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Investigations on the Rat Lungworm, *Angiostrongylus cantonensis*, in the Ryukyu Islands

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Introduction

Being primarily a parasite of wild rats, the rat lungworm, *Angiostrongylus cantonensis* has been known to invade the human central nervous system and produce menigoencephalitis as reported by Nomura and Lin (1945), Rosen *et al.* (1962), and Jindrák and Alicata (1965). Moreover, *A. cantonensis* is considered to be the most probable etiologic agent of eosinophilic meningoencephalitis in the Pacific and Southeast Asia. For this reason, surveys on *A. cantonensis* in wild rats present an intriguing field for research.

Epidemiologically, information on the incidence of *A. cantonensis* in the Ryukyu Islands is important because this parasite has already been reported to be present in Iriomote-jima (Nishimura *et al.*, 1964).

In August 1965, at the suggestion of Dr. J. E. Alicata, the author visited some of the Ryukyu Islands to know the distribution and prevalence of *A. cantonensis* in wild rats and to find the species of mollusks acting as local intermediate hosts. The study covered the islands of Okinawa, Miyako-jima, Ishigaki-jima, and Iriomote-jima. Partial results of this study from the island of Okinawa has been reported by Alicata and Nishimura (1965).

In this paper the final results of the investigation done in all of the above mentioned islands will be presented.

Materials and Methods

The rats were mostly captured with "Victor" snap-traps. The traps, baited with sweet potato, were set late in the afternoon and collected early next morning. Although most of the traps were set in sugar cane fields, some were placed around houses or storehouses. In addition, some of the rats were collected after being killed by rat poison.

The rats were identified as to species and then the body length of each rat was measured from the tip of the nose to the base of the tail. The lungs and the heart were removed from the thoracic cavity and were observed for gross pathological lesions. An examination for adult worms was made by carefully dissecting the lungs to avoid injury to the worms. The auricles and ventricles of the heart were opened and examined. The parasites were identified by their gross morphological structures, and the total number of worms found in each rat was counted.

Mollusks were captured alive and brought to the Ryukyu Hygienic Laboratory. They were passed through a meat grinder, and the minced tissue was digested artificially in a solution containing 1 % pepsin and 1 % HCl at 37°C. for $1\,^1/_2$ hours. The sediments were examined for larvae with a microscope. The larvae of the rat lungworm were identified by their characteristic morphological features. Actively moving larvae were orally fed to laboratory mice. Several days later, the entire brain of each mouse was examined by press preparation under the microscope to ascertain the possible presence of developing larvae.

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Results

A. cantonensis *in Rats:*

The rats were trapped from Okinawa, Miyako-jima, Ishigaki-jima, and Iriomote-jima.. The following species and numbers of rodents were found in these islands: Norway rats, *Rattus norvegicus*, 120; roof rats, *Rattus rattus*, 10. As shown in Table 1, *A. cantonensis* was found in *R. norvegicus* from every island. The 120 Norway rats examined from the four islands were harboring *A. cantonensis* in 15.8 % on the average. Eight out of 55 Norway rats from Okinawa showed the lungworm (14.5 %). As for *R. rattus*, of only 10 rats secured from the island of Okinawa, 3 showed *A. cantonensis*. Table 2 shows the prevalence of *A. cantonensis* by sex in *R. norvegicus*. No gross difference in prevalence was noted between male and female rats. Table 3 shows the prevalence of *A. cantonensis* by size and species of the rats examined. The prevalence of *A. cantonensis* in the larger rats was found to be higher in *R. norvegicus*. A marked difference was noted between two groups, namely below 179 mm. and over 180 mm. in body length ($\chi^2=15.21$, $p<0.01$). The total number of *R. rattus* was too small to note any difference between the two species of rats. The lowest number of the parasite found in a rat was a single worm in *R. norvegicus* and the highest was 37 worms in *R. rattus*. There were slightly more female than male worms in both rats. In gross observation, the lungs of rats harboring *A. can-*

