Tropical Medicine and Health Vol. 33 No. 1, 2005, pp. 7-11

Epidemiological study on *Centrocestus armatus* metacercariae in the Chikusa River, Hyogo Prefecture, Japan

DAISUKE KIMURA and SHOJI UGA
Accepted 11, January, 2005

Abstract: The prevalence of *Centrocestus armatus* metacercariae in fresh water fishes were examined in Hyogo Prefecture, western Japan for a period of one year from April 2003 to March 2004. Three species of cyprinoid fish, namely *Zacco temminckii, Zacco platypus,* and *Pseudogobio esocinus,* were found to harbor metacercariae of *C. armatus.* The infection rate of metacercariae in *Zacco temminckii, Zacco platypus,* and *Pseudogobio esocinus* was 99% (371/377), 100% (8/8), and 100% (2/2), respectively. The mean number of metacercariae recovered was 232 for *Z. temminckii,* 2,123 for *Z. platypus,* and 20 for *P. esocinus.* However, we focused on *Z. temminckii* in this study because it was found to be the most predominant fish species in the Chikusa River and to have high a metacercariae prevalence as well. *In Z. temminckii,* metacercariae were distributed in the brain (37%), viscera (35%), muscles (29%) and gills (0%). No metacercariae were recovered from the scales and fins. This is the first report of the occurrence of *C. armatus* metacercariae in the brain region of the second intermediate fish. The prevalence of *C. armatus* metacercariae was almost constant throughout the year, and no positive correlation was found between the prevalence and seasonal changes. However, a positive relationship was observed between prevalence of metacercariae and fish length, i.e., the intensity of infection increases with the size of the fish host ($p < 0.05$). People in the survey area sometimes eat fish raw, indicating enlightenment as well as the countermeasures to prevent *C. armatus* infection is needed.

Keywords: *Centrocestus armatus,* metacercariae, epidemiological study, freshwater fish, Chikusa River, Japan

INTRODUCTION

The fluke genus *Centrocestus* is a group of minute intestinal trematodes belonging to the family heterophyidae. *Centrocestus* is widely distributed in Japan, Taiwan, Republic of Korea, and Southeast Asia. Four species of *Centrocestus,* namely *C. armatus* [1], *C. formosanus*[2], *C. nycticoracis* [3] and *C. asadai* [4], have been reported to be distributed in Japan. These species normally infect fish-eating birds. They have also been reported to infect mammals, including man except for *C. nycticoracis* [5]. Moreover, *C. formosanus* var. *kurokawai, C. caninus,* and *C. armatus* have been reported to infect humans in Japan, Thailand, and Korea [6, 7, 8].

The first intermediate host of *Centrocestus* is a fresh water snail belonging to genus *Semisulcospira* [9], the second intermediate host fresh water fish, and birds such as *Nicticorax nycticorax* the final host [1]. About 20 species of fresh water fish including *Zacco platypus, Pseudorasbora parva,* and *Carassius carassius* are known to serve as second intermediate hosts of *C. armatus* [1, 10].

Rim *et al.* [11]reported that metacercariae of *C. armatus* were found at a rate of 33-100% in five species of fresh water fish from three rivers in Korea. Furthermore, Sukontason *et al.* [12] reported the presence of *Centrocestus* metacercariae in six species of cyprinoid fish in northern Thailand. The prevalence of metacercariae in different locations, seasons, and fish species had also been reported. However, the number of fish examined by Rim et al. was limited to only 1-15 samples. Moreover, the prevalence of fish infection was not described and no precise description was provided regarding the identification of metacercariae.

In the present study, therefore, the state of *Centrocestus* metacercariae infection in freshwater fish of the Chikusa River in Hyogo Prefecture, Japan was investigated from April 2003 to March 2004. The correlation of seasonal changes and fish length to metacercariae prevalence was also determined.

