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Gnathostomiasis in Frog-eating Snakes from Japan

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ABSTRACT: *Gnathostoma doloresi* parasitizes the gastric wall of wild (boars) and domestic (pigs) swine (*Sus scrofa*). Its larvae cause cutaneous larva migrans in humans. Amphibians, reptiles and a freshwater fish are infected with the advanced 3rd stage larvae. Prevalence of *G. doloresi* larvae were surveyed in several snakes, especially in a common frog-eating snake (*Rhabdophis tigrinus*). All species of snakes examined were infected with *G. doloresi* larvae suggesting that snakes are important reservoir hosts. Prevalence of *G. doloresi* larvae in frog-eating snakes was lower than that found in mammal-eating snakes. Thus, as a source of infection to snakes, small mammals may be more important than frogs in the natural life cycle of *G. doloresi* in Japan.

Key words: *Agkistrodon halys, Elaphe quadrivirgata, E. conspicillata, Gnathostoma doloresi*, nematode prevalence, *Rhabdophis tigrinus*, transmission.

Gnathostoma doloresi occurs in the gastric wall of wild (boars) and domestic (pigs) swine (*Sus scrofa*) in Asia. This large nematode (Gnathostomatidae) causes ulcerative or granulomatous lesions in the stomach of those host animals. *Gnathostoma doloresi* is, like other *Gnathostoma* spp. such as *G. spinigerum, G. hispidum,* and *G. nipponicum,* known as an important zoonotic pathogen causing cutaneous larva migrans in humans (Nawa, 1991). Since the first case of gnathostomiasis by *G. doloresi* infection was discovered in Miyazaki Prefecture (Nawa et al., 1989), about 30 cases have been seen in the vicinity of Miyazaki (Maruyama et al., 1996). While the definitive hosts of *G. doloresi* are domestic pigs in most Asian countries, in Japan wild boars (*S. scrofa leucomystax* and *S. riukiuensis*) are important hosts (Miyazaki, 1960). Cyclops are the first intermediate host (Ishii, 1956). Advanced 3rd stage larvae have been found in various amphibians (Miyazaki and Ishii, 1952; Hasegawa et al., 1981, 1982), reptiles (Miyazaki and Kawashima, 1962; Tada et al., 1969; Toshioka, 1970; Hasegawa et al., 1981; Mako and Akahane, 1985; Imai et al., 1988) and a freshwater fish (Nawa et al., 1993). However, the relative importance of these intermediate and/or paratenic hosts in the life cycle of *G. doloresi* has never been studied in detail. Advanced 3rd stage larvae of *G. doloresi* are concentrated in a poisonous snake (*Agkistrodon halys*) which is a favored food item of wild boars (Imai et al., 1988). Since the major prey items of *A. halys* are small mammals (about 60%) and frogs (about 20%) (Uchida and Imaizumi, 1939), it is of interest to identify which is more important as the source of infection of *G. doloresi* larvae to snakes. Because *Rhabdophis tigrinus* is a common frog-eating snake, we examined the prevalence of *G. doloresi* larvae in this host, caught in the same locality as our previous survey was conducted on *G. doloresi* infection in *A. halys* (Imai et al., 1988). In addition, several other species of snakes caught at this locality also were examined.

In August and October 1996, 17 snakes were captured around Shiromi Village (32°16′N, 131°15′E) at Saito city (Japan). They were anesthetized by hypothermia on ice. After measuring the body length and weight, their heads were cut off and the skin peeled. Viscera were removed and the muscles with bones were minced and homogenized by a blender with 10-fold volumes of artificial gastric juice consisting of 0.1% pepsin (1:10,000; NACALAI TESQUE Inc., Kyoto, Japan) and 0.7% conc. HCl in 1 L of distilled water, then digested at 37 C overnight with gentle stirring. The residues were examined for larvae under a dissecting microscope.

The parasite specimens we collected in this study were deposited in the Department of Parasitology, Miyazaki Medical

College, Japan; (Accession number GdL960806).

Thirteen *R. tigrinus* (mean body length ± SE = 82 ± 4 cm, range (*r*) = 53–102 cm; mean body weight = 87 ± 12 g, *r* = 26–165 g), two *A. halys* (54 and 48 cm, 86 and 84 g each), one *Elaphe quadrivirgata* (110 cm, 170 g) and one *E. conspicillata* (54 cm, 21 g) were examined. Six of 17 snakes (35%) were infected with *G. doloresi* larvae. The prevalence and the intensity of infection in *R. tigrinus* was low; 3 of 13 (23%) had only a few larvae (1, 2 and 3 larvae each). In contrast, one of two *A. halys* had 49 larvae of *G. doloresi*. *Elaphe quadrivirgata* and *E. conspicillata* also were infected with *G. doloresi* larvae (2 and 4 larvae each), although only one each of the species were examined. It should be noted that four *G. doloresi* larvae were found from *E. conspicillata* in spite of its small size.

In our previous survey on the prevalence of *G. doloresi* larvae in *A. halys* captured around Shiromi Village, all (6/6) *A. halys* were infected with the larvae (mean intensity = 25 ± 6, *r* = 11–48) (Imai et al., 1988). Compared with this previous data in *A. halys,* the present results clearly show that the prevalence and the degree of infection with *G. doloresi* in the frog-eating snakes *R. tigrinus* were low. Unexpectedly, a very small *E. conspicillata* was infected with 4 larvae. According to Uchida and Imaizumi (1939), *A. halys* eats mostly small mammals and some frogs, *R. tigrinus* consumes only frogs, and *E. conspicillata* ingests only small mammals. Thus, transmission of *G. doloresi* larvae from frogs to snakes might occur less frequently than that from small mammals to snakes in the natural life cycle of *G. doloresi* in Japan. A more extensive survey on larger numbers of samples is required to substantiate this hypothesis.

In the present study, despite the large variance in the prevalence and the density, all species of snake that we examined were infected with *G. doloresi* larvae. Because snakes are one of the favorite prey items of wild boars (Imai et al., 1988), they might be an important source of *G. doloresi* infection for wild boars in addition to frogs (Hasegawa et al., 1981; 1982) and salamanders (Miyazaki and Ishii, 1952). The prevalence of *G. doloresi* in wild boars has been extremely high in the mountainous areas of Kyushu District, Japan (Miyazaki, 1960; Sakaguchi et al., 1985; Nawa and Imai, 1989). The natural life cycle of *G. doloresi* seems to be maintained by a complicated predator/prey relationship among intermediate and/or paratenic hosts.

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