Table 1. Prevalence of *A. cantonensis* among *R. norvegicus* examined in the Ryukyu Islands

Island	Number examined	Number positive	Per cent positive
Okinawa			
Ginama	2	0	
Toguchi	1	0	
Nago	15	3	
Sedaka	4	0	
Ginosa	8	0	
Ishikawa	1	0	
Yomitan	2	0	
Koza	10	1	
Ishimine	9	3	
Naha	2	1	
Kokuba	1	0	
Totals	55	8	14.5
Miyako-jima			
Hirara (1)	9	4	
Hirara (2)	5	1	
Totals	14	5	35.7
Ishigaki-jima			
Ishigaki (1)	17	0	
Ishigaki (2)	11	4	
Totals	28	4	14.3
Iriomote-jima			
Ohara	12	0	
Otomi	11	2	
Totals	23	2	8.7
Grand Totals	120	19	15.8

Table 2. Prevalence of *A. cantonensis* by sex of *R. norvegicus* examined in the Ryukyu Islands

Island	Male			Female		
	Number examined	Number positive	Per cent Positive	Number examined	Number positive	Per cent positive
Okinawa	29	5		26	3	
Miyako-jima	8	3		6	2	
Ishigaki-jima	8	0		20	4	
Iriomote-jima	10	1		13	1	
Totals	55	9	16.4	65	10	15.4

Table 3. Prevalence of *A. cantonensis* by size of rats examined

Species & body size in mm.	Number examined	Number positive	Per cent positive
R. norvegicus			
< 160	34	0	0
160–179	37	3	8
180–199	29	6	21
200–219	16	6	37
220+	4	4	100
R. rattus			
< 160	3	1	33
140–159	5	1	20
160–179	2	1	50

tonensis were usually remarkably enlarged and the characteristic circumscribed consolidations were found in the affected lobes.

Infective Larvae of A. cantonensis *among Mollusks :*

A species of wild slug identified as *Laevicaulis alte* (Férussac) was commonly found in sugar cane fields where the infected rats had been trapped on the islands of Miyako-jima, Ishigaki-jima, and Iriomote-jima. However, this slug was not found in Okinawa. The giant African snail, *Achatina fulica*, was very common in Okinawa and Miyako-jima, but none was seen in Ishigaki-jima. By artificial digestion method, a number of metastrongylid larvae were found in each of 10 slugs collected from each of the above mentioned islands. However, the larvae recovered from the giant African snails from Okinawa were notably less in number. These larvae agreed morphologically with the infective larvae of *A. cantonensis*. All the laboratory mice which had been fed the larvae, recovered from the slugs and snails, showed many living larvae in the brain 4 days later.

Discussion

A. cantonensis *in Rats :*

Recently, the geographical distribution of *A. cantonensis* has been reviewed by several investigators (Alicata and McCarthy, 1964 ; Kuntz and Myers, 1964 ; Alicata, 1965 ; Wallace and Rosen, 1965). At present, the areas in which *A. cantonensis* has been found in rats are as

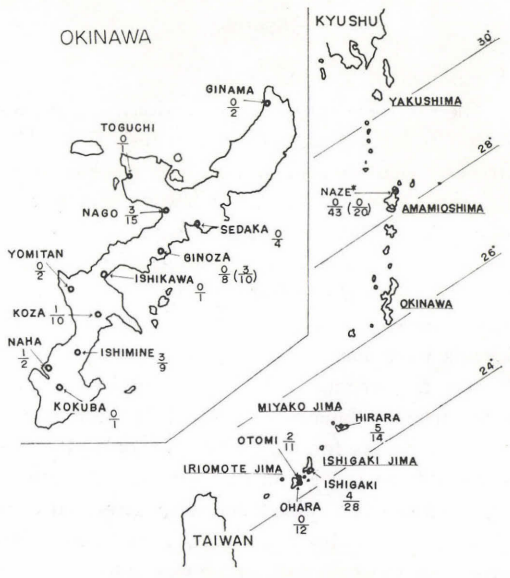

Fig. 1. Each denominator expresses total number of rats examined, and the numerator expresses positive number of the rats. Each fractional number in the parentheses shows *R. rattus* and others are *R. norvegicus*. [* Kawashima *et al.* (1965)]

