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Gnathostoma doloresi: Development of the Larvae Obtained from Snakes, *Agkistrodon halys*, to Adult Worms in a Pig

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Abstract

One hundred advanced third-stage larvae of *Gnathostoma doloresi* obtained from snakes, *Agkistrodon halys*, were experimentally infected in a pig. The eggs began to appear in the feces on day 58 post-infection and the egg count per gram of feces rose rapidly with time. By autopsy on day 87, a total of 21 worms were recovered from the stomach. The adult worms in the stomach and the eggs in feces had morphological characteristics of *G. doloresi*. These results show that the *G. doloresi* larvae obtained from *A. halys* could develop to mature adults in the final host.

Key words: *Gnathostoma doloresi*, experimental infection, pig, the advanced third stage larvae, snake, *Agkistrodon halys*

Introduction

Gnathostoma doloresi is naturally a parasite of wild boars and pigs, and is widely distributed in the southern and western part of Japan (Miyazaki, 1960). Recently, however, human cases of infection with the advanced third-stage larvae of *G. doloresi* have been found in Miyazaki Prefecture, Kyushu (Nawa *et al.*, 1988; Ogata *et al.*, 1988; Nawa *et al.*, 1989). Thus, this parasite is now considered as one of important pathogens causing zoonoses. Although the exact route of infection to human or the life cycle of this parasite in the endemic area has not yet been determined, we have found that the poisonous snakes, *Agkistrodon halys* (Common Japanese name: Mamushi) captured in the endemic area harboured the encysted larvae, which were identified morphologically as the advanced third stage larvae of *G. doloresi*, with a 100% prevalence (Imai *et al.*, 1988). To confirm further our identification of the larvae and to examine their infectivity, the larvae recovered from snakes, *A. halys*, were inoculated into a pig which is known as the final host for *G. doloresi* in nature (Miyazaki, 1960) and in experiments (Ishii, 1956; Mako and Akahane, 1985; Horiuchi *et al.*, 1988).

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Materials and Methods

The snakes, *A. halys*, were captured in Shiromi-Village, Saito City, Miyazaki Pref., where is located in the center of the endemic area of human gnathostomiasis doloresi. To recover the larvae, the carcases of the snakes were minced and digested in an artificial gastric juice (pepsin 1 g, conc. HCl 7 ml, distilled water 1000 ml) at 37°C for 3 hr. The advanced third-stage larvae were collected, washed extensively with saline, and kept in saline at 4°C until used.

A young female pig (Berkshire strain, 45 day-old and weighing approx. 3.0 kg) was infected with one hundred *G. doloresi* larvae by intra-peritoneal inoculation through a small midline in-

cision on the abdominal wall under intramuscular anesthesia with Ketamin-HCl. The pig was kept under a clean, conventional condition in the Animal Center, School of Medicine, Fukuoka University.

Daily fecal egg count was monitored using AMS-III method from 6 weeks afterwards. The pig was starved for 24 hr before autopsy on day 87 post-infection. The stomach was cut open along the greater curvature and was inspected macroscopically. The worms were removed carefully from the stomach and fixed in 10% buffered formalin. Some worms were further processed for scanning electron microscopic observation as described previously (Imai *et al.*, 1988).

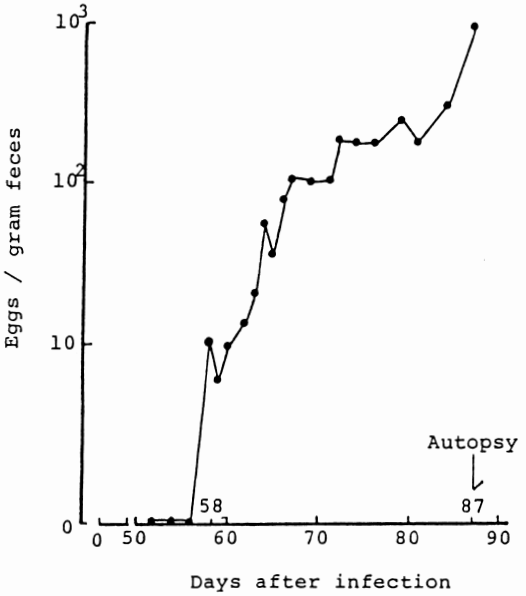


Fig. 1 Daily egg count in feces after infection.
A Berkshire pig was infected with 100 advanced third stage larvae of *Gnathostoma doloresi* obtained from snakes, *Agkistrodon halys*.

