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An epidemiological survey on *Angiostrongylus cantonensis* and angiostrongyliasis in the Southwest Islands, Japan.

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Angiostrongylus cantonensis, primarily a parasite of rodents, has attracted notice as an important agent of eosinophilic meningitis or meningoencephalitis on many Pacific islands and in Southeast Asia.¹⁻⁷⁾ It is generally speculated in the Pacific and Asian tropical and subtropical zones that the parasite has extended its geographic range to adjacent regions by introduction of the infected intermediate hosts, mainly *Achatina fulica*, or by the stowaway rats.⁸⁾

The Southwest Islands are in a unique position in Japan consisting of many small islands, so-called the Ryukyu and Amami Islands, which are located in a subtropical zone drawing an arc from the northeast to the southwest along the east coast of the Asian Continent and are separated only by a narrow strait northwestward from Taiwan (Fig. 1). *A. fulica*, a most important intermediate host, is believed to be introduced from Taiwan and/or the South Pacific Islands in relatively recent years, and now the snails are distributed widely on the Islands. The adult parasites were first recovered from a wild rat in 1964⁹⁾ and the larval stages were from *A. fulica*, in next 1965.¹⁰⁾ Successively, human cases suspected of angiostrongyliasis were reported in 1970¹¹⁾ and until now, 16 cases have been known from the Islands.^{12),13)}

Several surveys on the distribution and incidence of the parasite have been done by many workers¹⁴⁻¹⁸⁾ but the data available on the incidence and distributional pattern of the parasite are still insufficient. The authors have conducted an epidemiological survey on animal infections and human angiostrongyliasis since 1972 and this paper deals with these results obtained on 13 islands of the Ryukyu Islands (Okinawa Prefecture) and 2 of the neighboring Amami Islands (Kagoshima Prefecture).

