

琉球大学学術リポジトリ

沖縄のリュウキュウジャコウネズミ (*Suncus murinus riukiuanus*) におけるトキソプラズマ抗体の調査(畜産学科)

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Prevalence of *Toxoplasma gondii* Antibody in House Shrews (*Suncus murinus riukiuanus*) in Okinawa*

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I INTRODUCTION

Toxoplasmosis is one of the most widespread zoonotic diseases in the world, for the serologic evidence of infection is abundant in human beings and in a large number of wildlife and livestock species^{2,7,8,12,13,17}). Especially, toxoplasmosis in meat-producing animals is of public health concern because the parasite can be transferred to human beings who eat or handle raw meat³).

In Okinawa Prefecture, prevalence of swine toxoplasmosis is somewhat higher than those of other prefectures in Japan^{1,5,16}). Therefore, the swine toxoplasmosis became the center of public attention in Okinawa from view point of the public health significance and of the economic loss in the animal.

To understand the reason of high prevalence of swine toxoplasmosis in Okinawa, our experiment was designed to investigate toxoplasma antibodies among several wild animals including stray cats that live around pig-premises.

The present paper, as the first trial, reported the prevalence of *Toxoplasma gondii* antibody in the house shrews (*Suncus murinus riukiuanus*) belonging to order *Insectivora*⁶), collected from pig-premises.

II MATERIALS AND METHODS

1 House shrews tested

A total of 157 house shrews were captured by live trapping at 10 different pig-premises and 2 residential farm houses (table 4). They were anesthetized by use of chloroform-saturated cotton in a glass jar and blood samples were collected by cardiac puncture. The serums obtained were kept frozen until used.

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2 Serological examination

Antibody to *Toxoplasma gondii* was determined by the indirect hemagglutination test using commercial sensitized blood cell antigen ("Toxotest" Eiken, Tokyo). The microtiter system was applied. The serum samples were inactivated at 56°C for 30 minutes and were absorbed non-specific antibodies by unsensitized blood cells. Two-fold serial dilutions of serum from 1:32 to 1:2048 were made.

The serums giving a positive reaction at 1:512 dilution or higher, at 1:256, and at 1:128 or lower were recorded as positive, suspicious positive and negative, respectively.

3 Isolation of toxoplasma in mice

Portions of brain, lungs, liver, and mesenteric lymph nodes were taken from each animal. The tissue specimens of each animal were pooled, then ground using a mortar and pestle, and finally suspended in 0.85% saline solution containing 100 µg of ampicillin and of streptomycin per ml of saline solution. Then 0.5 ml of a 20% tissue suspension thus made was inoculated intraperitoneally into each of 4 mice. The mice were observed for evidence of toxoplasma infection for 4 weeks. Those that survived for 4 weeks observation periods, were exsanguinated and their pooled brains were passaged intraperitoneally into 4 mice, those were subsequently observed for 4 weeks. Brains of all mice were examined microscopically for toxoplasma cysts. The serum samples from all mice inoculated were tested for toxoplasma antibody.

III RESULTS

1 Prevalence of antibody to *Toxoplasma gondii*

A serological survey on indirect hemagglutination antibody against toxoplasma was carried out in 157 house shrews.

The distribution of antibody titers was shown in table 1.

Table 1. Prevalence of antibody to *Toxoplasma gondii* in 157 house shrews

Antibody reciprocal titer	≤ 32	64	128	256	512	1024	2048	≥ 4096
	Negative			Suspicious	Positive			
Number of serums	20	20	20	15	41	19	21	1
	60				82			
Percent	12.7	12.7	12.7	9.6	26.1	12.1	13.4	0.6
	38.2				52.2			

Of these 157, 82 (52.2%) were seropositive, showing reaction at 1:512 or higher serum dilution. Fifteen (9.6%) had a titer of 1:256 which was judged as suspicious positive. Other 60 (38.2%) which had a titer of 1:128 or lower were negative.

