ANGIOSTRONGYLUS CANTONENSIS INFECTION IN *AMPULLARIUS CANALICULATUS* (LAMARCK) IN KYUSHU, JAPAN

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INTRODUCTION

Angiostrongylus cantonensis is primarily a parasite of wild rodents, but it is now generally recognized as the causative agent of human eosinophilic meningoencephalitis widely observed in the Pacific, Southeast Asia and even some parts of Northeast Asia. In Japan, *A. cantonensis* was first found in rodents captured in Iriomote Island of the Ryukyu Islands (Nishimura *et al.*, 1964). Since then, it has been well established that the parasite is commonly distributed in the Ryukyu Islands. The distribution of *A. cantonensis* in the Amami Islands in Kyushu is also recognized (Ishida *et al.*, 1977; Yamashita *et al.*, 1978; Sato *et al.*, 1982). Moreover, presence of the parasite in rodents has been recorded from as far north as Hokkaido (Hattori *et al.*, 1982).

A. cantonensis requires a molluscan intermediate host for completion of its life cycle. Various species of terrestrial and aquatic snails and terrestrial slugs are known as the intermediate host (Alicata and Jindrak, 1970).

Ampullarius canaliculatus is a large freshwater snail belonging to Family Ampullariidae (Fig. 1). The original habitat of the snail is said to be Argentina. In 1981, this species of snail was brought to Japan for food. However, the farming of the snail did not become as popular as expected for economical reasons. Since then, those which grew wild have propagated in rice fields, irrigation canals, ponds and lakes, and are causing damage to the rice plant or other aquatic plants by feeding on them. The damage is considerable, especially in Kyushu, and now it is a serious problem in agriculture (Miyahara *et al.*, 1986).

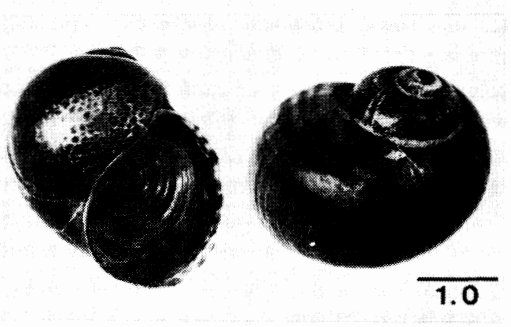

Fig. 1—*Ampullarius canaliculatus*(Lamarck)collected from Saga Prefecture in Kyushu, Japan. (Scale in cm)

If *A. canaliculatus*, which is growing in number everywhere in Japan proved to be a suitable intermediate host for *A. cantonensis*, it would be a valuable species from the parasitological point of view. For this reason, the present study was undertaken to find out whether *A. canaliculatus* would be a suitable intermediate host for *A. cantonensis*.

MATERIALS AND METHODS

In order to examine whether natural infection with *A. cantonensis* is present in *A. canaliculatus*, snails were captured alive

and brought to the laboratory. The shell of each snail was crushed and the muscular portion of the foot, together with visceral organs was removed. The tissure was minced and digested artificially by being kept in a solution containing 1% hydrochloric acid at 37°C for 1 and a half hours. Except for examination of an individual snail, 13 to 50 snails were minced together and artificially digested. The sediments were examined for larvae with a microscope. Larvae of *A. cantonensis* were identified by their characteristic morphological features. Actively moving third-stage larvae were fed orally to albino rats. Autopsy of each rat thus treated was performed 43 or more days after the infection. The lungs and the heart were removed from the thoracic cavity and were examined for gross lesions. An examination for adult worms of *A. cantonensis* was made by carefully dissecting the lungs to avoid injury to the worms.

As to the method of experimental infection of *A. canaliculatus* with *A. cantonensis*, living snails were collected from an irrigation canal in Saga Prefecture where it was known that no naturally infected snails with the parasite were present. A fresh rat fecal pellet containing first-stage larvae of *A. cantonensis* was fed to each snail following a fast. The infected snails were bred in water at 25°C. All snails experimentally infected with the parasite were examined for second-stage and third stage larvae of *A. cantonensis* from 35 to 91 days after the infection, by the above-mentioned artificial digestion procedure. As a control group, 20 snails collected at the same place were bred in the same condition and examined for larvae of *A. cantonensis*. Actively moving third-stage larvae of *A. cantonensis* recovered from experimentally infected snails were also fed to albino rats; later at autopsy, they were examined for adult worms together with lung pathology.

RESULTS

Natural infection: The results of examination for third-stage larvae of *A. cantonensis* in *A. canaliculatus* are summarized in Table 1. Natural infection with *A. cantonensis* was confirmed in snails collected from 5 different places in 4 hamlets in Okinawa and those from Ishigaki Island (Fig. 2). The highest number of larvae found in a given group of snails was 288, and the lowest was only 3. No larvae of *A. cantonensis* were found in snails collected from other places in the Southwest Islands (Nansei Islands) and from the main island of Kyushu.