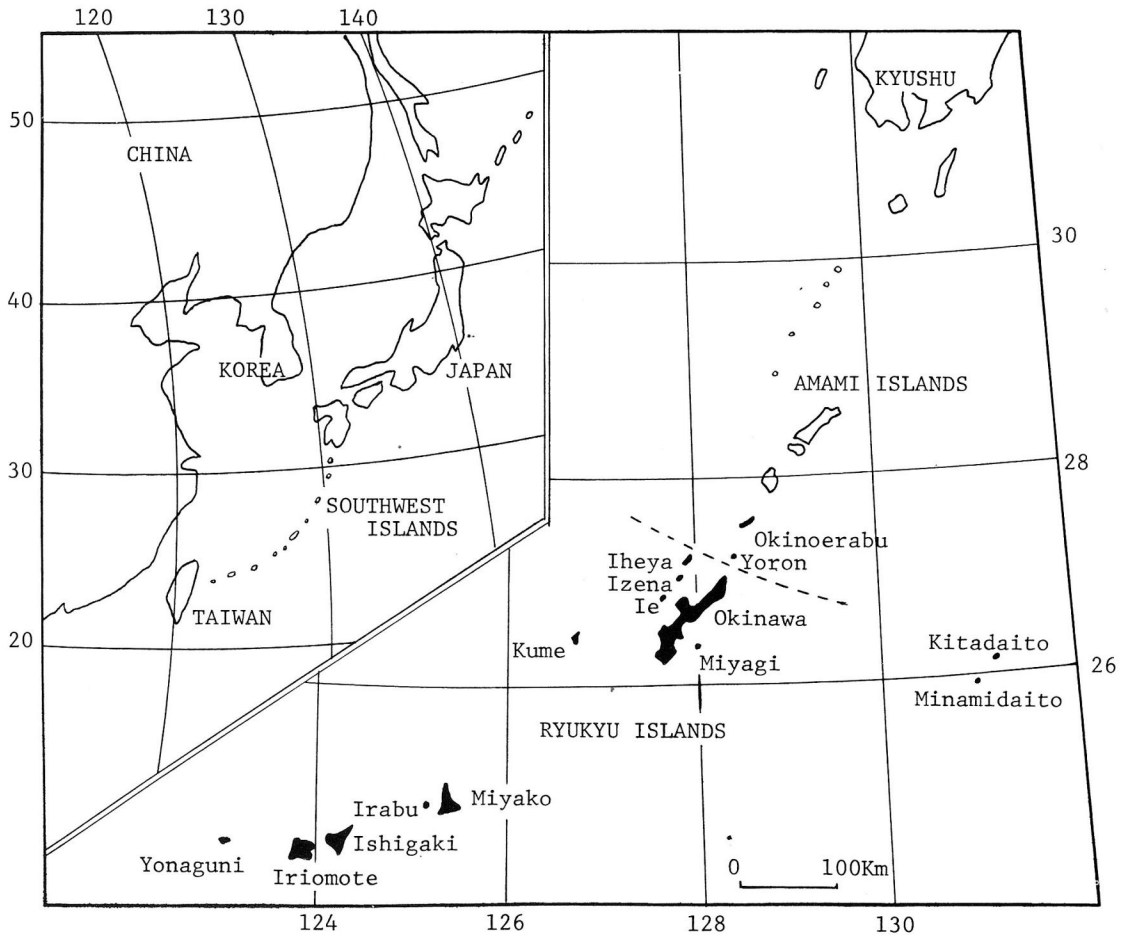


Fig. 1 Map of the Southwest Islands (the Ryukyu and Amami Islands) of Japan. The islands surveyed are expressed as black shading.

Materials and Methods

1) Survey of the definitive hosts:

A total of 799 small mammals consisting of 5 species (343 *Rattus norvegicus*, 314 *Rattus rattus*, 117 *Suncus murinus riukiuanus*, 16 *Herpestes edwardsii* and 9 *Mus caroli*) were collected and the pulmonary arteries and brains of these mammals were examined as to the infection with *A. cantonensis*.

2) Survey of the intermediate and paratenic hosts:

Six species of terrestrial snails (3,684 *A. fulica*, 621 *Bradybaena circulus*, 519 *Fruticicola despecta*, 260 *Satsuma mercatoria*, 163 *Cyclophorus turgidus* and 100 *Assiminea japonica*) and 5 species of slugs (360 *Laevicaulis alte*, 58 *Incilaria bilineata*, 50 *Limax flavus*, 22 *Vaginulus plebeius* and 16 *Incilaria fruhstorferi*) were collected. In the case of

A. fulica, the mantle pieces were sandwiched between glass slides and examined for the 3rd-stage larvae in the tissues under a microscope. The other small snails and slugs were chopped into small pieces and digested for 2 to 3 hours at 37 C by an aqueous solution containing 0.6% pepsin and 0.8% hydrochloric acid. The digested materials were examined microscopically for the 3rd-stage larvae.

As for the paratenic host, a species of fresh water prawn (86 *Macrobrachium longipes*) and 3 species of land crabs (4 *Eriocheir japonicus*, 6 *Potamon dehaani* and 2 *Cardisona carnifex*) were collected and examined in the same manner as small snails and slugs.

3) Identification of the parasites:

For the identification of adult and larval stages, the morphological features were observed microscopically and the results of their microscopic measurements, such as body length, body width, lengths from cephalic apex to nerve ring and excretory pore, length from tail tip to anus and so on, were compared with those of *A. cantonensis*. The larvae were further administered to albino rats and it was examined whether the adult worms could be recovered from the rats or not.

4) Indirect hemagglutination test:

The antigen employed was purified from *A. cantonensis* adults as described in the previous paper.²⁰ Sheep red blood cells prepared to a 3.0% suspension were treated with a tannin solution and sensitized with the antigen. For the estimation of antibody titers, a micro-titer plate with V-bottom was used. The sensitized cells were suspended in PBS containing 0.6% normal rabbit serum to make a 0.8% suspension and added in each of wells in which the test sera were diluted in a 2-fold-dilution. The plate was shaken to suspend the cells and allowed to stand for 2 hours at room temperature before the resulting patterns of cells settled on the bottoms were read.

Results

1) Incidence of *A. cantonensis* among mammalian hosts:

The results obtained were summarized in Table 1. Two species of rats were incriminated as reservoirs of the parasite. The average infection rates in the Ryukyu Islands were 42.8% in *R. norvegicus* and 18.3% in *R. rattus*. The infection rates of *R. norvegicus* on all islands were more than 33% and were always higher than those of *R. rattus* except on Ishigaki-Island where a *R. rattus* was trapped and positive (100%). *R. norvegicus* were also found infected on 2 of the Amami Islands.

