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SOME COMMON SPECIES OF PULMONATE SNAILS AS THE EXPERIMENTAL INTERMEDIATE HOST OF *ANGIOSTRONGYLUS CANTONENSIS* IN JAPAN

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The occurence of *Angiostrongylus cantonensis*, generally considered as a causative agent of human eosinophilic meningoencephalitis in Pacific islands, was reported in Ryukyu Islands by Nishimura *et al.* (1964) and Nishimura (1966). As pointed out by many workers (Alicata, 1962 ; Ash, 1962 ; Lim *et al.*, 1962 ; Richards, 1963 ; Lim *et al.*, 1965), a variety of molluscs was reported to serve, experimentally and naturally, as the intermediate host of the parasite. In view of data from these reports investigation on the common species of snails in Japan as a potential vehicle for its transmission is urgently needed.

The present paper deals with experimental infection of some common species of snails with *A. cantonensis.*

Materials and methods

Species of pulmonate snails used and their localities are as follows : *Zonitoides arboreus*, Tokyo ; *Allopeas kyotoensis*, Tokyo ; *Fruticicola despecta sieboldiana*, Tokyo ; *Euhadra peliomphala*, Tokyo ; *Euhadra quaesita*, Saitama prefecture ; *Physa acuta*, Saitama prefecture. Small-sized young *E. quaesita* shown in Table 4 and 5, were all laboratory-reared from eggs laid by the field-collected adults.

A. cantonensis used was given by Dr. Alicata in Honolulu and has been maintained in the author's laboratory using freshwater snails, *Australorbis glabratus* and albino rats, *Rattus norvegicus* according to the method of Richards (1966).

Motile first-stage larvae of the parasite were isolated from feces of experimentally infected rats by means of Baerman apparatus. Lettuce leaves on which concentrated larval suspension was placed, were fed to the land snails that were previously starved for two days. After 2-hour exposure at room temperature, they were washed with water and kept at 25-27°C in earthenware dishes with soil. During this 2 hour's period snails, when crept out, were replaced on leaves. The dishes were further placed in a shallow glass cylinder containing water to moist the dish. They were fed daily with lettuce occasionally on Standen's Ca-arginate (1951) for hervivorous gastropodes. In the case of fresh water snails, *P. acuta* starved previously for two days, was directly exposed to feces containing motile first-stage larvae in water. At the end of 1-day exposure they were washed with water and reared at 25-27°C with lettuce.

Examination of exposed snails for the larvae was made by direct microscopic observation on press preparation of snail soft tissue and/or by peptic digestion (1.0 % HCl and 1.0% pepsin). Infection of rats with third-stage larvae thus isolated was performed by means of a stomach tube after counting. The rats were examined on their heart, pulmonary artery, and lungs when died or killed for another purpose.

Table 1 Experimental infection of *Z. arboreus* and *A. kyotoensis* with the first-stage larvae of *A. cantonensis* and the infectivity of the third-stage larvae isolated to rats

Snail No.	Size at infection	Days after infection	Larvae recovered by: Pressure preparation 2nd.	Larvae recovered by: Pressure preparation 3rd.	Larvae recovered by: Peptic digestion 3rd.	Experimental infection of rats: No. rats used	No. larvae given	Days after infection	No. worms recovered ♀ : ♂
Zonitoides (Zonitellus) arboreus									
1	5.3mm	18	+	+	+	1	28	181	12 : 3
2	5.6	22		+	+}	3	53	171	11 : 13
3	5.6	22		+	+}		55	171	19 : 18
4	5.9	30		+	+}		59	47	12 : 11
5	6.0	30		+	+}	1	100	29	Many worms in brain
6	6.1	30		+	+}				
7	5.1	44		+	+}	8	53	46	20 : 15
8	5.6	44		+	+}		39	162	8 : 9
9	5.7	44		+	+}		58	162	11 : 10
							51	46	20 : 20
							58	87	unexamined
							57	67	13 : 21
							46	162	14 : 11
							59	29	14 : 9(8 : 2 in brain)
Uninfected									
10	6.0	44	−	−	−				
11	5.5	44	−	−	−				
Allopeas kyotoensis									
1	7	18	+	+	+}	3	51	181	4 : 11
2	7	18	+	+	+}		67	181	14 : 19
							82	42	15 : 9
Uninfected									
3	6	18	−	−	−				
4	8	18	−	−	−				

Brain of the rats dying within 50 days after infection was also examined.

