by *C, nipponensis* with 0.6 %, *C. incerta* with 0.49% and cercaria of *Metagonimus* spp. with 0.43%. In the three kinds of undetermined cercariae, the infection rates varied from 0.11% to 0.22%. Note that no *P. westermani*

Table 3. Incidence of infection of larval trematodes in *Semisulcospira libertina* (1997-1998)

Larva trematodes	Size of *Semisulcospira* snail(mm)											
	10-14		15-19		20-24		25-29		30-34		Total	
	No.*	%	No.	%	No.	%	No.	%	No.	%	No.	%
N. magnivatus	0		1	0.20	4	1.08	1	1.12	0		6	0.33
C. orientalis	5	0.58	7	1.39	6	1.62	2	2.25	1	5.88	21	1.14
Metagonimus sp.	0		3	0.60	4	1.08	1	1.12	0		8	0.43
C. armatus	1	0.12	2	0.40	1	0.27	0		0		4	0.22
C. yoshidae	0		0		3	0.81	0		0		3	0.16
C. nipponensis	0		2	0.40	9	2.43	0		0		11	0.60
C. incerta	0		3	0.60	5	1.35	1	1.12	0		9	0.49
P. westermani	0		0		0		0		0		0	
Cercaria A	0		0		2	0.54	2	2.25	0		4	0.22
Cercaria B	0		0		1	0.27	1	1.12	0		2	0.11
Cercaria C	0		0		1	0.27	1	1.12	0		2	0.11
Total	860(0.70)**		503(3.58)		371(9.70)		89(10.11)		17(5.88)		1,840(3.80)	

* No. means number of snails infected. ** Number in the Parentheses means percent liberated.

cercaria was found.

In the liberation rate for cercariae to the length of snails, it was found to be 0.70% in the snails between 10-14 mm shell length, and 3.58% in the snails between 15-19 mm shell length. The rates subsequently increased and reached a maximum of the 10.11% in the snails between 25-29 mm shell length, and then dropped to 7.14% in the snails more than 30 mm shell length.

Table 4 summarizes the monthly variations of cercariae from the *Semisulcospira* snails. In general, the larval trematodes began to emerged from May, and peak in its emergency was found in July. There was a gradual decrease from August, with a few number of them emerged until October of the year studied.

The cercarial infection rates of *C. orientalis* in May was 0.52%, and the rates remained at the similar values, 0.78% in June, 0.70% in July, and 0.77% in August. It increase to

Table 4. Monthly variations of cercariae from *Semisulcospira* snail (1997-1998)

Cercaria	May (385)*	June (128)	July (574)	Aug. (259)	Sept. (258)	Oct. (236)
N. magnivatus	0	0	3(0.52)**	2(0.77)	1(0.39)	0
C. orientalis	2(0.52)	1(0.78)	4(0.70)	2(0.77)	6(2.33)	6(2.54)
Metagonimus sp.	0	3(2.34)	5(0.87)	0	0	0
C. armatus	1(0.26)	0	2(0.35)	1(0.39)	0	0
C. yoshidae	0	0	2(0.35)	1(0.39)	0	0
C. nipponensis	2(0.52)	4(3.13)	1(0.17)	3(1.16)	1(0.39)	0
C. incerta	3(0.78)	0	1(0.17)	2(0.78)	2(0.78)	1(0.42)
P. westermani	0	0	0	0	0	0
Cercaria A	1(0.26)	1(0.78)	2(0.35)	0	0	0
Cercaria B	1(0.26)	0	1(0.17)	0	0	0
Cercaria C	0	0	2(0.35)	0	0	0
Total	10(2.60)	9(7.03)	23(4.01)	11(4.35)	10(3.88)	7(2.97)

* Parenthesis means the number of snails examined.

** Number in parentheses means the percentage of snails infected with cercariae.

2.33% in September and reached the peak, 2.54% in October. The cercaria of *Metagonimus* spp. were found from June to July.