Among the infected rats, the worms were most frequently found in the pulmonary arteries only. The rats harboring the worms in their brains only were 9.0% (12/134) in *R. norvegicus* and 14.0% (8/57) in *R. rattus*. The parasites were found in both of the brain and pulmonary arteries in 3.0% (4/134) of *R. norvegicus* and 1.8% (1/57) of *R. rattus*

Table 1 Incidence of *A. cantonensis* in small mammalian species collected in the Southwest Islands.

Species	Island	No. posit./No. exam.(%)
<u>Rattus norvegicus</u>	Okinawa(Ryukyu)	48/138(34.8)
	Izena(")	72/144(50.0)
	Kume(")	1/3(33.3)
	Ishigaki(")	1/2(50.0)
	Iriomote(")	1/1(100)
	Yonaguni(")	1/2(50.0)
	Yoron(Amami)	7/49(14.3)
	Okinoerabu(Amami)	3/4(75.0)
<u>Rattus rattus</u>	Okinawa(Ryukyu)	35/204(17.2)
	Izena(")	15/75(20.0)
	Kume(")	1/10(10.0)
	Ishigaki(")	1/1(100)
	Iriomote(")	3/9(33.3)
	Yonaguni(")	2/12(16.7)
	Okinoerabu(Amami)	0/3(0)
<u>Suncus murinus</u> <u>riukiuanus</u>	Okinawa(")	0/70(0)
	Izena(")	0/19(0)
	Kume(")	0/4(0)
	Iriomote(")	0/1(0)
	Yonaguni(")	0/16(0)
	Yoron(Amami)	0/7(0)
<u>Mus caroli</u>	Okinawa(Ryukyu)	0/9(0)
<u>Herpestes edwardsii</u>	Okinawa(Ryukyu)	0/16(0)

2) Incidence of *A. cantonensis* among intermediate and paratenic hosts:

As seen in Table 2, 8 of molluscan species were found infected. The highest prevalences were *A. fulica* and *L. alte* being 34.1% and 22.5%, respectively. The lowest were observed in *B. circulus* and *F. despect* with the rates of 1.4% and 0.6%. The infection rates of *A. fulica* were more than 30% on 5 islands but lower than 5% on the other 3 islands. The high prevalences were also observed in *S. mercatoria*, *I. bilineata* and *L. flavus* on 4 islands.

From 4 species of crustaceans, as paratenic host, the larva considered to be *A. cantonensis* could not be detected.

On Yoron-Island, a total of 668 *A. fulica* was examined in 4 areas through a period from 1974 to 1979. The infected snails were first observed in 2 restricted areas in 1977 and the average infection rate in this year was 20.0%. In the other 2 areas where the positive result could not be obtained in the surveys before 1977, the high prevalences being 53.3% and 65.0% were observed in the next 1979 and the average rate rose to 45.0%, showing that the parasite has been extended rapidly its distribution on the Island.

Table 2 Incidence of *A. cantonensis* in intermediate and paratenic hosts in the Southwest Islands.