2 Prevalence of toxoplasma antibody, by sex and pregnancy

Inasmuch as the sex of all house shrews tested was known, this factor was analyzed for significance. Of the 157, 91 were males and 66 were females.

As shown in table 2, the antibody prevalences for males and females were 47.3 (43 out of 91) and 59.1% (39 out of 66), respectively. On the other hand, 38.5 (35/91) of the males and 37.9 (25/66) of the females were seronegative. No significant relationship was observed between the rate of seropositive and the sex of the house shrews examined.

Table 2. Prevalence of toxoplasma antibody in house shrews, by sex

Reaction *	Male	Female	Total
Positive	43 (47.3%)	39 (59.1%)	82 (52.2%)
Suspicious	13 (14.3)	2 (3.0)	15 (9.6)
Negative	35 (38.5)	25 (37.9)	60 (38.2)
Total	91 (100)	66 (100)	157 (100)

* Positive : $\geq 1:512$; Suspicious : 1:256 ; Negative : $\leq 1:128$

Of the 66 females, 23 were pregnant when tested, therefore, the results were tabulated as shown in table 3 to examine whether or not the antibody prevalence was influenced by pregnancy. There was no significant difference in the antibody prevalence of house shrews by pregnancy, for about equal percentages of pregnant (56.5%) as non-pregnant (60.5%) females were seropositive.

Table 3. Prevalence of toxoplasma antibody in female house shrews, by pregnancy

Reaction *	Pregnant	Non-pregnant	Total
Positive	13 (56.5%)	26 (60.5%)	39 (59.1%)
Suspicious	1 (4.4)	1 (2.3)	2 (3.0)
Negative	9 (39.1)	16 (37.2)	25 (37.9)
Total	23 (100)	43 (100)	66 (100)

* See footnote of table 2

3 Prevalence of toxoplasma antibody, by location

To examination whether there was any difference in prevalence of toxoplasma antibody according to pig-premises from which the house shrews were captured, the data were grouped by location of premises, as shown in table 4. The house shrews were captured from 10 pig-premises and 2 residential farm houses. The size of pigs keeping, condition of feed and management of pigs in the premises were also tabulated in the table.

Table 4. Prevalence of toxoplasma antibody in house shrews, by location

Location	No. of pigs feeding	Condition of		No. of tested	Positive	
		feeding*	management		No.	percent
Pig-premises						
A	200	G	good	48	17	35.4
B	100	F + G	bad	24	8	33.3
C	400	F + G	good	21	12	57.1
D	60	G	bad	17	12	70.6
E	70	F + G	good	13	8	61.5
F	30	F	good	6	5	83.3
G	60	G	bad	4	4	100
H	20	G	bad	3	2	66.7
I	30	G	bad	1	1	100
J	100	F	good	1	1	100
Total				138	70	50.7
Residence						
K				15	10	66.7
L				4	2	50.0
Total				19	12	63.2
Grand total				157	82	52.2

* F : commercial formula feed only ; G : mainly garbage ; F + G : combine commercial formula feed with garbage

Firstly, if the prevalence of seropositive was compared between the animals from pig-premises and those from residential houses, the prevalence for 138 former was 50.7% and for 19 latter, 63.2%. However, no significant difference in prevalence between them was observed.

Secondary, the prevalence among house shrews by location (pig-premises) was compared. Among 8 (A ~ H) premises from which 3 or more house shrews were captured, the prevalences of seropositive animals ranged from 33.3% in the premises B to 100% in the premises G. However, the wide range of variations of antibody prevalence for house shrews in individual premises indicated that the differences among premises were not significant.

And it was also shown that the prevalence of antibody in house shrews was not influenced by condition of premises, such as number of pigs feedings, state of feeding and management of pigs in these premises.

