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Epidemiological Observations on Angiostrongyliasis in Taiwan. 1. Results of Indirect Hemagglutination Test for Angiostrongyliasis among Suspected Japanese Encephalitis Cases

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Eosinophilic meningoencephalitis which is prevalent throughout the Pacific Islands and Southeast Asia is now believed to be caused by accidental infection with rat lung worm, *Angiostrongylus cantonensis*.

Human infection with this nematode was first reported in Taiwan by Nomura and Lin (1945). However, little attention had been paid to this parasitic disease until Rosen *et al*. (1962) found again the worm in the brain of an autopsy case in Hawaii and emphasized the significance of this disease. Consequently, several studies on the disease have been carried out in Taiwan, and it was elucidated that the disease is very prevalent and widely distributed in Taiwan, especially in the southern and eastern areas of the Islands (Cross, 1967). Now, this nematode is generally recognized to be one of the important etiologic agents which cause pathological conditions of the central nervous system in Taiwan.

Soms characteristic clinical symptoms of this parasitic disease are helpful in differentating it from other diseases. However, particularly in atypical cases, this disease is often confused with other central nervous system disorders, especially with Japanese encephalitis (JE), which is more prevalent in Taiwan.

From an epidemiological point of view, therefore, it is necessary to determine whether or not this disease is actually involved in suspected cases of JE. To answer this question, a serologic examination for angiostrongyliasis was conducted on serum specimens of suspected JE cases in Taiwan.

Materials and Methods

Serum specimens were randomly selected from suspected cases of JE which broke out during the period from 1967 to 1971 throughout Taiwan (Table 1). These specimens were collected for the sero-epidemiological studies of JE by WHO JE Research Unit in Taipei and stored at −70°C in Taiwan Provincial Vaccine and Serum Laboratory. The suspected JE cases were serologically calssified into three groups, as described below, by the Unit on the basis of the titers of a JE hemagglutination inhibition test (HI-test) which was performed twice at intervals of 3 weeks;

(1) The confirmed group: This group is composed of 1,265 cases, in which HI-test gave a titer of 1 : 256 or more at the first

and second examinations.

(2) The equivocal group: This group includes 1,005 cases. HI-test showed positive reaction, but the titer was below 1 : 256 at the first examination and a slight rise of the titer, within two-fold, or an unchanged titer was shown at the second examination.

(3) The excluded group: This group is composed of 140 cases, in which HI-test showed negative reaction at the both examinations.

In the present study, the antibody for angiostrongyliasis was titrated on the serum specimens from 800 cases, which consisted of 288 from the confirmed, 408 from the equivocal and 104 from the excluded groups respectively.

Indirect hemagglutination test (IHA-test) for angiostrongyliasis was performed after the method of Jacobs and Lunde (1957) for toxoplasmosis. The antigen employed was prepared from the adults of *A. cantonensis*. The worms collected from the pulmonal vessels of experimentally infected rats were washed several times with isotonic phosphate buffer saline (PBS), pH 7.2 and ground with a mortar in PBS. The supernatant yielded by centrifugation of the emulsion at 3,000 rpm for 10 minutes was dialysed against isotonic phosphate buffer (PB), pH 6.4 for 48 hours at 4°C. The emulsion was centrifuged again and the supernatant thus obtained was adjusted to centain 400 μg of protein per ml and stored at −70°C until use. Sheep red cells were stored at 4°C in 3 volumes of Alsever's solution. The cells were washed 3 times with PBS and diluted to a final concentration of 2.5 %. The cells were treated by adding an equal volume of 1 : 40,000 tannic acid solution and incubated in an ice-bath for 15 minutes with occasional shaking. After centrifugation of the suspension at 2,000 rpm for 5 minutes, the supernatnat was discarded, and the cells were washed once with PBS. The cells were then resuspended in the original volume of physiologic saline, and the same volume of antigen was added. The mixture was incubated in a water-bath at 37°C for 30 minutes with occasional shaking. The sensitized cells were then washed 3 times with PBS containing normal rabbit serum in 0.1 % (NRS), and resuspended in the original volume of NRS. The cells treated with PB instead of the antigen were utilized as a control. All sera tested were inactivated at 56°C for 30 minutes, and absorbed by adding an equal volume of 4 % sheep red cell spspension at 4°C for 12 hours. After centrifugation at 2,000 rpm for 5 minutes, the supernatant obtained was diluted five-fold with NRS. Starting with 1 : 10 dilution, two-fold serial dilutions of each serum were made. To 0.5 ml of sera, 0.05 ml of sensitized cell suspension were added, and the tubes were vigorously shaken. Titers were read after 12 hours. Agglutination titer was expressed as the reciprocal of the highest dilution of the sera which gave three plus reactions. Sera which showed the titers of over 1 : 40 were determined to be positive.

