# SEROLOGIC STUDY OF TOXOPLASMOSIS IN TAIWAN

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#### INTRODUCTION

Little information is available regarding the prevalence of toxoplasmosis in Taiwan. Only two papers on the subject have been published and both elude to the low prevalence of infection among Chinese on the island (Yeh, 1969; Tsai and Cross, 1972). Between 1971 and 1973, we conducted a number of serologic surveys in both man and animals in various parts of Taiwan in order to determine the prevalence of *Toxoplasma* antibodies in these different populations. The purpose of this paper is to report the results of those surveys.

# MATERIALS AND METHODS

Whole blood from man and animals was usually collected by venipuncture, allowed to clot, and the separated serum was kept frozen until tested at the NAMRU-2 laboratories in Taipei. In a few cases blood was collected on 5 mm  $\times$  30 mm Nobuto's filter paper strips (Toyo Roshi Kaisha Ltd., Tokyo, Japan) which were allowed to dry at room temperature and stored at 4°C until tested. Filter papers were eluted in physiologic saline at an initial dilution of 1:8 before testing. Sera were tested by either the indirect hemagglutination (IHA) test as described by Jacobs and Lunde (1957) or the dye test using the method described by Wallace (1969). Sera for the IHA test and the dye test (DT) were inactivated at 56°C and 60°C, respectively, for 30 minutes prior to In the IHA test serial two-fold testing. dilutions of inactivated test sera were made in phosphate buffered saline containing 0.125% bovine crystalline albumin with a beginning dilution of 1:4. In the dye test inactivated test sera were screened at an initial dilution of 1:4 and positive sera were titrated to their end points.

Blood samples were collected from man and animals in Miaoli County in northern Taiwan, in Pingtung County in southern Taiwan, and from cats in Taipei City (Fig. 1).

Blood samples were obtained from 12 persons and 12 cats living in the village of Din-tapu near Chunan township in northern Taiwan and from 325 pigs, 11 feral cats, 20 rats and 114 employees of the adjacent swine farm of the Taiwan Sugar Corporation (TSC). The village of Din-tapu is located approximately 1/8 mile from the swine farm and is a typical rural Taiwanese village. The majority of persons living in this small village are farmers, while a few work at the swine farm. In Din-tapu village only persons residing in

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Fig. 1—Map of Taiwan indicating areas surveyed for Toxoplasmosis.

houses containing a serologically positive cat were bled. The swine farm raises approximately 40,000 head of pure bred and cross bred swine utilizing excellent husbandry practices. Swine are raised both for market and to supply breeding stock for other TSC swine farms. All swine are under cover, on concrete floors, and fed a high quality prepared feed. No garbage of any sort is fed. Feral cats and rats were trapped in the vicinity of the swine barns and/or the feed mill. Some of the employees at the farm reside in a compound on the farm premises, a few reside in Din-tapu village and others in townships of Hsinchu, and Miaoli Counties.

Two separate surveys were conducted in Pingtung County; the first at the aborigine village of Santimen and the second at another large TSC swine farm. The village of Santimen is located in the mountains of southern Taiwan and is populated by members of the Paiwan Tribe of aborigines.

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Blood samples were collected as part of a general medical survey (Durfee and Tsai, 1972) from a stratified random sample of 121 persons living in the village and from 73 elderly people who volunteered to give us a blood sample in exchange for a physical examination. Six pigs, 3 dogs, 1 cow and 9 rats were also bled.

Blood samples were obtained from 102 adult pigs and from 26 employees at the TSC swine farm near Pingtung during the course of the investigation of an outbreak of pseudorabies at this farm (Yang *et al.*, 1972). No cats are present on the premises and employees reside in the nearest village which is 5 km away.

At the request of the authors, the National Taiwan University School of Veterinary Medicine and the US Air Force Veterinarian stationed in Taipei collected blood samples from a total of 35 pet cats presented to their clinics in 1972 and early 1973. In addition, 5 feral cats trapped in the Taipei area were tested.

#### RESULTS

Six (50%) of the 12 cats tested in Din-tapu village had positive dye test titers to *T. gondii* (Table 1). These were all adult cats and resided in 6 different houses scattered throughout the village. Owners of these animals were contacted and all volunteered to give us a small blood sample on filter

#### Table 1

Distribution of Toxoplasma gond	<i>lii</i> antibody
in pet cats in Taipei City and	Din-tapu
Village, Chunan, Taiwa	ın.