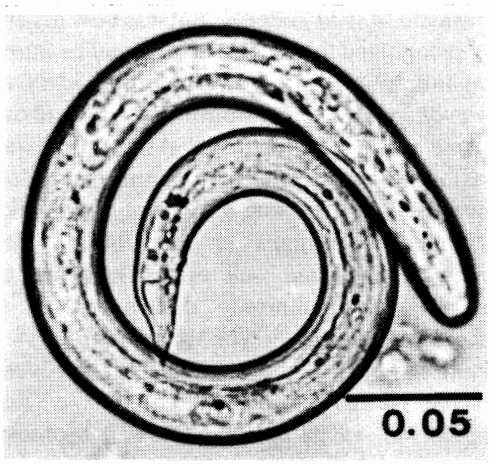

Fig. 2—Third-stage larva recovered from *A.canaliculatus* collected in Okinawa. (Scale in mm)

Fifty larvae recovered from the snails collected from a hamlet in Okinawa were fed to each of 2 rats. At autopsy 43 days after the infection, the rats showed 35 and 10 living adult worms of *A. cantonensis* in the pulmonary artery, respectively. In addition, 5 larvae recovered from the snails collected from another hamlet in Okinawa were fed to one rat, and 15 larvae recovered on Ishigaki Island were fed to another. The rats showed 3 and 6 adult worms of *A. cantonensis*, respectively. All 4 rats infected with *A.*

Table 1

Natural infection with third-stage larvae of *Angiostrongylus cantonensis* in *Ampullarius canaliculatus* collected in the Southwest Islands (Nansei Islands) and the main island of Kyushu, Japan.

Locality	Collect. Date	No. Snails	Size ($\bar{X}$ ± S.D.)	No. larvae
Okinawa Is.	Dec. 11, 85	30	35.6 ± 6.9	104
Okinawa Is.	Dec. 11, 85	30	31.4 ± 2.4	3
Okinawa Is.	Dec. 11, 85	30	33.8 ± 1.8	5
Okinawa Is.	Dec. 19, 85	30	31.8 ± 3.4	0
Okinawa Is.	Feb. 23, 86	30	26.7 ± 3.6	5
Okinawa Is.	Mar. 8, 86	25	35.5 ± 6.0	288
Ishigaki Is.	Dec. 16, 85	20	30.2 ± 2.3	15
M. Daitou Is.	Jan. 12, 86	35	40.9 ± 5.5	0
Tokashiki Is.	Feb. 17, 86	35	25.4 ± 4.9	0
Kume Is.	Feb. 14, 86	18	34.2 ± 4.4	0
Miyako Is.	Feb. 17, 86	19	31.7 ± 4.5	0
Yonaguni Is.	Feb. ? 86	13	45.9 ± 5.5	0
Iriomote Is.	Mar. 13, 86	13	30.5 ± 4.2	0
Iheya Is.	Mar. ? 86	29	26.3 ± 12.7	0
Tokuno Is.	Feb. ? 86	30	39.2 ± 3.3	0
Amami Is.	Feb. 20, 86	30	37.3 ± 2.7	0
Saga, Pref.	Nov. 13, 85	20	–	0
Saga, Pref.	Nov. 20, 85	20	–	0
Saga, Pref.	Dec. 6, 85	50	–	0
Fukuoka Pref.	May 1, 86	30	38.7 ± 2.7	0
Nagasaki Pref.	May 8, 86	23	23.1 ± 1.8	0
Miyazaki Pref.	May 27, 86	30	29.4 ± 3.0	0
Kagoshima Pref.	May 30, 86	30	40.8 ± 3.7	0
Ooita Pref.	May 29, 86	30	25.6 ± 8.8	0

cantonensis showed the typical features of lung pathology in murine angiostrongylosis as described by Nishimura (1966).

Each of 40 snails collected from a hamlet in Okinawa where the infection had been confirmed was examined for larvae of *A. cantonensis*. Among them, 4 were so infected. Thus, prevalence of *A. cantonensis* in *A. canaliculatus* in the area was 12.5%. The highest number of the larvae found in an individual snail was 1827, while the lowest was only 1. The average number of larvae in these 4 snails was 578.

Experimental infection: Recovery of larvae of *A. cantonensis* from experimentally infected *A. canaliculatus* is shown in Table 2. All snails infected with first-stage larvae of *A. cantonensis* showed second-stage and third-stage larvae at the time of examination. The highest number of third-stage larvae confirmed in a snail was 1233, and the lowest was only 1. No larvae of *A. cantonensis* were found in the control group. Actively moving third-stage larvae, recovered from 4 different snails, 31, 20, 15 and 10 in number, were fed to 4 albino rats. At autopsy 58 days

Table 2

Recovery of larvae of *A. cantonensis* from experimentally infected *A. canaliculatus*.