Results

During the period from 1967 to 1971, a total of 3,068 with symptoms of menigoencephalitis was reported in Taiwan. Among these, 2,401 cases (78.3 %) were examined for HI antibody of JE and classified into three groups according to the criteria of WHO JE Research Unit (Table 1).

As shown in table 2, 48 (6.0 %) out of 800 cases examined showed positive reaction for angiostrongyliasis by means of IHA-test.

An apparent geographic difference was recognized in distribution of the positive cases, as it is shown in table 2 and figure 1. The highest prevalence (20.7 %) was seen in the eastern area, while in the southern, central and northern areas the incidences of the positive cases were as low as 7.6, 3.2 and 1.8 % respectively. On the other hand, as illustrated in figure 1, most of the positive cases were found in the southern part of the Island, namely the southern area and southern half of the eastern area. Although the southern part of the Island includes four prefectures, Tainan, Kaohsiung, Pingtung and Taitung, they are essentially similar in

Table 1. Results of HI-test in suspected JE cases (1967–1971)

Year	No. of cases reported	No. of cases sero-examined	No. of cases (%)		
			Confirmed	Equivocal	Excluded
1967	1,024	563	273 (48.5)	269 (47.8)	21 (3.7)
1968	610	528	277 (52.5)	210 (39.8)	41 (7.8)
1969	502	442	279 (63.1)	135 (30.5)	28 (6.3)
1970	577	540	269 (49.8)	227 (42.0)	44 (8.2)
1971	355	328	158 (48.2)	164 (50.0)	6 (1.8)
Totals	3,068	2,401	1,256 (52.3)	1,005 (41.9)	140 (5.8)

quoted from "Taiwan's Health" (1972)

Table 2. Incidence of positive reaction of IHA-test for *A. cantonensis* among suspected JE cases, in relation to antibody titer for JE and geography of the cases

JE group	Confirmed		Equivocal		Excluded		Totals	
Area	No. of cases examined	No. of positives (%)	No. of cases examined	No. of positives (%)	No. of cases examined	No. of positives (%)	No. of cases examined	No. of positives (%)
Northern	100	0	149	3 (2.0)	32	2 (6.3)	281	5 (1.8)
Central	74	2 (2.7)	94	1 (1.1)	22	3 (13.6)	190	6 (3.2)
Southern	79	0	125	4 (3.2)	33	14 (42.4)	237	18 (7.6)
Eastern	35	3 (8.6)	40	3 (7.7)	17	13 (76.5)	92	19 (20.7)
Totals	288	5 (1.7)	408	11 (2.7)	104	32 (41.4)	800	48 (6.0)

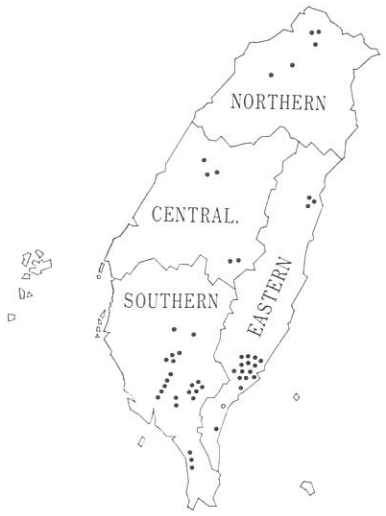

Figure 1. Geographic distribution of positive cases in IHA-test for *A. cantonensis*.

many respects, i.e. geographical characteristics, climate, living custom of the inhabitants, etc.