follows : Australia (Mackerras and Sandars, 1955), New Caledonia (Alicata, 1963), New Hebrides (Alicata, 1963), Tahiti and Society Islands (Alicata, 1962 ; Wallace and Rosen, 1965), Oahu, Hawaiian Islands (Ash, 1962 ; Wallace and Rosen, 1965), Solomon Islands (cited by Wallace and Rosen, 1965), Palau Islands (cited by Wallace and Rosen, 1965), Mariana Islands (Alicata, 1965), Caroline Islands (Jackson, 1962), Loyalty Islands (Alicata, 1963), Cook Islands (Alicata and McCarthy, 1964), Malaya (Schacher and Chee-Hock, 1960 ; Lim *et al.*, 1965), Thailand (Punyagupta, 1965), Canton, China (Chen, 1935), Formosa (Matsumoto, 1937 ; Kuntz and Myers, 1964; Chiu, 1964), Manila, Philippines (Nishimura and Yogore, 1965 ; de Leon *et al.*, 1965) Madagascar (Brygoo and Chabaud, 1964 ; Alicata, 1965), Iriomote-jima and Okinawa, Ryukyu Islands (Nishimura *et al.*, 1964 ; Alicata and Nishimura, 1965), Mauritius (Alicata, 1965), Ceylon (Alicata, 1965) and Sarawak (Alicata, 1965).

Wallace and Rosen (1965) reported that *A. cantonensis* was not found in rats from Majuro,

Marshall Islands ; Viti Levu, Fiji ; Tongatapu, Tonga ; Savaii and Upolu, Western Samoa ; Tutuila, American Samoa ; or Molokai Hawaii.

Other areas where *A. cantonensis* has not yet been found in rats examined are as follows : Wallis Islands (cited by Wallace and Rosen, 1965), Auckland, New Zealand (Alicata and McCarthy, 1964), England (Sandars, 1957), New Orleans, Puerto Rico (cited by Wallace and Rosen, 1965), Brazil, Netherlands, Germany (cited by Alicata, 1965), Egypt, Yemen, Sudan, Turkey (Kuntz and Myers, 1964), Amami Islands (Nishimura *et al.*, 1964 ; Kawashima *et al.*, 1965), and Kyushu, Japan (unpublished). This would indicate that *A. cantonensis* is present primarily in tropical and subtropical areas, as in the Pacific and Southeast Asia including the islands in Indian Ocean.

The Ryukyu Islands consist of 140 islands and islets and form a chain of islands located between 24°N. and 27°N. latitude. The northern-most island is close to Amami Islands, and the southern-most island of the chain is 40 miles east of Formosa. Rainfall is heavy with a yearly average of 82 inches. The yearly temperature averages 72°F ranging from 89° in summer to 61° in winter. The relative humidity averages 76 per cent.

According to Uchida (1963), the following are species of rodents found in the Ryukyu Islands : *Rattus rattus rattus*, *Rattus norvegicus norvegicus*, *R. norvegicus norvegicus* form. *hibernicus*, *Mus molossinus yonakuni*, *Mus caroli caroli*, *Diplothrix legata*, *Tokudaia osimensis osimensis*, and *Tokudaia osimensis muenninki*. However, among these rodents only *R. norvegicus*, *R. rattus*, and *Mus caroli* were trapped. *R. norvegicus* was commonly found in the sugar cane fields. All *R. rattus* were captured in a single house in Okinawa. Numbers of *R. rattus* collected were too few to allow a valid comparison of prevalence by host species.