Results

As shown in Table 1 and 2, all of four species of snails, *Z. arboreus*, *A. kyotoensis*, *F. despecta sieboldiana*, and *E. peliomphala* were found to harbor the third-stage larvae except one of *E. peliomphala* that died as early as 3 days after exposure but showed a number of the first-stage larvae in its tissue. The third-stage larvae isolated from these snails proved to be infective to rats, in which they developed to mature but rarely to immature adults when host animals died early.

In the experimental infection of adult *E. quaesita* (Table 3) 5 of 7 snails were positive for the third-stage larvae 23–137 days after exposure. The isolated larvae were used successively to infect rats. Two negative snails (No. 2 and 5) are somewhat larger in size than other positive snails. In another experiment (Table 4) young *E. quaesita* measuring 8.7 mm in average shell diameter, and raised from eggs laid by the adults in the laboratory, were exposed to the first-stage larvae in the same manner as previously described. All of the 5 snails exposed were found to be infected with the third-stage larvae by examination at 29–31 days after infection. Isolated larvae from them proved to be infective to the definitive hosts. Table 5 indicates comparison of susceptibility in young *E. quaesita* with that of other small-sized but adult species of snails under the same condition. It is likely that the susceptibility of young *E. quaesita* is the same as that of *Bradybaena similaris* serving naturally and experimentally as the intermediate host of the

Table 2 Experimental infection of *F. despecta sieboldiana* and *E. peliomphala* with the first-stage larvae of *A. cantonensis* and the infectivity of the third-stage larvae isolated to rats

Snail No.	Size at infec-tion	Days after infec-tion	Larvae recovered by: Pressure preparation 2nd.	Larvae recovered by: Pressure preparation 3rd.	Larvae recovered by: Peptic digestion 3rd.	Experimental infection of rats: No. rats used	No. larvae given	Days after infection	No. worms recovered ♀ : ♂
Fruticicola despecta sieboldiana									
1	adult	74	–	+	+	6	62	230	17 : 15
							46	55	14 : 13
							45	282	15 : 10
							64	33	12 : 12(7 in
							43	51	25 : 16brain)
							50	282	15 : 14
2	adult	80	–	+	+}	2	75	94	30 : 28
							68	45	21 : 25
3	adult	80	–	+	+}				
Uninfected									
4	adult	80	–	–	–				
Euhadra peliomphala									
1	29mm	23	+	+	+	1	25—40	252	2 : 1
2*	30	3	–	–	–				
3*	32	52		+	+	2	59	89	18 : 21
							49	31	20 : 20
Uninfected									
4	24	23	–	–	–				
5	28	52	–	–	–				

* Those with asterik were maintained at room temperature (Jun. 16, to Aug. 7 in 1965)

Table 3 Experimental infection of *E. quaesita* with the first-stage larvae of *A. cantonensis* and the infectivity of the third-stage larvae isolated to rats

Snail No.	Size at infec-tion	Days after infec-tion	Larvae recovered by: Pressure preparation 2nd.	Larvae recovered by: Pressure preparation 3rd.	Larvae recovered by: Peptic digestion 3rd.	Experimental infection of rats: No. rats used	No. larvae given	Days after infection	No. worms recovered ♀ : ♂
Euhadra quaesita									
1	12mm	23	+	+	+	2	75	29	8 : 9(in brain)
							75	42	21 : 29
2	47	28	+	+	–				
3	36	55		+	+	2	10-15	371	3 : 3
							47	51	13 : 11
4	18	61			+	1	80	212	8 : 2
5	35	137		–	–				
6*	39	23	+	+	+	2	50	40	25 : 22
							50	43	16 : 17
7*	26	55		+	+	5	58	139	9 : 10
							61	117	16 : 10
							57	133	12 : 13
							69	45	12 : 14
							70	133	6 : 9
8*	39	84	–	–	–				
Uninfected									
9	45	84	–	–	–				
10	42	55	–	–	–				