Species	Island	No. posit./No. exam.(%)
Snails:		
<u>Achatina fulica</u>	Okinawa(Ryukyu)	955/2,206(43.3)
	Miyagi(")	13/43(30.2)
	Iheya(")	1/120(0.8)
	Kume(")	61/193(31.6)
	Kitadaito(")	1/20(5.0)
	Minamidaito(")	1/201(0.5)
	Miyako(")	71/130(54.6)
	Ishigaki(")	60/103(58.3)
	Yoron(Amami)	93/668(13.9)
<u>Satsuma mercatoria</u>	Okinawa(Ryukyu)	35/235(14.9)
	Izena(")	1/5(20.0)
	Okinoerabu(Amami)	0/20(0)
<u>Fruticicola despecta</u>	Okinawa(Ryukyu)	3/303(1.0)
	Izena(")	0/53(0)
	Miyako(")	0/41(0)
	Yonaguni(")	0/30(0)
	Yoron(Amami)	0/17(0)
	Okinoerabu(")	0/75(0)
<u>Bradybaena circulus</u>	Okinawa(Ryukyu)	3/435(0.7)
	Izena(")	0/13(0)
	Yoron(Amami)	6/90(6.7)
	Okinoerabu(")	0/83(0)
<u>Cyclophorus turgidus</u>	Okinawa(Ryukyu)	0/163(0)
<u>Assimineia japonica</u>	Okinawa(Ryukyu)	0/100(0)
Slugs:		
<u>Laevicaulis alte</u>	Okinawa(Ryukyu)	24/66(36.4)
	Izena(")	19/60(31.7)
	Miyako(")	24/44(54.5)
	Iriomote(")	11/85(12.9)
	Yonaguni(")	3/105(2.9)
<u>Incilaria bilineata</u>	Okinawa(Ryukyu)	0/24(0)
	Yoron(Amami)	4/22(18.1)
	Okinoerabu(")	3/12(25.0)
<u>Incilaria fruhstorferi</u>	Okinawa(Ryukyu)	0/16(0)
<u>Vaginalus plebeius</u>	Okinawa(Ryukyu)	2/22(9.1)
<u>Limax flavus</u>	Okinawa(Ryukyu)	9/50(18.0)
Fresh water prawn:		
<u>Macrobrachium longipes</u>	Okinawa(Ryukyu)	0/86(0)
Land crabs:		
<u>Eriocheir japonicus</u>	Okinawa(Ryukyu)	0/4(0)
<u>Potamon dehaani</u>	Okinawa(Ryukyu)	0/6(0)
<u>Cardisona carnifex</u>	Okinawa(Ryukyu)	0/2(0)

3) Incidence of *A. cantonensis* among rats and *A. fulica* on Okinawa-Island:

The infection levels among rats and *A. fulica* in different areas on Okinawa-Island, the most large island in the Southwest Islands, were further shown in Table 3. Generally, high infection rates in both species were shown in the areas of the middle part. The highest average rates of *R. rattus* (30.8%) and *A. fulica* (55.4%) were shown in the middle part, while the rate of *R. norvegicus* (68.8%) was highest in the northern part. The rates of *A. fulica* in the northern and southern parts were nearly the same (23.4% and 28.7%, respectively), though a significant difference was seen in the case of rats. The average rate of rats in the northern part was 29.8% against 10.9% in the southern part, notwithstanding that *R. rattus* was trapped more often than *R. norvegicus* in the northern part and the ratio reversed in the southern part. No marked difference, however, was observed between town and agricultural districts.

Table 3 Incidence of *A. cantonensis* in rats and *A. fulica* on Okinawa-Island.

Locality	No. positive/No. examined(%)		
	<i>Rattus norvegicus</i>	<i>Rattus rattus</i>	<i>Achatina fulica</i>
Northern	Janagusuku		14/162(8.6)
	Ogimi	0/2(0)	1/11(9.1)
	Shioya		4/36(11.1)
	Yako		0/20(0)
	Bise	3/3(100)	2/12(16.7)
	Yamagawa	0/3(0)	9/59(15.3)
	Gogazi		46/92(50.0)
	Goga		11/29(37.9)
	Nago	19/24(79.2)	2/7(28.6)
	Kayo		3/32(9.4)
	Kiyoda		11/21(52.4)
	Ginoza		27/67(40.3)
Middle	Ishikawa	4/8(50.0)	5/21(23.8)
	Gushikawa	3/5(60.0)	6/12(50.0)
	Koza	1/2(50.0)	2/5(40.0)
	Atsuta	0/6(0)	1/3(33.3)
	Futenma	4/10(40.0)	1/8(12.5)
	Touma	2/6(33.3)	3/3(100)
	Ginowan		2/13(15.4)
Southern	Serikyaku		1/20(5.0)
	Yonabaru	9/19(47.4)	0/14(0)
	Naha	1/15(6.7)	1/9(11.1)
	Tomigusuku	1/15(6.7)	0/14(0)
	Tomori		100/301(33.2)
	Itoman	1/20(5.0)	0/13(0)

4) Survey on the inhabitants in the Ryukyu Islands:

In a screening survey with the indirect hemagglutination test, the sera of 2,937 persons collected randomly from 21 areas on 6 islands were tested. As seen in Fig. 2, 9.3% to 63.2% of the sera showed positive antibody levels over 1:16 in every area, but only 1.7% in Niigata Prefecture, a nonendemic control area in Japan. On Okinawa-Island, the highest positive rates were observed in the northern agricultural districts. In Nakijin and Kushi areas, the positive rates were as high as 63.2% and 53.1%. Higashi, Ginoza, Nakagusuku, Sashiki and Kanegusuku areas, all belonging to the agricultural districts, showed positive rates over 25%, while in the urban districts such as Nago and Naha areas, the positive rates were only 10.8% and 13.7%. On the other islands, the high positive rates ranging from 45.2% to 53.1% were observed on Miyako- and Irabu-Islands, but the rates were not so high on Ishigaki-, Kume- and Ie-Islands showing 25.2%, 26.1% and 14.6%, respectively. The sera collected from pupils of junior high school in Yonagusuku of Okinawa-Island showed positive rate lower than 10%. On Kume-Island, the positive rate of pupil group (9.3%) was also considerably lower than that of the adult group (26.1%).

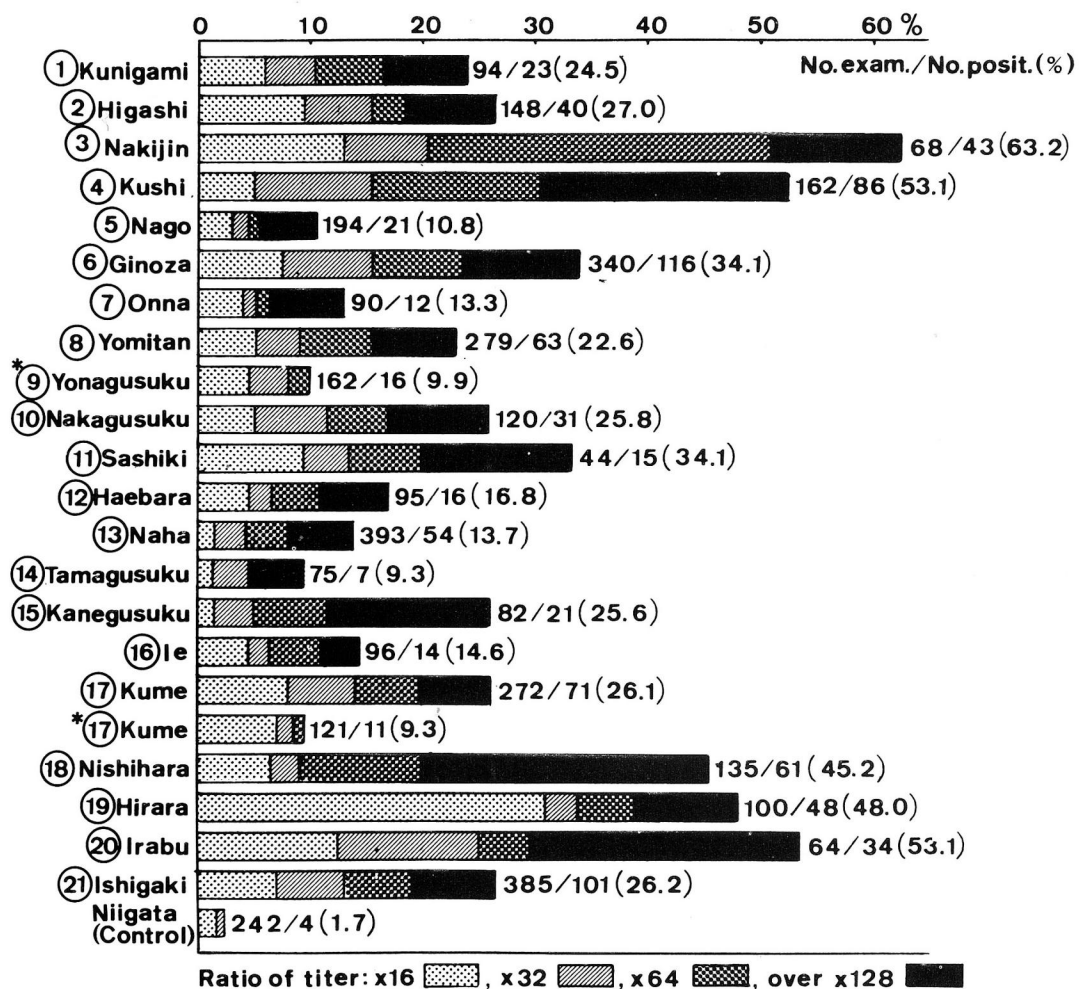


Fig. 2 Positive rates of antibody and ratios of sera classified by their antibody titers on the indirect hemagglutination test for angiostrongyliasis in sera of inhabitants of the Ryukyu Islands. Areas 1-15) Okinawa-Island (1-7 northern, 8-10 middle, 11-15 southern); 16) Ie-Island; 17) Kume-Island; 18, 19) Miyako-Island; 20) Irabu-Island; 21) Ishigaki-Island; *Result of pupil group.

Among these positive sera, the ratios of sera classified by their titers were also represented in Fig. 2. In areas where high positive rates over 40% were shown, the ratios of sera with high titers over 1:128 were 41.9% in Kushi, 57.4% in Nishihara and 44.1% in Irabu, but only 18.6% in Nakijin and 18.8% in Hirara areas. In addition to the low positive rates, the sera with these high titers could not be seen among the pupil sera and sera in Niigata Prefecture.

Discussion

In the present survey, the prevalence of *A. cantonensis* in the intermediate, paratenic and definitive hosts was surveyed on 15 islands of the Southwest Islands (the Ryukyu and Amami Islands). On these islands, 799 small mammals consisting of 5 species, 5,853 mollusks of 11 species and 98 crustaceans of 4 species were examined for the infection with *A. cantonensis*.