Since there may be no analogous antigenicity between both causative agents, no common-reacting antibodies may be produced by infections with these agents. Of 104 cases which had no JE antibodes, 32 (41.4 %) showed positive IHA reaction against *A. cantonensis*, whereas only 5 (1.7 %) out of 288 cases of the confirmed and 11 (2.7 %) out of 408 cases of the equivocal groups were positive for *A. cantonensis*. This result indicates that 66.7 % of the positive cases were detected from JE excluded group. Furthermore, it is shown that, in the southern and eastern areas, antibody for *A. cantonensis* was found in more than half of the patients who manifested symptoms of meningoencephalitis without having JE an-

Table 3. Comparison of autibody levels at the first and second examinations

JE group	No. of positives	FE<SE	FE=SE	FE>SE
		No. of cases (%)	No. of cases (%)	No. of cases (%)
Confirmed	5	3 (60.0)	0	2 (40.0)
Equivocal	11	9 (81.8)	2 (18.2)	0
Excluded	32	26 (81.3)	4 (12.5)	2 (6.3)
Totals	32	38 (79.2)	6 (12.5)	4 (8.3)

FE: Antibody titer of serum obrum obtained at the first examination.
SE: Antibody titer of serum examined 3 weeks later.

tibody.

Compaison of *A. cantonensis* antibody titers at the first and second examinations resulted increase of the titer at the latter examination in 38 (79.2 %) out of 48 positive cases. On the other hand, very few cases showed decrease of the titer at the second examination comparing with that of the first examination. These results may indicte that, at the earlier stages of angiostrongyliasis manifesting symptoms of moningoencephalitis, the antibody titer has a trend to increase gradually during the course of the infection.

Discussion

In regard to the dispersal of *A. cantonensis*, Alicata (1969) represented an interesting hypothetic view that the parasite which originated somewhere in Eact Africa, possibly Madagascar, was spread to the southern and eastern parts of the Pacific during the past two centuries associating with the dispersal of the optimal intermediate snail host, *Achatina fulica* or the final host, some species of rats. In terms of the dispersal to Taiwan, this hypothesis can be accepted as pertinent: According to Chiu (1964), *A. fulica* was first introduced to Taiwan from Singapore in 1932; thereafter, this snail became well established all over the Islands. In 1935, three years after the snail was introduced, *A. cantonensis* in rats was first recorded in Taiwan by Matsumoto (1937). In 1946, the first human case infected with this parasite was reported in Taiwan by Nomura and Lin (1946). Nowadays, this snail is found throughout the Islands and causes not only excessive damage to vegetables but also a risk of *A. cantonensis* infection in the inhabitants.

Besides *A. fulica*, the land snail, *Bradybaena semilaris*, the land slug, *Vaginalus plebius* and the aquatic snail, *Cipangopaludina chinensis*, are known to serve as indigenous natural intermediate hosts of *A. cantonensis* in Taiwan (Cross, 1967). Up to the present time, hower, no reliable information about the infection rates of these mollusks with *A. cantonensis* has appeared. According to the preliminary survey performed by the present authors (unpublished data), incidence of the infection in the snails collected from some areas of the main island of Taiwan is sufficiently high. It is said that only one place where the infection in the snails was not detected in Taiwan was Penghu Islands (Chen *et al.*, 1973).

Kuntz and Myers (1964) examined for the worm in rodents trapped in Taiwan and the offshore islands, and reported that *Bandicota indica nemorivaga*, *Rattus coxinga coxinga*, *R. losea*, *R. norvegicus*, *R. rattus mindanensis* and *R. rattus* subsp. served as reservoir hosts.