	No. Tested	% Positive	Dye test titers		
			16-32	64-128	256-512
Din-tapu Village	12	50.0	2	3	1
Taipei City	35	20.0	1	3	3
Total	47	27.7	3	6	4

paper strips. Additional household members were bled if possible. On the average, 2 blood samples per house-hold were collected. Of the 12 persons from whom blood samples were obtained in this village, none had IHA antibodies to *T. gondii*.

At the adjacent swine farm, 115 of 325 swine (35.4%) were found to have IHA antibodies while no antibodies were found in 20 rats or 11 feral cats tested. Of the 114 employees tested, 3 (2.6%) had positive titers (2 @ 1:64; 1 @ 1:128.)

Of the 194 sera tested from persons residing in Santimen Village, none reacted to the IHA test. Of the 19 animal sera tested, 2 pigs (33.3%) were found to have positive titers of > 1:128. Of the 102 adult swine bled at the TSC swine farm near Pingtung City, 15(14.7\%) had positive IHA titers while none of the 26 employees were positive.

Of the 35 pet cats from Taipei City tested by the dye test, 7(20.0%) had positive titers (Table 1). None of the 5 feral cats had positive titers.

### DISCUSSION

Toxoplasmosis appears to be a fairly common parasitic disease of animals in Taiwan; however, prevalence of infection in humans is extremely low. Studies of Chinese in Hong Kong (Ludlam et al., 1969) has shown the prevalence there to be 6%. Previous studies in Taiwan (Yeh, 1969; Tsai and Cross, 1972) have reported the prevalence of antibodies in healthy adults to be between 1.6% and 3.6%. Our findings of a total prevalence of 1.97% in the 152 Chinese tested adds further evidence that infection with this parasite in Chinese is a rare event. Only one case of acute clinical toxoplasmosis in man has ever been reported from Taiwan (Tsai, 1972). The reason for the low prevalence of infection in Chinese is

unknown; however, dietary habits probably play a large part. A recent study of the prevalence of antibody titers in Chinese and Tahitians living in Tahiti (Wallace et al., 1974) indicates that there is no genetic resistance to infections in Chinese as they had a higher prevalence of antibodies than did native The main dietary protein of Tahitians. Chinese on Taiwan is either pork, fish, or chicken, supplemented occasionally with beef or mutton. It has been stated that the Chinese use all of the pig except the squeal and that all of this is thoroughly cooked prior to consumptions as Chinese do not eat raw meat. This would effectively eliminate the chance of individuals becoming infected through ingestion of tissue cysts or of oocysts which might have contaminated food prior to cooking. As from 20% to 50% of pet cats tested had T. gondii antibody, we can conclude that the organism and its oocysts are present in the environment. The low prevalence of infection in man living in this environment would suggest to us that infection of Chinese by oocysts is an uncommon event.

Our findings of none of 194 aborigines with T. gondii antibodies is interesting but not Another study of T. gondii surprising. antibodies in Taiwan aborigines showed that 16.5% had positive IHA titers (Tsai and Cross, 1972). These persons were living in an extremely primitive village deep in the mountains of central Taiwan. While no cats were observed in this village these people still hunted wild animals for their food and readily admitted the consumption of raw meat, especially livers from freshly killed animals. The residents of Santimen, however, have become acculturated to the point where they no longer hunt animals as a food source and have adopted Chinese culinary habits. A few cats were observed in this village and the presence of antibodies in swine in this village suggests that the organism is being transmitted at low levels in the area.

This is the first survey of cats to be conducted in Taiwan and even though the numbers are few, the results do indicate that domestic cats are not uncommonly infected with this parasite. Of the 47 owned cats tested, 13 (27.7%) had positive DT titers. The fact that none of 11 feral cats from the swine farm were positive is an interesting These animals live exclusively on finding. wild caught rodents and one would expect a high prevalence of antibodies if rodents in this area were infected with the organism. One explanation for the absence of infection in those cats might be that coprophagous rodents prefer the faeces of other animals to those of cats. In this instance the availability of pig faeces far out weighed the availability of cat faeces. The total absence of antibodies in 20 rats trapped at this farm also suggests the prevalence of infection in that species is low. This same phenomena was reported by Wallace et al., (1972) on a small Pacific island in which he noted that the prevalence of antibodies on one atoll which contained only rats and cats was considerably higher than on an atoll containing rats, cats, and pigs. Rodents residing in the village of Din-tapu and in Taipei City do not have access to swine or other large domestic animal faeces and thus must rely on the faeces of dogs and cats to satisfy their coprophagous cravings. It appears that most Chinese in Taiwan keep cats more as perambulating mouse catchers than as household pets and as very little, if any, animal protein is ever discarded, we can assume that most feline infections with Toxoplasma are probably the result of consuming infected rodents.