4 Attempted isolation of toxoplasma

Of the 157 house shrews 26 were used for isolation of toxoplasma in mice. Antibody titers of these house shrews and the location of premises from which they originated were shown in table 5. Of 26 house shrews examined, 3 were seropositive (1:512 or higher), 2 suspicious positive (1:256) and 21 seronegative (1:128 or lower).

Table 5. House shrews tested for toxoplasma isolation and their hemagglutination antibody titers

Location*	Reciprocal antibody titers					
	≤ 32	64	128	256	512	1024
A	6	5				
B	3	3	2	2	2	1
C	1					
K	1					
Total	21			2	3	

* Same with those of table 4

Toxoplasma was not isolated in all mice inoculated tissue suspensions of these 26 house shrews. And also no toxoplasma antibody was found in serums of these mice inoculated.

IV DISCUSSION

Toxoplasma gondii can be transferred to a wide variety of host species including meat-producing animals via oocyst from infected cats²⁾. One cat can shed millions of oocysts in her feces, which in turn are potentially capable of infecting a large number of animals⁴⁾. In soil, the sporulated oocysts can survive for long periods, up to one year, depending on

temperature, and moisture^{14,18}). The prevalence of infection appears to be greater in warm moist areas than in cold or hot dry areas⁷).

Now, the antibody prevalence of swine toxoplasmosis (45%) in Okinawa Islands, located in subtropical and moist areas, seemed to be somewhat higher than that (20 ~ 24%) reported in any other district in Japan^{1,5,16}). The high rate of swine toxoplasmosis in Okinawa appears to be due to such climatic conditions, that suitable for survival of the oocysts. To confirm this fact, the prevalence of toxoplasmosis among stray and feral cats, living around pig-premises, should be firstly examined. But inspite of our efforts, these cats could not be captured from the premises. In the present experiment, therefore, the authors tested for other wild animal, namely house shrews, collected from the pig-premises.

This animal mainly lives in premises of livestock and feeds not only on grain, garbage but also on earthworm, snail, insect, mouse, bird, and so on^{6,11}).

Several stray or feral cats were observed in and about the all pig-premises tested except one (the premises J, in table 4). In a few premises, their owners kept several house cats to exclude rodents from livestock feeding and feed storage areas. Though there is no report about feline toxoplasmosis in Okinawa, but it has been reported in other districts in Japan indicating high antibody prevalence such as 50.5%⁹) and 69%¹⁵). Sakurai et al¹⁵) have reported that the rate of seropositive was very high in cats which always ran around a piggery or a slaughterhouse. It is likely that the infection is widespread among cats in Okinawa. Supposing that, the infection in house shrews is more likely to result from ingesting either sporulated oocysts or small animals that eat oocysts shed by infected cats.

Ruiz et al¹⁴) reported the isolation of toxoplasma oocysts from soil. Therefore, it appears that earthworms and snails can serve as transport hosts for insectivora. In fact, as described above, house shrew feeds on these animals.

In the present survey, a high antibody prevalence (52.2%) was demonstrated in the house shrews, which appears to be due to recent toxoplasmic infection and to high environmental contamination. Because, some investigators reported that toxoplasma antibody prevalence increased with age²), and that antibody titers occurring in small animals that survived not longer than one year could indicate recent infection or ongoing spread of infection¹⁷). From above facts, the authors supposed that the house shrews were infected with toxoplasma by ingesting oocysts directly or indirectly, for example, via earthworm in the pig-premises.

The behavior of house shrew for toxoplasmic infection and also epidemiological role for swine toxoplasmosis are still unknown, but it is necessary to clarify.

Since Nobuto et al¹⁰) found strikingly different rates of infection in various pig-breeding farms in Japan, the present authors tried to find similar results in the house shrews that captured from 10 pig-premises. The prevalence rates among individual premises ranged from 33 to 100%. But no significant difference was observed. In further experiment, the antibody prevalences of house shrews that captured from highly contaminated pig-premises should be necessary to compare with that of house shrews from toxoplasma free premises. High

prevalence was also observed among the house shrews captured around 2 residential houses. We observed stray cats in the areas, too. Therefore, prevalence of human toxoplasmosis in these areas should be examined.