* Those with an asterik were maintained at room temperature (Snail No. 6, Nov. 24, 1965 to Dec. 16, 1965; No. 7, Nov. 27, 1965 to Jan. 21, 1966; No. 8, Nov. 24, 1965 to Feb. 16, 1966)

Table 4 Experimental infection of laboratory-reared young *E. quaesita* with the first-stage larvae of *A. cantonensis* and the infectivity of the third-stage larvae to rats

Snail No.	Size at infection	Days after infection	Larvae recovered by: Pressure preparation 2nd.*	Pressure preparation 3rd.*	Peptic digestion 3rd.	Experimental infection of rats: No. rats used	No. larvae given	Days after infection	No. worms recovered ♀ : ♂
1	8.5mm	29	40	254 }		6	33	83	1 : 7
2	9.0	29	23	248 }			31	178	10 : 3
							31	〃	4 : 5
							50	〃	3 : 4
							25	〃	2 : 3
							33	〃	4 : 3
3	7.1	31	10	80 }		6	35	186	3 : 3
4	8.7	31	28	207 }			28	〃	6 : 2
5	10.0	31	20	138 }			32	〃	6 : 2
							33	〃	2 : 3
							26	〃	4 : 4
							32	〃	6 : 2

* The number of the larvae isolated from the whole body without hepatopancreas. In snail No. 1, 2, 3 and 4 3rd-stage and 2 and 3 2nd-stage larvae were found in hepatopancreas respectively. No larva was found in uninfected snails measuring 9.0-10.0 mm in length.

Table 5 Comparative susceptibility of small-sized land snails to the first-stage larvae of *A. cantonensis*

Species of snails	No. snails exposed	Snail size (mm): Average	Max-Min.	Days after infection	Total No. of worms recovered: 3rd-stage	2nd-stage (%)
E. quaesita	5	8.7	10.0-8.5	29-31	941	98(9.4)
A. kyotoensis	4	7.5	8.8-6.1	31	750	51(6.4)
Z. arboreus	4	4.9	5.4-4.7	31	186	0
B. similaris	1	12.4		31	288	34(10.6)
〃	1	12.4		40	348	2
〃	1	12.9		45	199	1

Two unexposed snails of each species were examined as control. No larva of *A. cantonensis* was recovered.

parasite (Ash, 1962 ; Alicata *et al.*, 1962 a, b).

Experimental infection of fresh water snails, *P. acuta* failed to obtain the third-stage larvae (Table 6) with an exceptional snail (No. 8 in the first series) from which three third-stage larvae were found 25 days after exposure. The isolated larvae showed an ability to establish their parasitic phase in an albino rat. As seen in the table it is generally noted that the first-stage larvae can develop to the second stage but soon or later die without reaching the infective third stage in the snails.

Discussion

The presence of the third-stage larvae of *A. cantonensis* was proved experimentally in the following species: *Z. arboreus*, *A. kyotoensis*, *F. despecta sieboldiana*, *E. peliomphala*, *E. quaesita*, and *P. acuta*. Susceptibility of these snails to the parasite, is considered to vary with species, provided that first-stage larvae used in each experiment are uniform in infectivity. On the basis of number of infected snails and the third-stage larvae recovered the first four species are more susceptible than the last two. *P. acuta* is far less susceptible than any other species tested. Difference in the susceptibility to the parasite between young and adult snails of *E. quaesita* may suggest occurrence of age-resistance to the parasite as recently reported by Sogandares-

Table 6 Experimental infection of *Physa acuta* with the first-stage larvae of *A. cantonensis* and the infectivity of the third-stage larvae isolated to rats