Results

After infection, eggs in feces became detectable on day 58 and then the egg count rapidly increased with time (Fig. 1). At the time of autopsy on day 87, the number of eggs in gram of feces was 982. The fertile eggs in feces were of bulge-shape, containing one or two round cells, and had a cap at the each end of the shell (Fig. 3a). Their average size was 62.6 ± 2.2 × 31.6 ± 0.7 μm (Mean ± SE, n=30).

When the pig was autopsied, 12 parasitic burrows were found in the stomach wall. All these burrows were located in the fundus gland region (Fig. 2) and some burrows were surrounded by a thick crater-like protuberance (Fig. 3b). In all but one burrows 1—5 worms were found penetrating their head into the wall and stretching their tail out to the lumen (Fig. 3b). Eighteen worms (Fig. 3c; 7 males, 6 females and

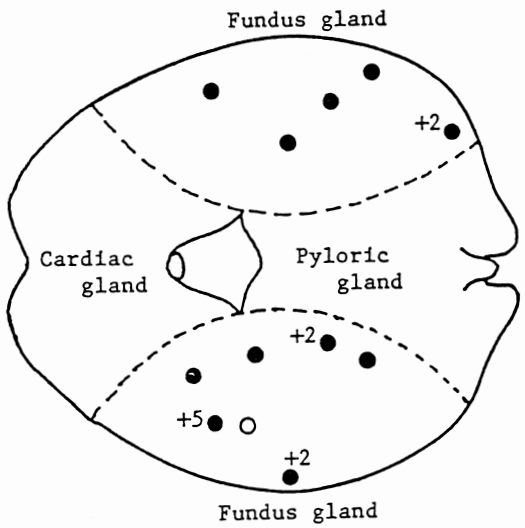
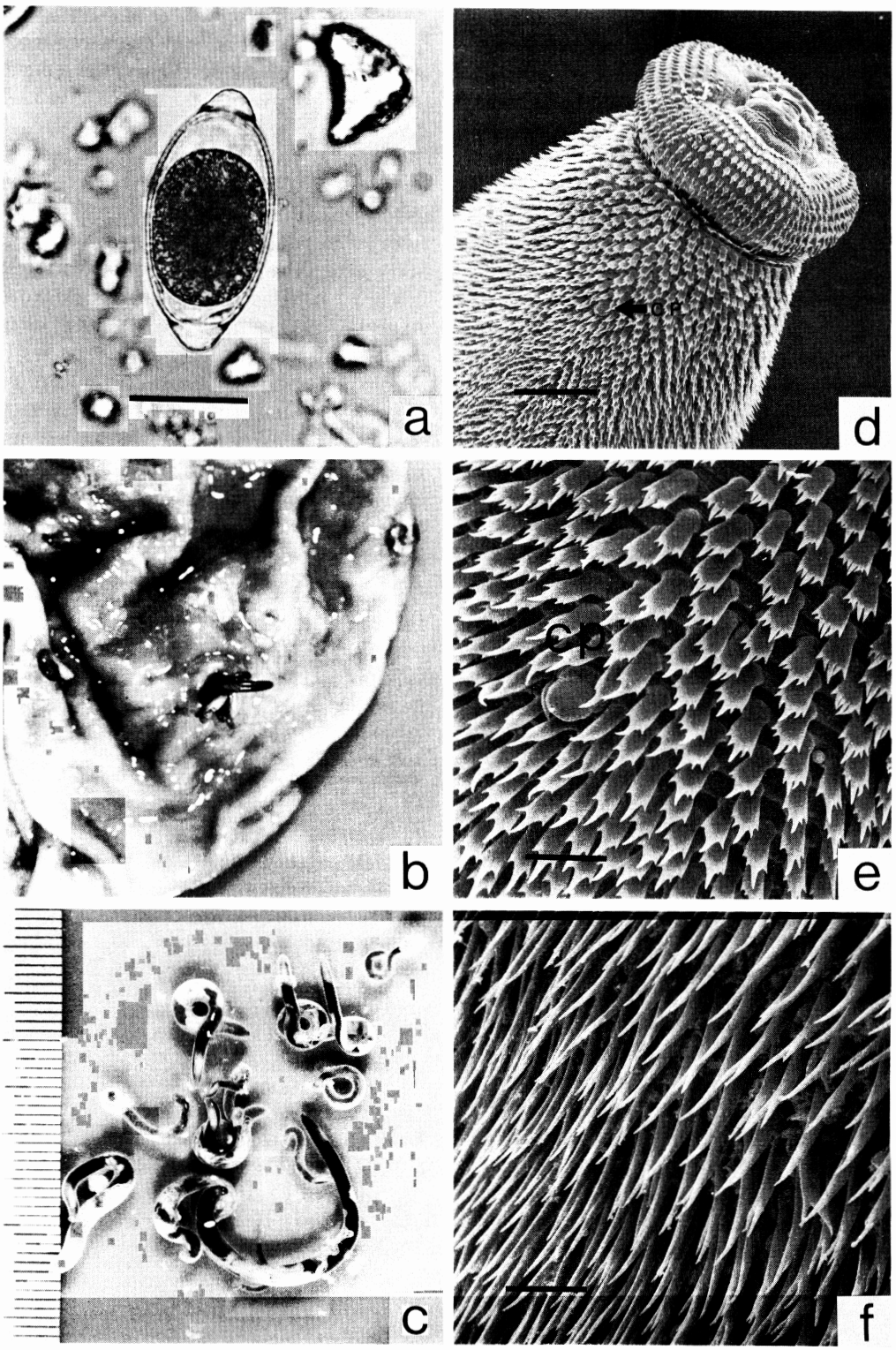