Discussion

The results of this study indicate that the infection rates of the fresh-water snail with cercariae of digenetic trematodes varied appreciably from snail to snail depending on the shell length. Larger snails were found to be more heavily infected than smaller ones. This variation may be correlated with the duration for which the larger snails were exposed to miracidia because, as also indicated by Kennedy (1975) and Kalantan *et al* (1997), size is often related to the age of the host and the older host has a long time period to make contact with the parasite.

In the studies of cercarial infection in the snail hosts in Korea, few factual works were done before the end of the Korean War, although *P. manchouricus* and *S. libertina* were thought to serve as intermediate hosts of intestinal, hepatic and/ or pulmonary flukes in Korea because some studies on the incidence of human flukes and their intermediate hosts were conducted by Japanese investigators (Matsumoto, 1915; Kobayashi, 1918; Shiba, 1933; Nishimura, 1943).

Since 1950s, the studies of infection rates of *Clonorchis* cercaria and other larval trematodes from *Parafossarulus* snails in Korea were carried out mostly in the vicinity of the rivers Han, Naktong, Kum, Mankyung, and Yeongsan and their tributaries, and the isolated rivers, such as Hyungsan, and Taewha (Chun, 1963; Kim, 1965; Kim, 1974; Joo, 1980; Soh & Ahn, 1980; Bae *et al*, 1983; Rhee *et al*, 1983; Choi, 1984; Chung & Soh, 1991; Joo & Hong, 1991; Joo *et al*, 1997).

As a result of previous studies, the infection rates of *C. sinensis* in the vector snails were found to be low, ranging from 0.09 to 3.10%.

In addition, the *Parafossarulus* snail has been existed in limited plain areas along the rivers. Chun (1963) found seven species of cercariae from *Parafossarulus* snails in Kimhae plain by crushing and natural emerging methods, and reported that *A. japonica* was the most frequently liberated cercaria being found in 9.7%, followed by cercaria of *Mucobuccalis, L. liberum*, and *E. japonicus* with the infection rates of 8.0%, 1.9%, and 1.6% in decreasing order. The less frequently liberated was *Clonorchis* cercaria with 0.7%.

Choi *et al* (1975) carried out a survey for *Clonorchis* cercariae in the snails collected from 10 well-distributed stations in the river Naktong and its tributaries of Kyongbuk Province, and reported that infection rates of the snail with *Clonorchis* cercaria was very low, ranging from 0.04 to 0.11%.

Subsequently Chung *et al* (1980) conducted the seasonal variation of *Parafossarulus* snail and larval trematode infections in the river Kumho, Kyongbuk Province, Korea, and reported that the population density of the vector snails and cercarial infection in the snail had gradually decreased.

In the study on the ecology of *Semisulcospira* snails and the infection patterns of the snails with the cercariae of digenetic trematodes, Han & Chun (1963) found six species of cercariae, cercaria of *Metagonimus* spp., cercaria of *P. westermani, C. yoshidae, C. longicerca, Cercaria acanthatrium, C. incerta*, and four kinds of undetermined cercariae from *Semisulcospira* snails which were collected in Kimhae plain, Kyongnam Province, Korea, and they also reported that *Metagonimus* species was the most frequently infected cercaria being found in 11.71%, followed by *C. yoshidae, C. longicerca*, and *C. incerta* with the infection rate of 3.88%, 1.77% and 0.28% in decreasing order.

They also observed that the correlation of cercarial infection rates to the shell length of snails were characterized by the maximum infection rates at 21-25 ㎜ sized snail group being found in 33.54%, followed by 26-30 ㎜-sized group with 15.36%. Both 16-20 ㎜-sized and 31-35 ㎜-sized groups were moderately infected with rates of 7.45% and 7.74%, respectively. The less frequently infected group was 10-15㎜-sized snail with rate of 0.81%.