Taking consideration into the trasmmision mode of *A. cantoenesis* to man, ingestion or handling including cooking of *A. fulica* is accounted for a main cause of the infection in Taiwan. Since *A. fulica* were originally introduced to make up for the meat deficiency in Taiwan, there is no doubt that it has been eaten by many people. Even now the snail

is consumed by large numbers of the inhabitants of Taiwan, especially in the southern and eastern areas. However, because the snail is seldomly eaten raw, the infection with this parasite is supposed to be caused through the contamination of hands, dishes or other foods with the larvae of the parasite during the process of cooking. In connection with the source of human infection, it is worth noting that Chang *et al.* (1968) reported a case of eosinophilic meningitis caused by ingestion of an aquatic viviparous snail, *Cipangopaludina chinensis*, from central Taiwan.

As for the detection method of the immuno-responses induced by *A. cantonensis* infection, Kamiya and Tanaka (1969) reported that an indirect hemagglutination test using crude worm extract is an useful diagnostic tool in rats infected experimentally. Recently, the present authors perfomed several experiments in this regard and revealed that this test was successfully applied to antibody detection in rabbits and monkeys : (1) The antibody was produced in rabbits infected experimentally with the parasites, but the production levels were generally lower than in the infected rats (Cheng *et al.*, 1973). (2) Most monkeys infected experimentally with these nematodes showed IHA titer of 1 : 40 or more at 3 weeks after the infection (Chen *et al.*, 1972). Based on the results of these experiments, the titer of 1 : 40 or more was defined as positive in the present study.

Because of impurity of the antigen used in the present experiment, there is a possibility that the present result includes cross reaction to other helminthic infections. Even though the possibility of non-specific reaction is not ruled out completely, some other facts support the probable presence of epidemic of *A. cantonensis* infection in Taiwan. The fact that a large quantity of *A. fulica* is still being consumed in the southern and eastern areas provides also a reasonable explanation for the higher incidence of the positive cases in these areas than the other areas. On the other hand, these results suggest that a considerable proportion of cases of meningoencephalitis of unknown etiology in Taiwan may be caused by *A. cantonensis* infection.

Summary

In order to estimate prevalence of angiostrongyliasis in Taiwan, a indirect hemagglutination test with crude *A. cantonensis* extract was performed on serum specimens which were obtained from patients with symptoms of meningoencephalitis during the period from 1967 to 1971. Among 800 cases examined, 48 cases (6.0 %) showed a titer of 1 : 40 or more. This examination also revealed that the majority of the positive cases were distributed in the southern area and southern half of the eastern area of the Island. Furthermore, 32 out of 48 positive cases (66.7 %) were found in the group of symptomatic patients with no demonstrable antibody of Japanese encephalitis by means of hemagglutination inhibition test. These facts suggest that angiostrongyliasis is prevalent in Taiwan, particularly in southern part of the Island.

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台湾における広東住血線虫症の疫学的調査
1. 日本脳炎疑似患者血清についての広東住血線虫症抗体調査成績

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広東住血線虫は台湾においては脳髄膜症状を引き起す重要な病原体の一つと考えられている．本線虫の感染による好酸球性脳髄膜炎はある程度特徴的な臨床症状を呈するものの決定的ではなく，しばしば他の中枢神経障害との鑑別が問題となると言われる，特に台湾では例年7～9月を中心に日本脳炎の流行がみられるから，この時期に発生した広東住血線虫症は日本脳炎と混同されることが充分考えられる．

台湾では WHO の協力を得て，1967年以来日本脳炎の疫学的調査を実施しており，疑似患者より採取した血清も −70°C で保存されている．今回の広東住血線虫症の抗体調査には，これらの保存血清の中から日本脳炎赤血球凝集阻止反応陽性，疑陽性，陰性の各群より計800例を選んで使用した．抗体は広東住血線虫成虫より抽出した粗抗原を用いた間接赤血球凝集反応によって測定し，1：40以上の抗体価を示すものを陽性とすると，800例中48例（6.0％）が陽性反応を示した．これを各群別にみると，日本脳炎抗体陽性群では288例中5例(1.7％)，疑陽性群では408例中11例(2.7％)，陰性群では104例中32例(41.4％)と陰性群に圧倒的に高い陽性率を示した．このことから，脳髄膜症状を呈しながら日本脳炎抗体の証明されない症例の中にはかなりの割合で広東住血線虫症が含まれている可能性があると思われた．