Fig. 2 Distribution of parasitic burrows in the stomach wall of the pig.

Fig. 3a A fertile egg in feces of the pig. (Scale; 30 μm)
Fig. 3b Adults of *G. doloresi* in the stomach of the pig.
Fig. 3c Adult worms recovered from the stomach.
Figs. 3d-f Scanning electron microscopic observation of an adult worm.
Fig. 3d Head bulb. CP: cervical papilla (Scale: 150 μm)
Fig. 3e Cuticular spines around cervical papilla (Scale: 45 μm)
Fig. 3f Cuticular spines on the posterior part of the body (Scale: 30 μm)


a
b
c
d
e
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GH
cp

5 damaged and unidentifiable) were recovered from the gastric wall. One burrow had no worms. One fully mature female worm was found free in the gastric contents. In addition, two immature worms having four rows of hooklets on the head-bulb were found in the subserosal connective tissue of the stomach. Thus, a total of 21 worms were recovered from the stomach with the recovery rate of 21%.

The body size of mature males ranged 13.5—20.5 × 1.0—1.8 mm (avg. 16.9 × 1.4 mm), while that of females ranged 19.8—29.2 × 2.0—2.9 mm (avg. 23.5 × 2.4 mm). The head-bulb of the adult worms was provided with 8—9 transverse rows of hooklets (Fig. 3d). A pair of cervical papillae were seen at the height of the 14—18th transverse rows of the cuticular spines (Figs. 3d, e). All over the body surface was covered with cuticular spines; those from the neck to the cervical papillae were pentadental and those from the cervical papillae to the middle part of the body were tridental, with the middle tooth being the longest (Fig. 3e). The posterior half was, to the end of the body, densely covered with long unidental spines (Fig. 3f).

Discussion

The results reported here show that the advanced third-stage larvae of *G. doloresi* obtained from snakes, *A. halys,* could mature into the adult stage in the stomach wall of a pig. The morphological characteristics of the adult worms in the stomach or of eggs in the feces were the same as those described by Miyazaki (1960) and also recently by Sakaguchi *et al.* (1985). The gross appearance and the localization of parasitic burrows in the stomach wall were essentially identical to those described on naturally infected pigs (Ashizawa *et al.*, 1967) or wild boars (Ashizawa *et al.*, 1969).

In the present study, excretion of eggs into the feces began on day 58 and the fecal egg count continuously rose till the pig was autopsied on day 87. Similar results were reported in experimental infection in wild boars (Miyazaki and Ishii, 1952) or in pigs (Ishii, 1956; Mako and Akahane, 1985; Horiuchi *et al.*, 1988). These results indicate that at least about two months are necessary for the development and maturation from the advanced third stage larvae of *G. doloresi* to the adult worms.

Since *G. doloresi* larvae were found in *A. halys* with a 100% prevalence in the endemic area in Miyazaki Pref. (Imai *et al.*, 1988) and since these larvae could develop to adults in a pig, *A. halys* seem to be an important paratenic host in this area. Akahane and coworkers (Mako and Akahane, 1985; Horiuchi *et al.*, 1988) already reported a successful infection in pigs with *G. doloresi* larvae obtained from *Dinodon semicarinatus* and emphasized the importance of snakes as the paratenic host. Since the major foods of *A. halys* are frogs and small mammals (Uchida and Imaizumi, 1939), these creatures in the endemic area should be examined as the source of infection to snakes.

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