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RESEARCH ARTICLE

Infection Status of Metacercaria in Adult Dragonflies from Republic of Korea

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ABSTRACT

The dragonfly serves as a second intermediate host of some trematodes. Seven species of dragonflies, *Sympetrum drawinianum, Orthetrum albistylum, Lyriothemis pachygastra, Sympetrum eroticum, Crocothemis servilia, Pantala flavescens and Sympetrum pedemontanum* were surveyed. The most abundant species among these dragonflies were *S. drawininanum, S. eroticum* and *C. servilia* (2,118 and 620 and 334 individuals, respectively). And, the least abundant dragonflies were *S. pedemontanum, L.pachygastra* and *O albistylum* (25, 57 and 62 individuals, respectively). Among these intermediate hosts, *S. eroticum* had the highest infestation rate of metacercaria per individual (11.71%). The infestation rates of two dragonflies, *S. drawinanum* and *S. pedemontanum* (8.58% and 4.56%, respectively) also were higher than those of the other four species. In artificial infection studies using animal hosts, we could identify the infections of adult *P. muris* and *P. japonicus* from only mouse, in which the infestation rates of *P. muris* and *P. japonicus* were 90% and 95% among 20-tested individuals, respectively. Interestingly, adult *L. liberum* was detected from only frog, *R. nigromaculata* and the rates of the infestation in frogs were 97.5% among 50-tested frogs. These results suggest that the population size of dragonfly is an important factor to carry high burden of metarcercaria. Moreover, we discussed their epidemiological implications for human and animal infections.

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INTRODUCTION

Tramatodes have five larval stages in the life cycle: miracidium, sporocyst, redia, cercaria, and metacercaria. Some of the stages may occur in a wide range of other invertebrate and sometimes in vertebrate, which serve as an intermediate host. Snails are the first intermediate host of digenetic trematodes, and then there are various second intermediate hosts, including arthropods and freshwater fishes. Dragonflies also serve as the second intermediate host of the species including the families of trematodes; *Prosthogonimidae, Microphallidae, Lecithodendriidae* and *Plagiorchiidae* (Asada *et al*., 1962). Since dragonflies are one of the predominant insects found in Republic of Korea (ROK) during summer, this may indicate the epidemiological importance of the trematode infections in the region.

In ROK, the trematodes in the genus *Plagiorchi* are the most abundant species, which include *P. koreanus, P. orientalis, P. corpulentus, P. vespertilionis, P. rhinolophi* and *P. kyushuensis* (Kifune *et al.*, 1983; Hong *et al*., 2006; Shin *et al*., 2008; Chai *et al*., 2009). In addition, *Plagiorchis muris* was one of the well characterized species in this region and frequently collected from wild and house rats captured in a range of collecting sites in ROK (Lee *et al.*, 1990; Seo *et al.*, 1964). So far, the distribution of *P. muris* is currently assumed to be prevalent in ROK. The other second intermediate hosts

such as dragonflies and freshwater fishes were also found to be infected by this trematode (Hong *et al*., 1998).

Regarding to other prevalent trematodes, *Loxogenus liberum* and *Pleurogenoides japonicus*, they were collected from frogs from the Han River in ROK, but their epidemiological impact to human and animal populations in ROK is still tentative *(Lee et al.*, 1976). These lines of evidences support the importance of intermediate hosts in transmission cycle of the trematodes. Therefore, in this study, we conducted to elucidate the infestation status of the dragonflies with trematodes.

MATERIALS AND METHODS

Collection of metacercaria in dragonflies and infection test in animal hosts

Dragonflies were caught near villages and streams in Daejeon city, Chungnam, Chonbuk province in ROK during June-August, 2009. Three collection sites within each village were chosen according to recommendations from local health department personnel (Fig. 1.). These dragonflies were identified according to method previously described (Shin, 1993) and grouped in species of dragonfly. After the discard of wings, each group of dragonflies was artificially digested for 2 hrs in artificial gastric juice (pepsin 6g, HCl 7ml in 1L D.W.) at 37 °C. The digests were washed and sedimented several times with 0.45% saline at room temperature. Six types of metacercaria harvested from dragonflies were classified as described previously (Park *et al.*, 2003). The metacercariae were collected from the sediment of digest under the dissecting microscopy and grouped under the light microscopy. Each grouped metacercariae was fed to mouse, rat, rabbit, chick, duck, dog and frog with a gastric needle. The number of tested host ranges from 20 to 100 individuals.