Department of Medical Technology, Faculty of Health Sciences, Kobe University School of Medicine, Kobe 654-0142, Japan
Correspondence: Uga Shoji
E-mail: ugas@ams.kobe-u.ac.jp
Tel & Fax: +81-078-796-4548

MATERIALS AND METHODS

Survey area

The study was carried out from April 2003 to March 2004 in the Chikusa River in Hyogo Prefecture, Japan. The survey area was a midstream section of the river 30 m in width and 0.1 to 1.5 m in depth. Various fish species were captured using a landing net once a month for a period of one year. The collected fish were kept alive in a cooled-box with aeration and brought to the laboratory. Taxonomic identification of the caputured fish species was carried out according to the fish atlas by Miyaji *et al.* [13]. In addition, water temperature was measured at the time of each survey.

Examination and observation of metasercariae

The fish brought to the laboratory were kept alive in an aquarium, with artificial aeration, until the examination of metacercaria. The body length of each fish sample was also measured and recorded. To obtain the metacercariae from each sample, fish was minced and digested using a digestive solution (0.1% each of pepsin and HCl in distilled water) at 37℃ for1hour. The digested material was filtered through a 325μm mesh to remove large particles and rinsed twice with saline solution (0.85% NaCl). The sediment was then centrifuged at 150 x g for 5 minutes. The *C. armatus* metacercariae collected were counted under a stereoscopic microscope.

In other experiments, organs such as the brain, muscles, gills, viscera, scales and fins were also dissected and digested artificially to determine the distribution of the metacercariae in the fish body. The counting of circumoral spines for identification of metacercaria was carried out using a light microscope and a scanning electron microscope (T-330A, JEOL, TOKYO). The size of different organs observed for metacercariae was also measured using a micrometer caliper (OLYMPUS, TOKYO) under a light microscope. In addition, the recovered metacercariae were excysted with artificial intestinal juice (0.4% sodium hydrogen carbonate, 1.0% trypsin, 0.85% NaCl) fixed with 10% neutral buffered formalin and observed under a scanning electron microscope to elucidate morphological detail.

Statistical analysis

The mean abundance was expressed as mean $\pm$ standard deviation (SD). The difference between length of fish and mean abundance was evaluated by one-way factorial analysis of variance (ANOVA), followed by Fisher's PLSD multiple comparison test to identify significant differences among the multiple samples. Differences in the prevalence of infection were tested using χ^2 test. A p value less than 0.05 was considered statistically significant.

RESULTS

Throughout course of the survey we caught 511 freshwater fish from Chikusa River. These consisted of 15 different species as shown in Table 1. Eight of the fish species belonged to the cyprinoid family. Table 1 shows the infection rate of each fish species examined and the mean number of metacercariae per fish. Metacercariae were obtained from only three cyprinoid fish species, namely *Z. temminckii, Z. platypus,* and *P. esocinus* with a high infection rate (99-

Table 1. Prevalence of metacercariae and mean number of metacercariae recovered in fish collected from the Chikusa River, Hyogo Prefecture, Western Japan

Species of fish	No. examined	No. of fish infected (%)		Mean No. of Mc** per fish
*Zacco temminckii**	377	371	(99)	229
Rhinogobius sp.	50	0	(0)	0
Plecoglossus altivelis	28	0	(0)	0
Silurus asotus	13	0	(0)	0
Cobitis biwae	12	0	(0)	0
*Zacco platypus**	8	8	(100)	2,123
Pelteobargus nudiceps	5	0	(0)	0
Coreopera kawamebari	5	0	(0)	0
Odontbutis obscurus	3	0	(0)	0
*Pseudogobio esocinus**	2	2	(100)	20
*Carassius carassius**	2	0	(0)	0
*Puntungia herzi**	2	0	(0)	0
*Squalidus gracilis gracilis**	2	0	(0)	0
*Tribolodon hakonensis**	1	0	(0)	0
*Cyprinus carpio**	1	0	(0)	0