Incidence of *A. cantonensis* in rats from the Ryukyu Islands is not as high as in the Hawaiian Islands (Wallace and Rosen, 1965), Society Islands (Wallace and Rosen, 1965), New Caledonia (Alicata, 1963), and Cook Islands (Alicata, 1964). However, the results suggest that *A. cantonensis* is widely distributed in the Ryukyu Islands.

Prevalence of *A. cantonensis* by sex and size of the host was previously reported by Wallace and Rosen (1965) in their studies of rats materials from Hawaiian and Society Islands. The present results are in agreement with the above report.

It is of interest to note that in general there were slightly more female than male worms in both rats. This result corroborates the findings by Weinstein *et al.* (1963) and Nishimura (1966) that the female worms could be recovered slightly more frequently than the male from experimentally infected albino rats.

Most rats harboring *A. cantonensis* were trapped in the sugar cane fields, but an infected rat was captured in a house located in the center of Naha City as well. Kuntz and Myers reported that the infection was found in rats captured in gardens or lots within the city limits of Taipei, Formosa. This is in agreement with the observations of the author who found some rats from the center of Honolulu severely infected with *A. cantonensis* (unpublished).

The relationship between the gross lesion of the lungs and the location of presence of *A. cantonensis* in the pulmonary artery was studied by the author (1966) in experimentally infected albino rats. The most frequent lesion is in the diaphragmatic lobe of the right lung. Adult worms were commonly present in the trunk of the pulmonary artery and in its branches in the diaphragmatic lobe. This relationship was observed also in those naturally infected wild rats from the Ryukyu Islands.

Infective Larvae of A. cantonensis *in Mollusks :*

Since Mackerras and Sandars (1955) found that the slug, *Agriolimax laevis* acted as an intermediate host for *A. cantonensis*, various kinds of slugs and land snails have been found to serve as intermediate hosts. Recently, the molluscan intermediate hosts for *A. cantonensis* were reviewed by Alicata (1965). As found in his review, *Laevicaulis alte* was reported as a natural intermediated host for *A. cantonensis* in Hawaii and New Caledonia under the name of *Veronicella alte* or *Veronicella leydigi* (Alicata, 1962, 1963). According to Kondo,

however, *Laevicaulis alte* is the latest accepted name of the slug (personal communication). From among the Ryukyu Islands, *Laevicaulis alte* is found in large numbers on the islands of Iriomote-jima, Ishigaki-jima, and Miyako-jima, but is not seen on Okinawa. On the other hand, numerous giant African snails are found in Okinawa and Miyako-jima. This snail is said to be present on the island of Ishigaki-jima, but not in Iriomote-jima. At present, therefore, it is most likely that the wild slug, *Laevicaulis alte* serves as a natural intermediate host for *A. cantonensis* on the islands of Iriomote-jima, Ishigaki-jima, and Miyako-jima, while the giant African snail serves as the intermediate host in Okinawa and Miyako-jima. It may be possible, however, that other mollusks would be recognized as the natural intermediate hosts in these areas.

As Wallace and Rosen (1965) have pointed out, it is possible that the third-stage larvae of many known and undescribed metastrongylid nematodes exist in the mollusks. As a matter of fact, the infective larvae of cat lungworm, *Anafilaroides rostratus*, can be found easily in the body of the giant African snail and are quite similar to those of *A. cantonensis*. Alicata (1963), additionally reported that the infective larvae of *A. rostratus* can be differentiated from those of *A. cantonensis* on the basis of the longer body and esophagus, and in some cases, the position of the genital primordium. Furthermore, the infective larvae of *A. rostratus* do not migrate to the central nervous system of rats or mice as do those of *A. cantonensis* (Alicata, 1963). Unfortunately, there were no available albino rats in Okinawa at that time and the author was compelled to feed larvae recovered from the mollusks to laboratory mice. Although *A. cantonensis* cannot mature in mice, the infective larvae do congregate in the central nervous system of the rodent (Mackerras and Sandars, 1955). No other metastrongylid nematodes, which migrate to the central nervous system, have been found in *Laeviculis alte* or in the giant African snail. Therefore, it is probable that the infective larvae recovered from the mollusks had migrated to the central nervous system in the mice are those of *A. cantonensis*. The first successful recovery of the larvae was made by the author in the Ryukyu Islands.