Light microscopy

The feces from animals were examined by Formaline-Ether method for the detection of eggs 4 days later. To validate the infection success, the small intestines of the infected animals were examined 19 days after the metacercarial infection. The recovered flukes were stained with Semichon's acetocarmine and identified using light microscopy.

RESULTS

Infection status of metacercaria in dragonflies and in animals

Seven species of dragonflies, *Sympetrum drawinianum, Orthetrum albistylum, Lyriothemis pachygastra, Sympetrum eroticum, Crocothemis servilia, Pantala flavescens* and *Sympetrum pedemontanum* were identified in this study. A total of 3,371 dragonflies were captured, in which 26,255 metacercaria were harvested. During the summer in 2009, the most abundant species among these dragonflies were *S. drawininanum, S. eroticum* and *C. servilia* (2,118 and 620 and 334 individuals, respectively, Table 1 and Fig. 2). And, the least abundant dragonflies were *S. pedemontanum, L.pachygastra* and *O albistylum* (25, 57 and 62 individuals, respectively, Table 1 and Fig. 2).

Six types of metacercarida, A, B, C, D, E and F types were observed in this study (Table 1 and Fig. 2). Three species of metacercaria were identified from A, B, and C types, which were *Loxogenes liberum, Pleurogenoides japonicus, Plagiorchis muris*. Three species were however, not characteristic among D, E and F types. Infestation rates of metacercaria also vary in a range from 0.47% to 11.71% (Table 1 and Fig. 2). Among these intermediate hosts, *S. eroticum* had the highest infestation rate per individual (11.71%, Table 1, Fig. 2 and Fig. 3). The infestation rates of two dragonflies, *S. drawinanum* and *S. pedemontanum* (8.58% and 4.56%, respectively) were also higher than those of the other species (Table 1, Fig. 2 and Fig. 3). Overall, there is a positive correlation between the population sizes of dragonflies and infestation rates of metacercaria. However, *S. pedmontanum* show the relatively high infestation rate even though they are scarce in the field (4.56% and 25 individuals, Table 1 and Fig. 2).

Infection rates among candidate animal hosts

In artificial infection studies using animal hosts, we could identify the infections of adult *P. muris* and *P. japonicus* from only mice, in which the infestation rates of *P. muris* and *P. japonicus* were 90% and 95% (each 20 individuals). Interestingly, adult *L. liberum* was harvested from only frog, *R. nigromaculata* and the rates of the infestation frogs were 97.5% among 50-tested frogs.

Fig. 1: Maps showing the three provinces (Daejeon, Chungcheongnam-do, Jeollabuk-do) sampled in Republic of Korea and village locations of the collection sites.

DISCUSSION

The dragonflies, *Sympetrum* species, were caught at rice paddies where they emerge from pupae and mature into adults. These dragonflies can be easily caught in the early morning or on rainy days. The other dragonfly, *P.*

Table 1: Prevalence of metacercaria in adult dragonflies from Republic of Korea

Species of dragonfly	No. of dragonfly	Species of metacercaria							No. of metacercaria infection per dragonfly
		P. muris	*L. liberum*	*P. japonicus*	D type	E type	F type	Total	
Sympetrum drawinianum	2,118	583	13,121	338	4,036	89		18,167	8.58
Orthetrum albistylum	62	29	18		26		30	103	1.66
Lyriothemis pachygastra	57	3	84		37			124	2.17
Sympetrum eroticum	620	85	1,143	28	5,973	31		7,260	11.71
Crocothemis servilia	334	25	102		29		2	158	0.47
Pantala flavescens	180	2	45		274	3	5	329	1.83
Sympetrum pedemontanum	25	72	5		37			114	4.56
Total	3,371	727	14,513	366	10,375	123	37	26,255	

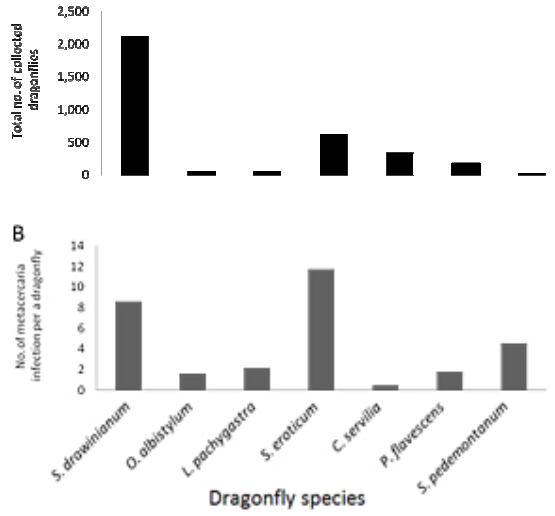

Fig. 2: Infection status of metacercaria in dragonflies. (A) Total number of specimens of dragonflies collected on three province areas (black bars). (B) Total number of metacercaria in each dragon species collected (grey bars). The dragonfly *S. drawininanum* is the most abundant species among seven dragonfly species collected from three regions in ROK. The dragonfly *S. eroticum* shows the highest average infection rate of metacercaria per an individual dragonfly.

