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Seasonal Variations of Metacercarial Density of *Clonorchis sinensis* in Fish Intermediate Host, *Pseudorasbora parva**

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INTRODUCTION

When the majority of host population are non-infected or infected with a few parasites, and a few hosts with larger worm burden, the negative binomial distribution is known to be accepted as a most appropriate statistical simulation not only in definitive host-adult parasite system (Li & Hsu, 1951; Pennycuick, 1971; Boxshall, 1974; Seo *et al.*, 1979; Lemly & Esch, 1984) but also in intermediate host-larvae system (Kang *et al.*, 1975). Kang *et al.* (1975) observed that the distribution pattern of metacercariae in crab host infected with *Paragonimus iloktsuenensis* was followed the negative binomial distribution. This kind of overdispersion with negative skewedness is considered as one of the basic mechanism of mutual coexistence of both host and parasite population.

To prove this pattern of the skewed distribution mathematically in experimental model, Anderson *et al.* (1978) performed an experiment in which the cercariae of the ectoparasitic trematode *Transversotrema patialense* was exposed to previously uninfected fish under the constant condition of temperature, light intensity, during fixed period of time. According to them, as the density and the duration of cercarial exposure increased, the proportion of infected host were led to change from the Poisson distribution in early time to negative binomial form in later stage of the experiment.

However, if the most of hosts are infected, and many with heavy burden, parasite population within host followed the lognormal distribution (Chai *et al.*, 1976; Kim *et al.*, 1979). Chai *et al.* (1976) described that the distribution of expelled *Enterobius vermicularis* by treatment with pyrantel pamoate in heavily infected children were fitted to the lognormal distribution. In addition, Kim *et al.* (1979) examined the metacercarial density of *Clonorchis sinensis* in *Pseudorasbora parva* which were collected from an endemic area in summer, and reported that the frequency distribution of metacercariae per fish was fitted to the lognormal distribution rather than to the negative binomial distribution.

P. parva is the most suitable second intermediate host and are heavily infected with metacercariae of *C. sinensis* among the cypriniid fish. Therefore, the intensity of metacercarial infection per fish has been used as one of the epidemiologic indices for the evaluation of human clonorchiasis in endemic areas. Even though *P. parva* are not consumed by the inhabitant, its epidemiological importance is that we can grossly evaluate the degree of water pollution with night soil containing *Clonorchis* eggs, degree of larval infection of snail hosts and degree of potential exposure of man to *Clonorchis* infection by eating other less susceptible fish hosts. However, the degree of metacercarial infection in *P. parva* is not so simple index, but a result of many intervening factors, such as life span of

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metacercariae of *C. sinensis*, degree of cercarial exposure and life span of fish themselves *etc.*

In this sense, the meaning of metacercarial burden of *Clonorchis* in *P. parva* need more attention and study for its proper understanding. This study was undertaken to describe the seasonal variations of metacercarial burden of *C. sinensis* in *P. parva* in a highly endemic locus in Korea.

MATERIALS AND METHODS

1. Collection of Fish and Area Subjected

From March, 1983 to February, 1984 about one hundred *P. parva* were collected in Sun-Am River which is located surrounding Kim-Hae City, Kyong-Sang Nam Do(=Province), Korea, every month. The locality is known hyperendemic area of human clonorchiasis, the prevalence being ranged from 50 to 60 percent (Song *et al.*, 1979). As shown in Table 1, a total of 788 fish was collected at one fixed point in Sun-Am River. The number of fish collected by season was as follows; 270 in spring (March to May), 169 in summer (June to July), 199 in autumn (September to November) and 150 in winter (February) respectively. The mean and standard deviation of fish length was 55.2± 6.8mm, and no significant differences were observed between collection month. The fish collection was always conducted at the first week end in every month.

2. Counting Metacercariae

The collected fish were transported to laboratory. Each fish was crushed by using a meat grinder separately and put it into 50 ml beaker, and digested using artificial gastric juice. Then, the supernatant in beaker was decanted and washed with saline solution (0.85% NaCl) for several times. Finally, the number of sedimented metacercariae of *C. sinensis* was counted under a dissecting microscope.

3. Data Analysis

The frequency of metacercariae per fish was counted by month. Then, observing the fitness of observed frequency to the negative binomial distribution, 'x' variable was divided by 50. The theoretical frequency was calculated by the methods of Bliss (1953). The equation of the negative binomial distribution was expressed by $(p-q)^{-k}$, where $q=1+p$, $p=m/k$. The main parameters, m(=mean) and k (=positive exponent) was computed by the maximum likelyhood methods proposed by Bliss. To check the degree of overdispersion pattern in observed data, the ratio of variance to mean was also calculated.