Snail No.	Snail size (mm)	Days after infection	Larvae recovered by press preparation 1st.	2nd.	3rd.	Experimental infection of rats: No. rats used	No. larvae given	Days after infection	Worms recovered ♀ : ♂
1		0	+	–	–				
2		5	–	–	–				
3		5	+	–	–				
4		6	+300*	–	–				
5	8	12		+	–				
6	9	21	+	+	–				
7	9	25	+*	+	–				
8	8	25		+*	+3	1	3	103	3 : 0
9	9	42	+*	+*	–				
Uninfected									
10	9	42	–	–	–				
11	10	42	–	–	–				
12	10	42	–	–	–				
1	7	28	+	–	–				
2	7	28	–	–	–				
3	10	28	+100*	–	–				
4	9	28	+20*	–	–				
5	7	28	+*	–	–				
6	8	34	+*	–	–				
Uninfected									
7	7	34	–	–	–				
8	9	34	–	–	–				
9	9	34	–	–	–				

Figures after sign + indicate the numbers of larvae recovered. Asterik represents the degeneration of the larvae.

Bernal (1965) regarding the susceptibility of *Pomatiopsis lapidaria* to American lung-fluke, *Paragonimus kellicotti*.

Physa sp. was reported by Richards to be less susceptible to the parasite than larger planorbid and lymneid snails tested. In the present work only one of 15 snails exposed was found to harbor only three third-stage larvae. It deserves further study on the susceptibility at younger stage.

Upon discussing the possibility that a new parasite can exist when unfortunately introduced into a non-contaminated area, information about the animals serving as intermediate and definitive hosts for the parasite is needed. *Rattus norvegicus* and *R. rattus* are common in Japan and the presence of snails that are able to serve as its intermediate host was clearly indicated by the present work. Duration of the infection in rats may be of the order of a year in author's experience while the larvae, according to the report of Richards (1963), survive 12 months in snail tissue. Although no report on the effect of low temperatures upon the larval development has been available, Mackarras & Sanders (1955) reported that 70–79° F was favorable for it. This range is usual in spring and autumn of Japan. Low temperatures in winter may have an inhibitory effect on the development but little effect on the infective larvae at quiscent stage as long as the host snail is alive. In view of these facts above mentioned the parasite can establish its whole life-cycle at least in spring to autumn in Japan. Irrespective of larval ability to pass the cold winter the adult worms can survive in rats to produce newly infected snails in coming spring.

Summary

Experimental infection of some com-

mon species of snails, *Zonitoides arboreus*, *Allopeas kyotoensis*, *Fruticicola despecta sieboldiana*, *Euhadra peliomphala*, *Euhadra quaesita*, and *Physa acuta* with the first-stage larvae of *Angiostrongylus cantonensis* was conducted. The presence of the third-stage larvae was observed in all of the 6 species exposed. These third-stage larvae isolated turned out to be all infective to the definitive host, *Rattus norvegicus*. The susceptibility of the snails varies with species. The first four species of snails are more susceptible than the last two. *P. acuta* is far less susceptible than *E. quaesita*. It is likely that difference of susceptibility is present between adult and young snails of *E. quaesita*.

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広東住血線虫 *Angiostrongylus cantonensis* の実験的中間宿主について

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本邦における陸棲貝の普通種，コハクガイ，オカチョウジガイ，ウスカワマイマイ，ミスジマイマイ，ヒダリマキマイマイ，および淡水棲のサカマキガイをつかつて，本線虫第1期幼虫の実験感染を試みた．

感染実験を行つた上記6種の総ての種類の貝において第3期幼虫が認められ，又これら感染貝より分離された第3期幼虫は終宿主に対して感染性を示した．

本線虫感染に対する貝の感受性は貝の種類によつて異る．前4種の貝は後2種の貝に比較して高い感受性を示し，後2者の内でもヒダリマキマイマイはサカマキガイよりも高い感受性を示した．

ヒダリマキマイマイの成熟貝と幼若貝ではその感受性に差があり，幼若貝で高く，成貝で低い．

以上の結果は上記本邦産普通種の貝が本線虫の中間宿主となり得る可能性を示すと共に，本邦に於ける本虫の将来の侵入の可能性をも示唆している．