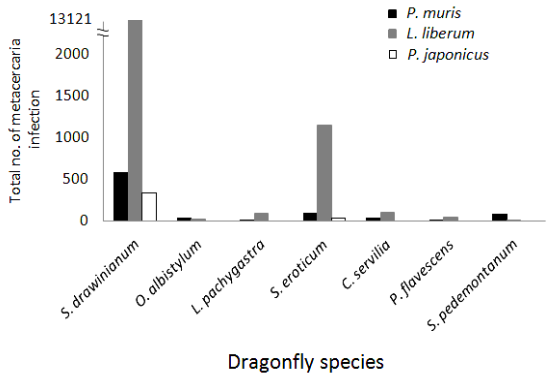

Figure 3: Total numbers of metacercaria infection from seven dragonfly species, the intermediate host. The trematode *L. liberum* was the most abundant metacercaria, especially in two dragonflies, *S. drawinianum* and *S. eroticum*. The other trematode *P. muris* is the second most abundant species, and mostly found in the dragonfly *S. darwinianum*.

flavoscens can be caught from a swarm of dragonflies flying in the air. Although there has been an implication of the involvement of dragonflies in transmission cycle of metacercaria in ROK (Hong *et al*., 1999), the impact on disease transmission cycle is still tentative. This study support the various dragonflies can be an important secondary host to transmit metacercaria to the other animal hosts. Interestingly, although three metacercaria species can infect all of seven dragonflies, there are different infestation capacities among dragonflies. The population size of dragonfly seems to be a significant factor to carry high burden of metarcercaria. Thus, understanding of ecological factors of two most abundant dragonflies, *S. drawinianum* and *S. eroticum* might elucidate the transmission cycle of metarcercaria, specially, in *P. muris* and *L. liberum*.

In previous epidemiological studies of *P. muris*, the metacercarial burden in the dragonflies in ROK appeared not to be closely related to the occurrence of the adult flukes in the rat (Seo *et al.*, 1964). There was, however, a disparity from a different survey (Hong *et al*. 1999); it was suggested that the occurrence of adult *P. muris* in house rats was related to the metacercaria in dragonflies. This result also suggests that field rats could be infected by feeding the infective dragonflies. The other host animal, bat, was recorded as a host animal of seven species of *Plagiorchis* (Kifune *et al*., 1983; Shin *et al*., 2008; Chai *et al*., 2009). These studies suggest that bats can be infected by infective insects such as dragonflies, mosquitoes, and chironomids which can be the second intermediate hosts of *P. muris* (Komiya, 1965; Tanabe, 1922). The aquatic insect larvae have also been thought to be a source of human infection of *P. muris* in Japan (Asada *et al.*, 1962). The surface water of springs, wells, ponds, streams and rivers contain a wide variety of aquatic arthropods which might be infected with *P. muris* metacercaria. Thus, by drinking the surface water without a proper treatment, human could also be infected by *P. muris* in ROK as was in Japan.

In previous epidemiological studies of *L. liberum*, this metacercaria is frequently found in frog, *R. nigromaculata* in ROK (Lee *et al.*, 1976). In present study, only *L. liberum* infection was found in the frog among candidate host animals. Among dragonflies in this study, *L. liberum* was the most abundant metacercaria, especially in two dragonflies, *S. drawinianum* and *S. eroticum*. Interestingly, the infestation rates of unclassified D-type metarcercaria were conspicuously

high in these two dragonflies. This infection pattern was not observed from the other unclassified E, and F-type metacercaria. This may, therefore, indicate that the unclassified D-type metacercaria could be a variety or other stage of *L. liberum* metacercaira in the intermediate host animals. This is a still tentative hypothesis, but clarification of this speculation will be important to understand the transmission cycle of *L. liberum* through the intermediate host, including dragonflies or other arthropods.

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