The lognormal distribution equation also tested in observed frequency. When the distribution of 'x' variable which replace 'ln x' followed the normal distribution, $N(\mu, \sigma^2)$, it would be called lognormal distribution. The parameters 'cv' (=coefficient variance), 'μ' and 'σ' was calculated by the index presented in Japanese Statistical Association (1972). And the theoretical frequencies were calculated by the square methods of normal distribution.

To test fitness between the observed frequencies and the theoretical ones in both negative binomial and lognormal distributions, chi-square probability was applied on the level of 5% confidence limit.

RESULTS

1. The Rate and Intensity of Infection of Metacercariae

The infection rates of metacercariae of *C. sinensis* per fish were presented in Table 1. Although both the rates and the intensities of infection showed wide variations during the course of the monthly observation, the metacercariae were always found in fish throughout the observation period. A total of 513(65.1%) out of 788 fish was found to be infected with metacercariae of *C. sinensis*. The infection rates ranged from 11.4% in March to 98.6% in June. In May, June, July and September, the fairly high infection rates were maintained in higher than 80%. The infection rates were relatively low in March (11.4%), in April

Table 1. The status of metacercarial infection of *Clonorchis sinensis* in *Pseudorasbora parva* by monthly observation in Kim-Hae City

Year	Month	No. of exam.	No. of fish infected(%)	Range of Mc* (in positive)	Total No. of Mc	Mean±SD
1983	March	70	8(11.4)	7~ 574	1,360	19.43± 87.31
	April	100	20(20.0)	3~ 491	1,179	11.79± 53.22
	May	100	98(98.0)	4~1,685	27,648	276.48±282.88
	June	69	68(98.6)	5~1,051	20,343	294.83±274.70
	July	100	82(82.0)	1~2,161	6,703	67.03±233.63
	September	99	92(92.9)	4~2,284	17,100	172.73±308.83
	November	100	48(48.0)	2~ 385	1,109	11.09± 41.97
1984	February	150	97(64.7)	1~ 639	5,739	38.26± 79.82
Total		788	513(65.1)	1~2,284	81,181	103.02±118.91

*: Metacercariae

Table 2. Frequency distribution of metacercarial density per fish by monthly observation

No. of Mc per fish	1983							1984
	Mar	Apr	May	Jun	Jul	Sep	Nov	Feb
0	62	80	2	1	18	7	52	53
1~50	4	15	20	19	64	23	44	67
51~100	1	3	11	4	4	12	2	11
101~150	0	0	9	3	1	19	0	10
151~200	0	1	10	7	2	13	1	4
201~250	0	0	8	2	3	8	0	0
251~300	1	0	5	3	4	7	0	2
301~350	0	0	3	3	1	3	0	0
351~400	1	0	6	4	1	2	1	1
401~450	0	0	5	5	0	2	0	1
451~500	0	1	1	0	0	0	0	0
501~550	0	0	4	8	0	0	0	0
551~600	1	0	3	1	0	0	0	0
601~650	0	0	3	2	1	0	0	1
651~700	0	0	1	1	0	1	0	0
701~750	0	0	4	2	0	0	0	0
751~800	0	0	2	0	0	0	0	0
801~850	0	0	0	0	0	0	0	0
851~900	0	0	0	0	0	0	0	0
901~950	0	0	0	2	0	0	0	0
951~1,000	0	0	0	0	0	0	0	0
1,000 over	0	0	3	2	1	2	0	0

(20.0%), in November (48.0%) and in February (64.7%).

The pattern of the infection intensity was similar to those of infection rates by monthly observation. Throughout a year, the mean number of metacerceriae per fish was 103.0 and standard deviation was 118.9. The maximum intensity per fish observed in this study was 2,284, found in September. Like the infection rates in May, June and September, the mean intensity were also higher when compared with those in March, April, November and February. The mean intensity was the highest in June (294.8).

2. Frequency Distribution of Metacercariae and Fitness to Statistical Models

As shown in Table 2 and Fig. 1, the frequency of metacercarial number of *C. sinensis* per fish showed the negative skewedness and not normally distributed. During one year, uninfected fish population was 275 (34.9%) out of 788 fish examined. According to the monthly observation, the negative rates were 88.6% in March, 80% in April, 52% in November and 35.3% in February whereas they were remarkably low; 2% in May, 1.4% in June and 18% in July. In infected fish population, 256(32.5%) had 1~50 metacercariae, 48(6.1%) had 51~100 metacercariae, 42(5.3%) had 101~150 metacercariae, 38 (4.8%) had 151~200 metacercariae and decreasing number of fish had the higher number of metacercariae.