As definitive host, 2 species of rats, *R. norvegicus* and *R. rattus* were found infected with the parasite and the average infection rate of *R. norvegicus* (39.1%) was more than 2 times as many as that of *R. rattus* (18.1%). The result was similar to those in the Pacific Islands and Southeast Asia, though the incidence in rodents of genus *Bandicota* was comparatively higher as compared with the species of *Rattus* in Southeast Asia.²¹⁻²³⁾ The high infection rate in *R. norvegicus* could be due to the different behavioral pattern and ecological niches of the species than that of *R. rattus*. Some of the species other than *Rattus* were trapped in the same areas but the parasite could not be detected among these species. As to *S. murinus riukiuanus*, however, the young adult worms have been found in the brain on Okinawa-Island.¹⁹⁾

In the previous surveys, 6 molluscan species, *A. fulica*, *B. circulus*, *F. despecta*, *S. mercatoria*, *V. plebeius*, *Deroceras laeve* and *Philomyces bilineata*, have been found to be natural intermediate hosts in the Ryukyu Islands. In addition to the former 4 species, the authors also proved the natural infections in the other 2 species, *L. alte* and *L. flavus*, in the present survey. The high prevalences were observed in *A. fulica* (34.1%), *L. alte* (22.5%), *L. flavus* (18.0%) and *S. mercatoria* (13.8%), but the positive rates in *F. despecta* and *B. circulus* were only 0.6% and 1.4%, respectively. On Okinawa-, Ishigaki- and Miyako- Islands, more than 40% of *A. fulica* and/or *L. alte* were found infected, while less than 5% on Iheya-, Kitadaito-, Minamidaito- and Yonaguni-Islands. The high infection rates of the intermediate hosts on Okinawa- and Miyako-Islands were presumably related to the facts that all human cases have occurred on these 2 islands. It is of interest that *A. fulica* was not seen on Izena-Island, though the infected rats were found to be as high as 39.7%. On the Island, 31.7% of *L. alte* and 20.0% of *S. mercatoria* were found infected suggesting that these species may be responsible for the murine infections. On Okinawa-Island, the infection rates of *A. fulica* were generally higher in the middle part as in the case of rats.

Yoron- and Okinoerabu-Islands are the southern islands of the Amami Islands, where the parasite has not been detected in the previous surveys.^{24,25)} In the present survey, *R. norvegicus* and 3 species of mollusks (*A. fulica*, *B. circulus* and *I. bilineata*) were found infected. On Yoron-Island, the infection was first observed in 2 restricted areas, but in the following 2 years, it became to be observed in all areas, along with a rapid increase in the infection rate, indicating that the parasite has been imported in relatively recent years and are extending its distribution on whole island.²⁶⁾ Similarly, the infections of molluscan species were proved on the other 2 islands of the Amami Islands and in 2 areas of the Kyushu provinces.²⁷⁾ These facts show that the parasite has already extended its distribution along the northern islands of the Southwest Islands further to Japan

proper, and the disease due to it is now one of the important public health problems in Japan proper, as well as in the Ryukyu Islands.