* : Cyprinoid fish
** : metacercariae

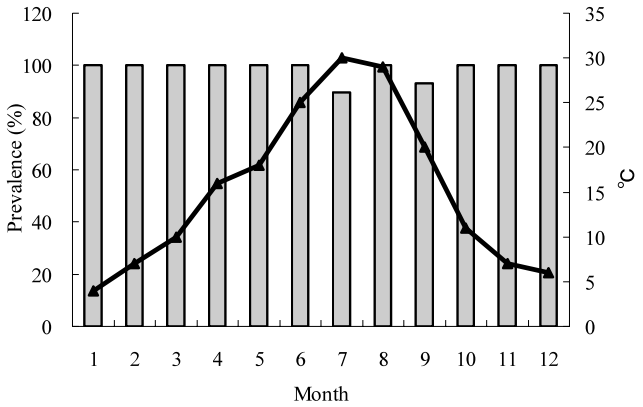

Fig. 1 Monthly changes in prevalence of metacercariae of *Centrocestus armatus* in *Zacco temminckii* (▒) and water temparature (▲).

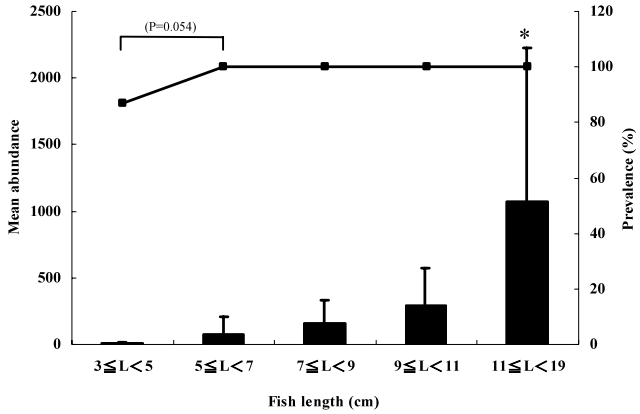

Fig. 2 Relationship of length of *Zacco temminckii*, mean abundance (■) and the prevalence (-■-) of *Centrocestus armatus* metacercariae in the Chikusa River, Western Japan. Data are expressed as mean ± SD (n: 16-65). *: $p<0.05$.

100%). *Z. platypus* showed the highest mean abundance of metacercariae. However, *Z. temminckii* was the most predominant fish species collected (74%) throughout the sampling period, and it also showed a high infection rate (99%). Therefore, further examinations were focused on *Z. temminckii*.

The monthly changes in the prevalence of metacercariae in *Z. temminckii* are shown in Fig. 1. The metacercariae infection rate of *Z. temminckii* was nearly 100% throughout the year. No positive relationship was observed between prevalence of metacerariae and seasonal changes and water temperature.

Throughout the survey, 84,921 metacercariae were recovered from infected 371 *Z. temminckii*, with a mean abundance of 229 (Table 1). Of the 11,760 metacercariae found in *Z. temminckii*, 37% (4,306) were observed in the brain; 0% (16) in gills; 35% (4,069) in viscera; and 29% (3,369) in muscles (Table 2). No metacercariae were found in scales and fins. The number of metacercaria was also computed per gram of fish meat. As a result, the brain showed the highest number of metacercariae per gram of meat (2,057/g), followed by viscera (262/g), muscles (20/g), and gills (5/g) (Table 2).

The relationship between fish length and the number of metacercaria per fish is also shown in Fig. 2. The prevalence levels were almost the same irrespective of fish length ($p > 0.05$). Even young fish of four centimeters or less in length showed an infection rate as high as 90%. The results also showed that the number of metacercaria per fish was directly correlated with fish length, i.e., there was an increase in the mean abundance of metacercaria as the fish length increased ($p < 0.05$). The number of metacercaria recovered from fish samples with smaller than 5 cm and more than11cm in length was8and1,069respectively ($p=0.017 \times 10^{17}$) (Fig. 2).

Table 2. The distribution of *Centrocestus armatus* metacercariae in various parts of fish

Parts of fish	Mean Weight (g)	Mean No. of Mc	Distribution rate (%)	No. of Mc/g
Brain	0.07	144	37	2,057
Gills	0.22	1	0	5
Viscera	0.52	136	35	262
Muscle	5.59	112	29	20
Scale, Fin	0.47	0	0	0

Thirty *Zacco temminckii*, 9-10 cm, were examined.