A prevalence of 14.7% to 35.4% of *T.* gondii antibodies in swine agrees generally with reports by others (Yeh, 1969; Liu *et al.*, 1973) who have reported prevalences using the IHA test to range from 0 to 38% in breeding boars and 28 to 59% in market

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swine. We have shown (Durfee et al., 1974) that the IHA test is a relatively insensitive measure of infections due to T. gondii in swine and so the true prevalence is probably higher than that indicated. The higher prevalence of antibodies in swine at the TSC farm at Din-tapu may be attributed to the presence of cats in the area; however, this was never proven. The TSC swine farm near Pingtung is located in the middle of sugar cane fields and there are no cats on or near the premises; however, many of the breeding sows on this farm originated at the Din-tapu breeding farm and may have been infected prior to arriving at the Pingtung farm.

## SUMMARY

Toxoplasma gondii antibody titers as determined by either the indirect hemagglutination test or the methylene blue dye test were determined for a group of aborigines and for Chinese as well as for swine, cats, rats, dogs and one bovine, all resident on the island of Taiwan.

None of the 194 aborigines and only 3 (1.97%) of the 152 Chinese were found to be positive. Of 433 swine tested 132 (30.5%) were positive. Thirteen (27.7%) of 47 domestic pet cats were positive while none of 11 feral cats were positive. None of the 29 rats, 3 dogs or the one bovine were positive. Possible reasons for the lack of infection in Chinese is discussed.

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#### REFERENCES

- DURFEE, P.T., LEKSANA, S.B., JOSEPH, S.W. and KOESHARJONO, C., (1974). The relationship between hemagglutinating antibody titers and infection with *Toxo*plasma gondii in swine. Southeast Asian J. Trop. Med. Pub. Hlth., 5:4.
- DURFEE, P.T. and TSAI, C.S., (1972). Intestinal parasites among aborigines at Santimen, Southern Taiwan. J. Formosan Med. Ass., 71:724.
- JACOBS, L. and LUNDE, M.N., (1957). A hemagglutination test for toxoplasmosis. J. Parasit., 43 : 308.
- LIU, K.H., CHEN, S.N., SUZUKI, T., LEE, S.Y. and TSENG, P.T., (1973). Toxoplasmosis in Taiwan: 2. Examinations of porcine (pig) meat and sera. *Chinese J. Microbiol.*, 6:37.
- LUDLAM, G.B., WONG, S.K.K. and FIELD, C.E., (1969). *Toxoplasma* antibodies in sera from Hong Kong. J. Hyg. Camb., 67:739.
- TSAI, C.S. and CROSS, J.H., (1972). Serologic observations of human toxoplasmosis in Taiwan. *Chinese J. Microbiol.*, 5:122.

- TSAI, D.H.J., (1972). An acquired toxoplasmosis. J. Formosan Med. Ass., 71:123.
- WALLACE, G.D., (1969). Sabin-Feldman dye test for toxoplasmosis. The use of sodium citrate in accessary factor, and a method for collecting and storing blood on paper discs. Amer. J. Trop. Med. Hyg., 18:395.
- WALLACE, G.D., MARSHALL, L. and MAR-SHALL, M., (1972). Cats, rats, and toxoplasmosis on a small Pacific island. *Amer. J. Epidem.*, 95: 475.
- WALLACE, G.D., LAIGRET, J. and KAEUFFER, H., (1974). A comparison of the prevalences of *Toxoplasma* antibody in Tahitians and Chinese living in Tahiti. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 5:350.
- YANG, J.P.S., DURFEE, P.T., MA, C.H., CHANG, C.P. and NEW, A.E., (1972). An epizootic of Aujeszky's disease in swine in Taiwan: virus isolation, identification, and seroepidemiologic studies. *Chinese J. Microbiol.*, 5:69.
- YEH, Y.C., (1969). Survey on Toxoplasma antibodies and its isolation. J. Nat. Taiwan Univ. Agric. Coll., 10:24.