From their studies on the larval trematodes from *Semisulcospira* snails in Kyongbuk Province, Korea, Choi *et al* (1982) reported that the most frequently emerged cercaria was *Metagonimus* species with 1.48%, followed by *N. magniovatus* with 0.87% and *C. nipponensis* with 0.61%. The least frequently emerged cercaria was *P. westermani*, with the rate of 0.04%. There was no correlation in the liberation rate for cercariae and the shell length of snails.

In the present study, the overall infection rate for larval trematodes in *Parafossarulus* snail was 3.08%, and 1,840 *Semisulcospira* snails were collected, from which 10 species of larval trematodes including 3 kinds of unidentified cercariae A, B, and C were found. Their infection rates varied appreciably in snails depending on the shell length, ranged from 0.11% to 1.14%.

Our figures are much lower than those reported by Chun (1963) in Kimhae plain, Chung *et al* (1980) in the River Kumho, Han & Chun (1963) in Kimhae plain and Choi *et al* (1982) in Judong stream.

The main causes of these lower values can hardly be explained. However, these findings are quite conceivable that low incidence of larval trematodes in snails collected in the River Cheongdo may be due to the water pollution by industrial and household wastes. Other causes are sprayed insecticides and pesticides for cultivating new breed of rice and land reclamation.

Such considerations were also recognized by Kim (1965), Soh *et al* (1980), Chung *et al* (1980), Joo & Hong (1991), and Joo *et al* (1997).

As for the seasonal variation of *Clonorchis* cercaria and other larval trematodes in the *P. manchouricus*, Kim (1974) observed that *Clonorchis* cercarial liberation in the snail was seen in nature in the period from May to October.

Li (1989) reported that in Liaoning Province, China, 4.8% of *P. manchouricus* were infected in June, whereas the infection rate had increased by July to 13.2% and decreased to 2.8% and 1.9% in August and September.

In this study, *Clonorchis* cercaria and the other larval trematodes emerged from May to October, and the higher liberation rates were observed in July. Similar seasonal variations were observed among *Parafossarulus* snails in Kimhae plain by Chun (1963), Chung *et al* (1980), and Zhu (1984).

As for the monthly fluctuations for larval trematodes in the *Semisulcospira* snail, Han & Chun (1963) reported that the monthly liberation rate of the cercariae parasitic to *S. libertina* were higher in June (29.0%) and July(30.14%) than in the other months of the year studied. Choi *et al.*(1982) also reported that the highest rate was encountered in May, and decreased in the period from June to November. On the basis of the previously reported data and our figures, it is concluded that in the temperate zones of Korea and Japan, the cercariae were emerged from May to October and earlier in subtropical zones.

In fact, in some areas of Asia including Korea rainy season occurs in July and August. Human and animal excreta are easily washed into rivers and their tributaries, and many natural and fish-breeding ponds and the snail infection rates reach their maximum levels during this period of time. Two or three months after the peak infection in the snail intermediate hosts, the peak level of cercarial shedding occurs. Such considerations was also recognized by Komiya & Suzuki (1964), Kim (1974), Rim (1986), Li (1989), and Chen *et al* (1994).

As a result of previous studies and our own figures, no infected *Parafossarulus* snails were found after October. The main causes can hardly be explained. However, there was no consensus on the viability of infection after aestivation.

Some investigators have presumed that *C. sinensis* miracidia that penetrated the snail before aestivation, arrested in the redia stage over-winter and then emerged cercariae around April (Komiya & Suzuki, 1964;

Chung *et al*, 1980; Rim, 1986; Chen *et al*, 1994). As also indicated by Rim (1986), it was suggested in this study that the cercariae and rediae were unable to survive aestivation in an area as cold as Korea, and new infections were initiated each year and emerged cercariae in late spring. Compared with the earlier reports available, *C. sinensis* and other larval trematodes in the snail intermediate hosts are now much less infective, due to ecological changes of the river. The destruction of the natural environment of the rivers can be attributed to the widespread uses of pesticides together with massive drainage of household wastes into the rivers.

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