In addition, the recovered metacercariae were measured under a microscope. The average length was about 200 μm and the average width about 100 μm. The metacercariae were mounted on glass slides for detailed observation. The oral sucker, ventral sucker, and excretory bladder of mature metacercaria were observed. Light and scanning electron microscopic observations revealed that the oral sucker was located at the anterior end, with 44 circumoral spines arranged in two rows around it (Fig. 3a, 3b). The ventral sucker was located in the midventral part and the excretory bladder was shown as an "X"-shaped organ located posterior to the ventral sucker (Fig. 3a). However, these organs were not observed in immature metacercariae except for the eyespot that was a remnant of the cercarial stage (Fig. 3c).

DISCUSSION

Seven out of 20 known freshwater fish species were observed in this study as second intermediate hosts of *Centrocestus armatus* [1, 10]. In the present study, metacercariae of *C. armatus* were detected in only 3 out of the 15 freshwater fish species obtained from Chikusa River,

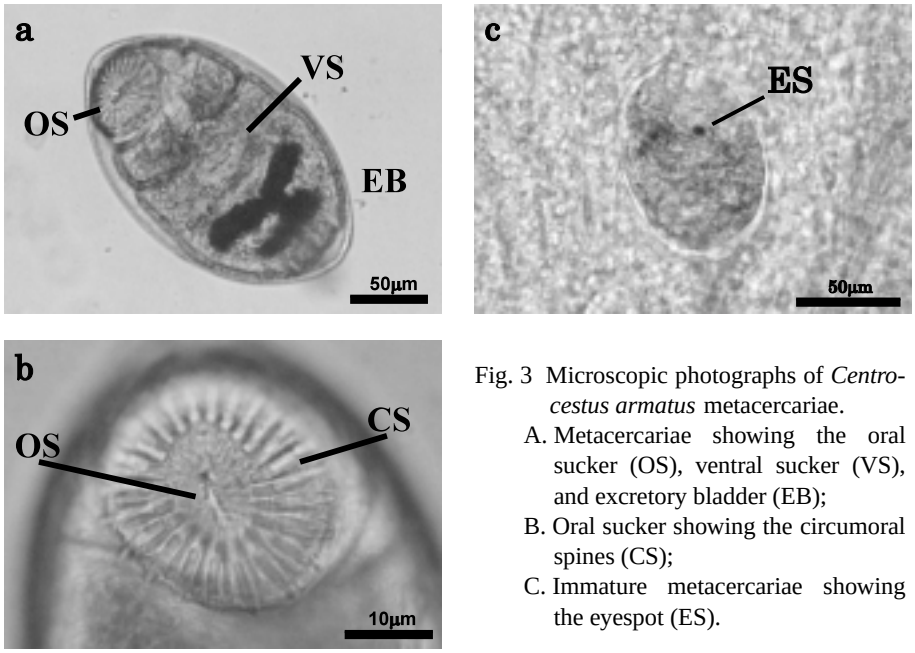

Fig. 3 Microscopic photographs of *Centrocestus armatus* metacercariae.
A. Metacercariae showing the oral sucker (OS), ventral sucker (VS), and excretory bladder (EB);
B. Oral sucker showing the circumoral spines (CS);
C. Immature metacercariae showing the eyespot (ES).

namely *Zacco temminckii, Zacco platypus,* and *Pseudogobio esocinus.* The prevalence of metacercaria in these three species of freshwater fish was almost 100%. Among the 3 species, *Z. platypus* showed the highest mean abundance of metacercaria. However, since *Zacco temminckii* was found to be the most predominant fish species collected from the river, it can be inferred that this fish played an important role as the second intermediate host of *Centrocestus armatus* in the Chikusa River.

