

EPIDEMIOLOGY OF SWINE TOXOPLASMOSIS IN TAIWAN

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Abstract. From July 1987 to June 1988, serum samples from 3,880 pigs from eight geographic locations in Taiwan were examined for *Toxoplasma gondii* antibodies using the latex agglutination test (LA test) and IgM-enzyme-linked immunosorbent assay (IgM-ELISA). A total of 1,073 samples (27.65%) were positive by the LA test. The percentage of positive reactions varied by location as follows: Taoyuan 44.44% (128/288), Taichung 27.60% (183/663), Tainan 22.28% (119/534), Kaohsiung 19.60% (98/500), Pingtung 17.92% (86/480), Hualien 33.95% (163/480), Ilan 31.66% (152/480), Taitung 31.64% (144/455). In the IgM-ELISA 1,828 of 3,880 samples (47.11%) were positive and the distribution of positive reactions were: Taoyuan 59.02% (170/288), Taichung 53.69% (356/663), Tainan 52.24% (279/534), Kaohsiung 54.60% (273/500), Pingtung 18.95% (91/480), Ilan 47.50% (228/480), Hualien 42.70% (205/480), Taitung 49.67% (226/455). On one farm, 20 of 120 sows experienced abortion and stillbirths due to *Toxoplasma gondii*. Lesions and *T. gondii* were found in lungs, liver, kidneys, heart, and placenta of one of the aborted fetuses.

INTRODUCTION

Toxoplasma gondii infection in pigs is of public health and economic importance (Dubey and Beattie, 1988). Humans can become infected with *T. gondii* by ingesting undercooked pork infected with *T. gondii*. Toxoplasmosis also causes neonatal mortality in pigs in Taiwan (Pan *et al.*, 1962) and in other countries (Dubey and Beattie, 1988). Limited serological studies indicate widespread exposure of pigs in Taiwan (Wu *et al.*, 1963; Lee *et al.*, 1973; Liu *et al.*, 1973; Kuo *et al.*, 1984). The purpose of the study reported here was to describe seroprevalence of *T. gondii* in 3,880 pigs from 8 locations in Taiwan. An episode of congenital toxoplasmosis in pigs on one farm is also reported.

MATERIALS AND METHODS

Serologic examination

From July 1987 to June 1988 serum samples from 3,880 pigs from 8 locations (Table 1) were examined for *T. gondii* antibodies in the IgM-enzyme linked immunosorbent assay (IgM-ELISA) (van Knapen and Panggabean, 1982) and the latex agglutination test (LA) (Tsubota, 1977). Positive and negative control sera were used with

each test. The positive control serum was obtained from pigs experimentally infected with *T. gondii*. The negative control serum was obtained from a specific pathogen free pig from the Pig Research Institute (Chu-Nan, Republic of China).

IgM-ELISA

The antigen was prepared at the National Pingtung Institute of Agriculture. *Toxoplasma gondii* tachyzoites of the TS-strain were aspirated from the peritoneal cavities of mice inoculated with tachyzoites 3 days previously. Tachyzoites (10^7 per ml) were washed three times with 0.15M NaCl solution centrifuged at 2,000xg for 15 minutes and finally suspended in 0.15M NaCl. The parasite suspension was filtered through a 3- μ m membrane filter (Nuclepore, CA, USA). The filtrate was centrifuged at 2,000xg for 15 minutes and the sediment of parasites was suspended in nine volumes of distilled water, frozen and thawed three times, then centrifuged at 14,000 g for 2 hours at 4° C. The supernatant was used as the soluble antigen and stored at -70° C until used. Protein concentration was estimated using Bio-Rad protein assay system (Bio-Rad, Richmond, CA, USA). Samples were analyzed at 595