To compare the observed and theoretical distributions calculated by the negative binomial, Poisson and lognormal probability, the statistics

Table 3. Statistics for fitting the observed frequencies to negative binomial, Poisson and lognormal probability distribution

Year	Month	Mean(m)	Variance/Mean (s^2/m)	k*	Negative binomial	Poisson	Lognormal
1983	March	19.43	392.33	0.050	$p>0.05$	$p>0.05$	$p<0.05$
	April	11.79	240.23	0.049	$p>0.10$	$p<0.05$	$p<0.05$
	May	276.48	289.43	0.959	$p<0.05$	$p<0.05$	$p>0.75$
	June	294.83	255.94	1.156	$p<0.05$	$p<0.05$	$p>0.10$
	July	67.03	814.31	0.082	$p<0.05$	$p<0.05$	$p>0.05$
	September	172.73	552.17	0.313	$p<0.05$	$p<0.05$	$p>0.10$
	November	11.09	158.83	0.070	$p>0.10$	$p<0.05$	$p<0.05$
1984	February	38.26	166.52	0.232	$p>0.10$	$p<0.05$	$p<0.05$

*: parameter in negative binomial probability distribution

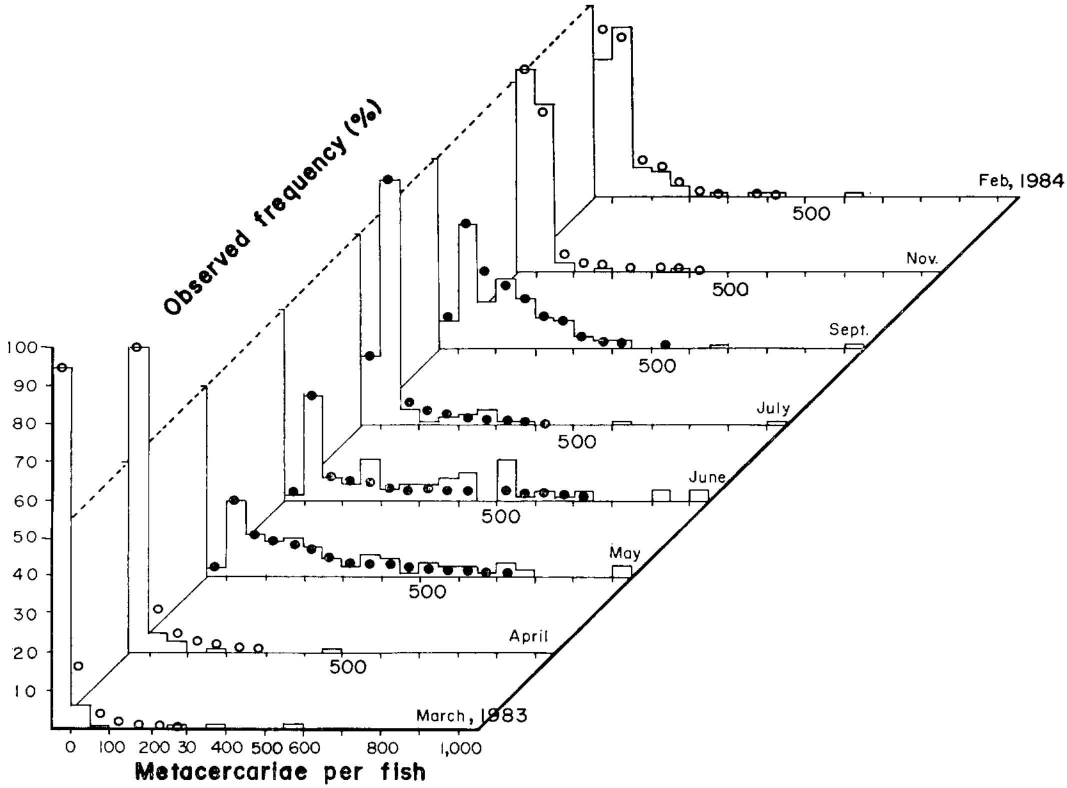

Fig. 1. Fitness of observed frequency to the negative binomial (○) and lognormal distribution (●).

and graphic illustration of these distribution were presented in Table 3 and Fig. 1. In March, distributions of negative binomial and Poisson probability were fitted to observed distribution ($p>0.05$). In April, November and February, negative binomial distributions were fitted to observed one on the level of $p>0.10$. However the observed distribution in May, June, July and September were more fitted the lognormal distribution ($p>0.05$, $p>0.75$) rather than the negative binomial and Poisson probability distributions.