The prevalence and intensity of trematodes infection to second intermediate hosts such as fish were said to be associated with seasonal changes [14]. Sukontason *et al.* [12] mentioned that trematode metacercariae levels were highest in winter and lowest in the rainy season. Several other studies showed that the emergence of cercariae from the first intermediate host increased as water temperature increased [15, 16]. A study was also conducted on prevalence of cercariae of *C. armatus* in the Chikusa River by Kimura and Uga [17]. They showed that the mean prevalence of cercariae in the snail *Semisulcospira libertina* was low at 6% (1-16%) during winter and spring seasons (October-May) when water temperature was low. In contrast, the period from June to September when water temperature was higher showed a very high prevalence. The mean prevalence value was 39% [17]. The authors conclude that the infection of cercariae to fish as the second intermediate host had occurred during summer when water temperature was high. However, the results of the present survey did not show any seasonal changes in prevalence of metacercariae. Chubb [14] also mentions that changes in the prevalence of metacercariae were not very evident at different climatic seasons of the year. He states that metacercariae could thrive for a long period of time inside the fish host and therefore that no drastic changes in metacercariae prevalence were observed at different seasons of the year.

Rim *et al.* [11] examined the distribution of metacercariae in the different organs of fish using 22 samples of *Z. temminckii* and *Z. platypus.* They reported that the metacercariae were distributed in the gills, muscles, intestinal organs, and scales and fins, and that the intestinal organs showed the highest mean abundance of metacercariae. In the present study, the brain, muscles, viscera, gills, and scales and fins of 30 *Z. temminckii* were examined for presence of metacercariae. The brain region showed the highest mean abundance (144), followed by the viscera and muscles with abundance of 136 and 112, respectively. However, the gills showed only a mean of 1, and 0 for scales and fins. These results were similar to the observation of Rim *et al.* [11] except for the presence of metacercariae in the brain region. In general, metacercariae of any fluke species can be found in the muscles and viscera of the fish body. In the present study, however, it is interesting that the infection of metacercariae of *C. armatus* was observed in the brain region of the second intermediate host. No related studies have reported the occurrence of metacercariae of *C. armatus* in the brain.

Poulin [18] reported the positive correlation of fish length and mean abundance of metacercariae. Dogiel *et al.* [19] also stated that, in fish populations, the intensity of infection by parasites increased with the age and size of the fish hosts. The same result was observed in the present study, i.e., the longer the fish length the higher the metacer-

cariae infection. At a fish length of 3-5 cm, the mean abundance was 8, but this increased gradually to 1,069 at a fish length of 11-19 cm ($p=0.017 \times 10^{-17}$). Since a fish with a larger length has a higher contact area, it is likely that cercariae have more chances for attachment to the fish body surface. These cercariae, in turn, could develop into metacercariae inside the fish body and accumulation could occur over time. That is why a higher number of metacercariae was observed in older fish with a greater fish length.

It was reported that metacercaria of the four species of *Centrocestus* distributed in Japan have a different number of circumoral spines: *C. armatus*, *C. formosanus*, *C. nycticoracis*, and *C. asadai* have circumoral spines of 44, 32, 42, and 38, respectively [10]. Throughout the present survey, the recovered metacercariae were found to have 44 circumoral spines and were identified as *C. armatus.*

In conclusion, the prevalence of *C. armatus* in *Z. temminckii* was high in the Chikusa River and the fish *Z. temminckii* is considered to be the most common second host. The questionnaire survey done in our study area [20] revealed that 52% of the inhabitants have eaten fresh water fish including *Z. temminckii.* Moreover, about 10% of inhabitants eat these fish raw, indicating possibility that the people living in this area have been infected by *C. armatus.* Further enlightenment as well as the countermeasures to prevent the infection is needed.

ACKNOWLEDGEMENTS

Dr. GV Paller kindly checked the English manuscript.