No.	Size (mm)	Period (days)	2nd-stage	3rd-stage
1	29.0	35	8	6
2	30.5	37	6	7
3	23.2	37	36	8
4	30.0	53	*	209
5	30.0	53	*	23
6	32.5	53	*	1233
7	35.0	53	*	192
8	37.0	53	*	11
9	34.6	53	*	544
10	34.0	70	*	3
11	34.0	70	4	55
12	32.8	70	1	1
13	32.8	70	2	8
14	32.3	91	28	101

* Present, but not counted.

after the infection, the rats showed 25, 11,9 and 2 adult worms of *A. cantonensis*, respectively, together with the typical features of lung pathology.

DISCUSSION

With regard to natural infection with *A. cantonensis* in *A. canaliculatus*, Chen simply mentioned *A. canaliculatus* as one of the natural intermediate host of *A. cantonensis* in Taiwan (Chen, 1985). However, the details of the one-year field survey were later reported by Chen when the authors' presentation on the present study was made at the Sino-Jopanese Symposium on Parasitic Zoonoses, Osaka. In Japan, natural infection with *A. cantonensis* in the snail was first reported by Nishimura and Sato (1986).

Our present study has shown that the locality of incidence of *A. canaliculatus* harboring *A. cantonensis* is limited only to the Ryukyu Islands. In the Southwest Islands, composed of the Ryukyu and Amami Islands, 8 molluscan species have been proven as natural intermediate hosts for *A. cantonensis* (Sato *et al.*, 1981). The highest prevalences of infection with the parasite were found in *Achatina fulica* and *Lavicaulis alte*, 34.1% and 22.5%, respectively (Sato *et al.*, 1981). The infection rates of *A. cantonensis* in *A. canaliculatus* were lower than that in *A. fulica*. This was also confirmed in Taiwan (Chen *et al.*, 1986). Neither *A. fulica* nor *L. late* is distributed in the main island of Kyushu. Although infection with *A. cantonensis* was confirmed in *Fruticicola despecta sieboldiana*, a terrestrial snail, collected in Kagoshima Prefecture (Ishida *et al.*, 1977), the parasite is uncommon in the main island of Kyushu. This may be related to failure in

finding the parasite in *A. canaliculatus* collected in various parts of the main island of Kyushu.

As to experimental infection with *A. cantonensis* in *A. canaliculatus*, Yen *et al.* first reported the results in 1985. They found that 70.4% of first-stage larvae had developed to the third-stage in the snails, examined 18 days after initial infection. Subsequently, an experimental infection with *A. cantonensis* to individuals of *A. canaliculatus* collected in Kagoshima Prefecture, Japan was successfully done by Uchikawa *et al.*, (1986). Out of 25 snails infected with first-stage larvae of *A. cantonensis*, 22 (88%) showed the third-stage larvae. Moreover, they fed the larvae to laboratory rats, and confirmed adult worms of the parasite in the lungs of the rats 27 or 28 days after infection. Sano and Dharejo (1986) reported that first-stage larvae of *A. cantonensis* did not develop to the third stage in the snail within 50 days after experimental infection. This is considered to be due to the effects of low temperature of water. Ishii (1984) studied the effects of temperature on the larval development of *A. cantonensis* in *Biomphalaria glabrata* under controlled conditions. He has proved that first-stage larvae develop to the third-stage under the condition between 20° and 31°C. Poor development of *A. cantonensis* larvae in molluscs under low temperature was also confirmed by Hori *et al.*, (1985). To the author's best knowledge, however, no reports regarding the effects of temperature on the larval development of *A. cantonensis* in *A. canaliculatus* have been published to date.

In the Ryukyu Islands, the infection rates of *A. cantonensis* in *A. canaliculatus* were not so high as that in *A. fulica*. However, our present study together with what was made clear by the previous investigators, indicates that *A. canaliculatus* is a suitable intermediate host for *A. cantonensis*. Thus, *A. canaliculatus* is not only a pest in agriculture but also a medically important snail. The snail will be a potential source of human infection with eosinophilic meningoencephalitis in the endemic areas. For this reason, close attention should be paid in eating *A. canaliculatus* raw or in handling the snail.

SUMMARY

Ampullarius canaliculatus is a large freshwater snail of which the original habitat is said to be Argentina. Recently, the snail which grew wild has propagated in various parts of Japan, and is causing damage to aquatic plants by feeding on them. The present study was performed to find out whether the snail can be a suitable intermediate host for *Angiostrongylus cantonensis*.

Natural infection with *A. cantonensis* was confirmed in the snails collected from 5 different places in 4 hamlets in Okinawa and in those from Ishigaki Island. All snails experimentally infected with first-stage larvae of the parasite showed second-stage and third-stage larvae developing at the time of examination later on. Thus, *A. canaliculatus* is a suitable intermediate host for *A. cantonensis*, and, if eaten raw, it can be a potential source of human infection with eosinophilic meningoencephalitis in the endemic areas.

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