The overdispersion, measured by the ratio of variance to mean($=s^2/m$), were highly varied in the course of observation throughout a year. And the greatest ratio was in July, and decreased about 5 folds in November and February, but slightly increased in March(Table 3).

DISCUSSION

In our observations, both the rates and the intensity of infection of *Clonorchis* metacercariae in fish host, *P. parva*, were significantly higher in May, June, July and September than in March, April, November and February. The rates and intensity increased abruptly from May, maintained higher rates in summer season, then showed lower rates since November.

This result suggested that our results on the seasonal variation of metacercarial infection coincided with that of snail population in Korea. Chung *et al.*(1980) reported that *Parafossarulus manchouricus*, the only snail host of *C. sinensis*, appeared in April, reached their peak population density in late June, disappeared in early or mid-November, in Kumho River, a branch of Naktong River. Furthermore, the cercarial shedding from *P. manchouricus* was observed only in May to August, during 5 years study. Their results explained our observations on seasonal variation of metacercarial infection in *P. parva* very well if the life span of metacercariae in fish is relatively short.

However, our results of the present study differed from our own past experiences on metacercarial collection, especially in aspect of infection status in winter and spring. Before the study, we did not expect such remarkable seasonal variation even if present, because we could collect the metacercariae from the fish in winter as much as in summer even though we did not count individual number.

Furthermore, Lee (1968) reported that the infection rates of *P. parva* in Kumho River were almost same in spring (85.4%) and autumn (100%). Recently Bae *et al.* (1983) also observed higher infection rates (85.9%) both in summer and spring. Huang and Khaw(1964) described the rates and intensity of infection in Kongkuan-Pei (=lake), Taiwan, as follows; 83.3%, 227 metacercariae per fish in spring, 100%, 418 in summer, 96.6%, 309 in autumn, and 80%, 96 in winter. The report showed that the rates observed by season were not different but the intensity of infection were described to increase suddenly in May; from 152 in April to 313 in May.

At present, we can not properly interprete the difference of our study results from reported previously. However, we can discuss the possible factors involved as follows. First of all, the behavioral pattern, fecundity and longevity of *P. parva* are concerned with frequency of cercarial contact. Those aspects of *P. parva* were recorded by Chung (1977). According to him, the fish matured to 40mm long within 5 months of hatching, then overwintered. Their length reached up to 100mm or more in 3～4 years in good environmental condition. Because the most of examined *P. parva* in this study was in range of 48～62mm long, many of the young fish have no chance of cercarial contact, especially after September. This belatedly hatched fish should be found not infected or infected in low density when examined in winter and spring. Secondly, the contamination of nearby river with human or animal excreta containing *C. sinensis* eggs, is expected to continue throughout a year. Therefore, this factor would not be a contributing factor of seasonal variation. In third, Rhee *et al.* (1984) reported that *P. parva* had no cercaricidal/metacercaricidal substance in their epidermal mucus which are found in *Cyprinus carpio*, *Ophiocephalus argus* and *Parasilurus asotus*. They thought that the absence of the material in epidermis is one of the reasons of high susceptibility of *P. parva* to the cercariae of *C. sinensis*. Rhee *et al.* (1973) reported also that the longevity of metacercariae in flesh of *P. parva* was 770 days or more in laboratory condition. They actually exposed the newly hatched fish to cercariae of *C. sinensis*, and observed the longevity of the metacercariae in the laboratory. We accept that the longest living metacercariae survive 2 or more years in *P. parva* as observed by them, but not all. There should be metacercariae dying before 2 years, but we do not know how the number of metacercariae are decayed during the period of 2 years. By experimental infection, it is very difficult to calculate the average span of metacercaria unless there are control fish which were exposed exactly same amount of cercariae at each time of counting the number of metacercariae. It is because the number of metacercariae per fish distributed in skewed distribution (Anderson *et al.*, 1978; Scott & Anderson, 1984).

Our study area, Kimhae has been known as one of the hyperendemic areas of clonorchiasis in Korea. Recently the ecologic situations have been changing; industrial factories near the river began to pollute water of the river extensively, and to damage the ecology. We do not know whether such unfavorable ecologic changes in sake of *Clonorchis sinensis* was manifested in the seasonal variations of metacercarial density in *P. parva*. Further studies on this matter are needed.