Although, the antibody prevalence was compared between males and females, and also between pregnant and non-pregnant females, no difference was observed.

Regarding to *Toxoplasma* isolation, 26 house shrews were tested by mouse inoculation. No parasite was isolated from all mice inoculated and also no serological evidence was confirmed in serums of the mice. Reason for failure to isolate *Toxoplasma* might be partly related to the selected population that was sampled, because only 3 of the 26 tested were seropositive.

In the present paper, we demonstrated high prevalence of *Toxoplasma* antibody among house shrews that live in and about pig-premises, whether house shrew play any role in the epidemiology of swine toxoplasmosis in Okinawa needs further investigation because only a small number of house shrews were examined for evidence of *Toxoplasma* infection. And also to understand further the epidemiologic features of swine toxoplasmosis, it is necessary to investigate toxoplasmosis in the cats and other wild mammals that share the habitat with pigs.

But it can be said from present study that house shrews should be excluded from pig-premise and feed storage areas to prevent swine toxoplasmosis.

V SUMMARY

House shrew (*Suncus murinus riukiuanus*) was tested for antibody against *Toxoplasma gondii*. A total of 157 animals, of which 138 were collected from 10 pig-premises and 19 from residential farm houses, were tested by the microtiter indirect hemagglutination method. Agglutination at 1:512 or higher serum dilution was considered to be a positive reaction.

Of 157 house shrews tested, 82 (52.2%) were seropositive for *Toxoplasma gondii*. Fifteen (9.6%) were suspicious positive with antibody titer of 1:256, and other 60 (38.2%) seronegative with 1:128 or lower.

No significant difference in the antibody prevalence of the house shrews by sex and pregnancy was observed. Also, there was no difference in prevalence according to pig-premises from which the house shrews originated.

No *Toxoplasma* parasite was isolated by mouse inoculation from 26 house shrews tested. But of these 26 tested, only 3 were seropositive, and of the remaining 23, 2 were suspicious positive and 21 seronegative.

The serological findings presented here indicated that the house shrews had been highly infected with *Toxoplasma gondii*. However, whether house shrews play any role in the epidemiology of toxoplasmosis in pigs needs further investigation.

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沖縄のリュウキュウジャコウネズミ
(*Suncus murinus riukiuanus*) における
トキソプラズマ抗体の調査

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要 約

沖縄県における豚トキソプラズマ症の発生は他府県のそれより高率である。その原因究明の手がかりを得るため、豚小屋周辺に棲息する野生小動物におけるトキソプラズマ症の浸淫状況を血清学的に調査することにした。

今回は食虫目に属するリュウキュウジャコウネズミを対象に、血球凝集反応(マイクロタイター法)によるトキソプラズマ抗体の検出を試みた。

供試したリュウキュウジャコウネズミは157匹で、うち138匹は10ヶ所の豚小屋より、19匹は2ヶ所の民家周辺より採取した。

157匹中、血球凝集抗体陽性例($\geq 1:512$)は82匹52.2%、疑陽性例($1:256$)は15匹9.6%、陰性例($\leq 1:128$)は60匹38.2%で、高率にトキソプラズマに汚染されていることが示唆された。

採取場所によって抗体分布に若干のばらつきはみられたが、有意差はなかった。また同様、抗体分布に性や妊娠による差も認められなかった。

26匹(陽性3, 疑陽性2, 陰性21)についてトキソプラズマの分離をマウスを用いて試みたが、全例陰性に終わった。

リュウキュウジャコウネズミにおける抗体分布は上記のようにかなり高率であるが、豚トキソプラズマ症の疫学上の役割については尚不明で、今後の研究を必要とする。

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