As far as known by local physicians, no case of eosinophilic meningoencephlitis has been noted among the inhabitants of the Ryukyu Islands.

Summary

One hundred and thirty rats of 2 species, *Rattus norvegicus* and *R. rattus*, from islands of Okinawa, Miyako-jima, Ishigaki-jima, and Iriomote-jima in the Ryukyu Islands were examined for the rat lungworm, *Angiostrongylus cantonensis*. This parasite was found in rats from all of the islands. Miyoko-jima and Ishigaki-jima are new localities of *A. cantonensis*. Of 55 rats, *R. norvegicus*, from the various parts of Okinawa, 8 showed *A. cantonensis* (14.5 %). The rats from four islands were found harboring *A. cantonensis* in 15.8 % on the average.

A wild slug, *Laevicaulis alte*, was commonly found on the islands of Iriomote-jima, Ishigaki-jima, and Miyako-jima. The giant African snail, *Achatina fulica*, was very common in Okinawa and Miyako-jima. From these mollusks, a number of the third-stage larvae, morphologically identical to those of *A. cantonensis*, were recovered by artificial digestion methods. Many of these larvae were found in the central nervous system of the laboratory mice which were fed with the third stage larvae 4 days prior to autopsy. From the above experimentation, it can be concluded that the probable infective larvae was that of *A. cantonensis*. The slug, *Laevicaulis alte*, and the giant African snail, *Achatina fulica*, therefore, serve as the natural intermediate hosts for the parasite in the Ryukyus. Up to the present, no case of eosinophilic meningoencephalitis has been observed in the Ryukyu Islands.

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琉球列島における広東住血線虫 *Angiostrongylus cantonensis* に関する研究

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広東住血線虫は人の中枢神経に寄生して，髄膜脳炎を起こし，太平洋地域に広く存在する好酸球性髄膜脳炎 eosinophilic meningoencephalitis の病原体と考えられている重要な寄生虫である．

著者は 1965 年 8 月，琉球列島に赴き，沖縄本島，宮古島，石垣島および西表島のドブネズミおよびクマネズミを採集して本線虫の寄生状況を調べた．沖縄本島各地から採集したドブネズミ 55 匹の中，8 匹 (14.5%) に本線虫寄生を認め，宮古島では 14 匹中，5 匹(35.5%)，石垣島では 28匹中，4 匹(14.3%)，西表島では 23 匹中，2 匹 (8.7%) に本線虫の寄生を証明した．上記の 4 つの島全体としてはドブネズミ 120 匹中，19 匹 (15.8%) に寄生が認められた．

クマネズミは沖縄本島のみから 10 匹採集され，その中 3 匹に寄生が認められた．

中間宿主の調査ではハワイやニューカレドニアにおいて本線虫の中間宿主であるアシヒダナメクジ *Laevicaulis alte* が西表島，石垣島および宮古島に数多く発見された．また，同じくハワイで普通の中間宿主であるアフリカマイマイ *Achatina fulica* は沖縄本島および宮古島に極めて普通にみられた．

このアシヒダナメクジとアフリカマイマイから消化法により，広東住血線虫の幼虫と形態学的に一致する多数の幼虫を得た．さらに，これらの幼虫をハツカネズミに経口的に与え 4 日後の剖検で脳に多数の幼虫を認めた．この実験から広東住血線虫と断定してまづ，間違いないと思われる．

以上，広東住血線虫は琉球列島全体に広く分布すると推定され，アシヒダナメクジおよびアフリカマイマイが主な中間宿主になると考えられる．現在，琉球列島においては好酸球性髄膜脳炎の臨床例は未だ確認されていないが，注意する必要がある．