To mention the statistical aspect of this study, the variance/mean ratio of metacercarial density fluctuated considerably during one year observation, which was the highest in July and the lowest in November. In model and stochastic system, parasite induced host mortality (=regulation of host population by parasite) has been demonstrated when the variance/mean ratio tend to decrease. According to Crofton (1971) and Gorden and Rau (1982), if parasites within host are greatly overdispersed and heavily infected, and if parasites are able to kill their host, the population of both parasite and host are stated to reach equilibrium. Both the lethal level and overdispersion of parasite depend mainly upon the number of parasite population. However, such phenomena have not been confirmed by field survey or in laboratory.

In our observation, the highest intensity per fish was 2,284. This suggests that the super-, cumulative infection is occurring in *P. parva* by the contact of *Clonorchis* cercariae. To confirm such infection dynamics, laboratory experiments are also needed. Anyway our study results indicated that the frequency distribution of the metaercariae per fish distributed not only in lognormal equation (Kim *et al.*, 1979) but also in negative binomial distribution.

SUMMARY

The seasonal variations of the rate and intensity of metacercarial infection of *C. sinensis* in *P. parva* were observed. The fish were collected at Sun-Am River which located in Kim-Hae City, Kyong-Sang Nam Do (=Province), Korea, from March 1983, to February 1984 every month. A total of 788 fish was examined. The number of metacercariae in each fish was individually counted after the individual digestion by artificial gastric juice.

The results were as follows:

1. During one year, 513 (65.1%) out of 788 fish were infected with metacercariae. In May, June, July and September, the infection rates ranged from 82.0% to 98.6% whereas the rates was relatively low in March, April, November and February ranging from 11.4% to 64.7%.

2. The intensity of infection was similar with those of infection rates. The mean intensity per infected fish was 103.0 and standard deviation was 118.9 throughout one year. The highest mean intensity was in June (294.8) and the lowest in November (11.1).

3. The observed frequency of fish with certain intensities of metacercariae were fitted to theoretical equations derived from negative binomial distribution in March, April, November and February ($p>0.05$). Meanwhile, the equation of lognormal distribution were fitted with the observed frequencies in May, June, July and September ($p>0.05$, $p>0.75$). The variance/mean ratio varied by month. The value was the highest in July (814.3) and the lowest in November (158.8).

Unlike our hypothesis, the metacercarial density of *Clonorchis sinensis* in its the most favorable fish host, *Pseudorasbora parva* showed considerable seasonal variations in the hyperendemic area. The possible factors were discussed.

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═국문초록═

간흡충 유행지역에서 참붕어내 피낭유충 감염밀도의 계절적 변동

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간흡충 유행지역에서 포획한 참붕어에 감염된 간흡충 피낭유충의 감염율을 관찰하고 아울러 감염밀도가 계절적으로 변화하는 양상을 파악하고 수리적 모델에 적용하고자 하였다.

1983년 3월부터 1984년 2월까지 경상남도 김해시에 위치한 선암강에서 포획한 참붕어 총 788마리를 대상으로 하여 1마리씩 인공소화시켜 피낭유충의 수를 관찰하였다. 그 결과를 요약하면 다음과 같다.

1. 일년동안의 간흡충 피낭유충의 총 감염율은 788중 513마리로 65.1%이었다. 월별로는 5월, 6월, 7월 및 9월에 포획한 참붕어의 감염율은 82.0%에서 98.6%의 범위에 있었으나 3월, 4월, 11월 및 2월의 감염율은 11.4%에서 64.7%이었다.

2. 월별로 관찰하였을 때 피낭유충 감염밀도의 변화는 감염율과 상응하는 양상을 보였으며 일년동안 한마리당 피낭유충 평균감염밀도는 103.0개이었고 표준편차는 118.9개로 나타났다. 평균 피낭유충 밀도는 6월에 가장 높아 294.8개이었으나 11월에서는 11.1개로 가장 낮은 수치를 보였다.

3. 3월, 4월, 11월 및 2월에 검사한 참붕어내 피낭유충의 분포양상은 음의 이항확율분포에 일치($p>0.05$)하였으나 한편 5월 6월 7월 및 9월에 검사한 것은 대수정규확율분포에 부합($p>0.05$ 및 $p>0.75$)됨을 알 수 있었다. 평균치에 대한 분산의 비율은 7월(814.3)에 가장 높았으나 11월에서는 가장 낮았다(158.8).

이상의 결과에서 농후한 간흡충 유행지역에서 참붕어내의 간흡충 피낭유충 감염율과 감염밀도는 계절적으로 영향을 받는 것으로 나타났다. 계절적 변동에 관여하리라고 생각하는 요인에 대하여 검토하였다.