In the survey of paratenic hosts, 4 out of 8 amphibian species, *Bufo asiaticus*, *Rana catesbeiana*, *R. Limnocharis* and *Rhacophorus leucomystax*, have been found naturally infected and the results were previously reported.²⁸⁾ On Miyako Island, 2 human cases infected by taking the raw livers of the toad, *B. asiaticus*, as a Chinese medicine have been reported.²⁹⁾ The other species, a species of fresh water prawn and 3 of land crabs, were further examined but none was found harboring the parasite.

For the epidemiological observations on the disease, a screening survey with the indirect hemagglutination test among the inhabitants were conducted and significantly higher positive rates of antibody compared with that in Niigata Prefecture, a nonendemic control area, were obtained in every area. The positive rates were generally higher on Okinawa and Miyako-Islands, and this result seems to correlated with the high prevalences of the parasite in animal hosts on these 2 islands. The similar result was obtained in the same survey conducted on Taiwan, in which an apparent geographic difference was also recognized in distribution of the antibody positive persons.¹²⁾ The high positive rates over 28% were seen in the southern and eastern areas, corresponding to the high prevalence of the parasite and the distribution of many patients. On Okinawa-Island, the positive rates were considerably higher among the inhabitants in the northern agricultural districts as compared with those in the middle and southern urban districts, though the infection rates in animal hosts were higher in the middle part. This result suggests that the human infection may be closely related with the ways of life as well as the prevalence of the parasite. The positive rates of the pupil group were significantly lower than those of the adult group. In Japan, only 3 out of 20 cases were of children. Similarly, in Thailand, more than 76% of suspected cases were in adult group, while the cases in age groups under 19-years-old were as many as 80% in Taiwan.^{30,31)} The difference in the positive rates between adults and pupils may reflect such difference in case distribution by age-groups. In previous paper, the authors examined immunologically 9 cases in Japan and indicated that the antibodies to the parasite were produced rapidly within a month after the suspected infection, then decreased slowly to a low level in the following 6 months and almost diminished in a year or more.^{13,32)} From the results, it was considered that the latent infections often occurred among the inhabitants in areas where the high positive rates were observed. In conclusion, the present survey reveals that the parasite is well established in the Ryukyu Islands and its distribution is extending throughout the Southwest Islands. It was also suggested that a considerable proportion of inhabitants in the Ryukyu Islands, as that in Taiwan, may be infected unknowingly with the parasite or mistaken for a variety of other diseases affecting the central nervous system. It is continuously necessary to carry out the observations on the incidence of the parasite and occurrence of the disease in Japan proper, as well as in the Southwest Islands.

Summary

An epidemiological survey on *Angiostrongylus cantonesis* and human angiostrongyliasis was undertaken on 15 islands of the Southwest Islands, Japan. A total of 799 mammals of 5 species, 5,853 mollusks of 11 species and 98 crustaceans of 4 species were examined, and 2 species of rats and 8 species of mollusks were found naturally infected with the parasite.

Rattus norvegicus was found to have a higher rate of infection compared with that of *Rattus rattus*, showing 39.1% and 18.1%, respectively.

The infection rates were 34.1% in *Achatina fulica*, 22.5% in *Laevicaulis alte*, 18.0% in *Limax flavus*, 13.8% in *Satsuma mercatoria*, 12.1% in *Incilaria bilineata*, 9.1% in *Vaginulus plebeius*, 1.4% in *Bradybaena circulus* and 0.6% in *Fruticicola despecta*.

In the screening survey of the inhabitants, 9.3% to 63.2% of sera from 3,937 persons showed positive antibody levels against *A. cantonensis* antigen in every area, suggesting that the disease may continuously occur in the Ryukyu Islands.

The obtained results reveal that the parasite is widespread and well established in the Southwest Islands.

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