REFERENCES

1. Tanabe H (1922) Studien uber die trematoden mit Susswasserfischen als Zwischenwirt. I. *Stamnosoma armatum* n.g., n.sp. Kyoto Igaku Zasshi 19 (3): 239-252 (in Japanese).
2. Nishigori M (1924) On a new species of fluke, *Stamnosoma formosanum* and its life history. Taiwan Igakkai Zasshi 234: 181-238 (in Japanese).
3. Izumi M (1935) A new study of a trematode belonging to the Genus *Stamnosoma* and its life cycle. Tokyo Iji Shinji 59 (2948): 2423-2430 (in Japanese).
4. Mishima T (1959) On a new species of the genus *Centrocestus* with its life history. Tokyo Iji Shinji 76 (5): 291-299 (in Japanese).
5. Yamaguti S (1958) Systema Helminthum, Vol. I. Digenetic Trematodes of Vertebrates, pp.705-707 & 872-873.
6. Kurokawa T (1935) On a new trematode of genus *Stamnosoma* proved from a man. Tokyo Iji Shinji 2915: 293-298 (in Japanese).
7. Waikagul J (1977) Human infection of *Centrocestus caninus* in Thailand. Southeast Asian J Trop Med Public Health 28 (4): 831-5.
8. Hong SJ, Seo BS, Lee SH, Chai JY (1988) A human case of *Centrocestus armatus* infection in Korea. Korean J Parasit 26 (1): 55-60.
9. Takahashi S (1929) A contribution to the life-history of *Stamnosoma armatum* Tanabe. Okayama Igakkai Zasshi 4 (8): 1759-1771 (in Japanese).
10. Komiya Y (1965) Metacercaria in Japan and adjacent territories. *Progress of Med. Parasit. in Japan.* Vol. II. Meguro Parasitological Museum, Tokyo. 135-143.
11. Rim HJ, Kim KH, Joo KH, Kim S J, Eom KS, Chung MS (1996) The infestation states and changing patterns of human infecting metacercariae in freshwater fish in Kyongsang-do and Kyonggi-do, Korea. Korean J. Parasit 34 (2): 95-105.
12. Sukontason K, Piangjai S, Muangyimpong Y, Methanitikorn R, Chaithong U (1999) Prevalence of trematode metacercariae in cyprinoid fish of Ban Pao district, Chiang Mai Province, northern Thailand. Southeast Asian J Trop Med Public Health 30 (2): 365-370.
13. Miyaji D, Kawanabe H, Mizuno N (1976) Colored illustrations of the freshwater fishes of Japan. Hoikusha Publishing CO., LTD Osaka (in Japanese).
14. Chubb JC (1979) Seasonal occurrence of helminthes in freshwater fishes. Part II. Trematoda. *In*: Lumsden WHR, Muller R, Baker JR (eds) Advances in parasitology, Vol 17. Academic Press, London, p141-313.
15. Taskinen J, Valtonen ET, Makela T (1994) Quantity of sporocysts and seasonality of two *Rhipidocotyle* species (Digenea: Bucephalidae) in *Anodonta piscinalls* (Mollusca: Bivalvia). Int J Parasitol 24: 877-886.
16. Lyholt HCK Buchmann K (1996) *Diplostomum spathaceum:* effects of temperature and light on cercarial shedding and infection of rainbow trout. Dis Aquat Organ 25: 169-173.
17. Kimura D, Uga S (2003) Epidemiological studies on *Centrocestus* spp. (Trematoda: Heterophyidae) in Chikusa River basin, Hyogo Prefecture: infection in the1 st intermediate host snail, *Semisulcospira libertina.* Jpn J Environ Entomol Zool 14 (2): 97-103 (in Japanese with English summary).
18. Poulin R (2000) Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. J Fish Biol 56: 123-137.
19. Dogiel VA, Petrushevski GK, Polyyanski YI (1958) Parasitology of Fishes. Oliver and Boyd, London (Translated by Z Kabata, 1961).
20. Kankawa, Y. and S, Uga. (2001) Epidemiological study on metagonimus spp. in Chigusa River, Hyogo Prefecture, Japan. *Jpn. J. Environ. Entomol. Zool,* 12 (3): 147-153. (in Japanese with English summary).