nm and compared to similarly tested bovine serum albumin in standard solution. The 96-well microtiter plate (Nunc, Denmark) was coated with 2 µg of *T. gondii* protein in 100 µl coating buffer in each well. The coating buffer contained 5nM Na₂CO₃ and 0.045M NaHCO₃ in 0.15M NaCl solution. The microtiter plate was left at 4° C overnight, then washed three times with 7.1 pH 0.1M phosphate buffer solution containing 0.5% tween-20 (PBST). Blocking buffer, 200 µl, which was 0.25% gelatin containing 0.15M NaCl and 0.05M EDTA-2Na (ethylenediaminetetraacetic acid disodium salt, Dotite, Japan) was added to each well and incubated at 37° C for 30 minutes. After washing three times with PBST, 100 µl of test serum was added to each well. The plate was incubated at 37° C for 30 minutes, and then washed 6 times with PBST. To the empty wells, 100 µl conjugate, goat anti-swine-IgM (u)-peroxidase (Kirkgaard and Perry Lab Inc, MD, USA) diluted 1:2,000 in blocking buffer was added. After 30 minutes at 37° C the microtiter plate was washed three times with PBST and 150 µl substrate was added to each well. The substrate contained 0.1% ABTS (2, 2' - azinobis (3-ethylbenz-thi-azoline-6-sulfonic acid) diammonium salt, Sigma, USA) and 0.1% H₂O₂ (30%, Merck, USA) in 0.1M citrate buffer, pH 4.2. The optical density (OD) was measured at 410 nm with an ELISA reader (MR 700 microtiter plate reader, Dynatech Lab, USA).

Latex agglutination (LA) test

The test was performed using the commercial kit (ToxoTest-MT, Eiken Chemical Co, Ltd, Tokyo, Japan). Round-bottom 96-well microtiter plate were used for the LA test. Sera were diluted 1:32 in the Eiken buffer and the agglutination patterns followed were those recommended in the kit. Twenty-five µl of serum in 1 to 2 serial dilutions with Eiken buffer were put in each well, and then 25 µl of latex reagent added to each well. Eiken *Toxoplasma*-positive serum control was used as a positive control and Eiken buffer was used as the negative control. The microtiter plates were left at 25° C overnight.

Clinical toxoplasmosis on a swine farm

Twenty of 120 sows on a farm in Tainan aborted in 1987. Sera from aborted sows and

tissues from one aborted fetus were examined for *T. gondii*. Fetal membranes and tissues of the fetus were fixed in 10% formalin solution, sectioned in paraffin, stained with hematoxylin and eosin, and examined microscopically.

RESULTS

Seropositive pigs were found in all 8 geographic locations (Table 1). A total of 1,073 serum samples (27.65%) in the LA test, and 1,828 samples (47.11%) in IgM-ELISA test were positive.

Of 3,880 sera, 1,697 were randomly chosen for seropositivity rates based on age. In pigs younger than 3-months, 25 of 433 sera (5.77%) were positive by the LA test whereas 149 of 433 (34.41%) were positive by IgM-ELISA. However, in the group older than 3 months, 375 of 1,264 pigs (29.66%) were positive by the LA test while 759 of 1,264 (60.04%) were positive by IgM-ELISA (Table 2).

The sera of 5 of 20 sows suffering from abortion had high titers of 1,024 or 2,048 by the LA test and all of them showed positive reactions in IgM-ELISA. Two of the aborted sows had high fever, constipation and respiratory distress. Based on histologic examination, toxoplasmosis was diagnosed in one aborted fetus. The placenta had a yellowish discoloration. Necrotic lesions associated with *Toxoplasma* tachyzoites were in the placenta, lungs, heart, liver, and kidney. Numerous tachyzoites were seen in necrotic chorionic villi of placenta. The lungs were edematous and diffusely infiltrated with mononuclear cells. The myocardial lesions consisted of necrosis and dystrophic calcification. The main hepatic changes were disruption of hepatic cords, dilation of sinusoids and multifocal necrosis. Both kidneys had few necrotic foci.

DISCUSSION

Little information is available concerning the specificity and sensitivity of different serologic tests for swine toxoplasmosis. There is a general agreement that IFA titers correspond well with dye-test (DT) titers (Waltman *et al*, 1984; Dubey, 1986). The modified agglutination test appears to be as sensitive as the DT. In the present study IgM-ELISA 47.11% (1,828/3,880) was much more sensitive than the LA test. The high prevalence of

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Table 1

Geographic prevalence of *Toxoplasma gondii* antibodies in pigs in Taiwan using latex agglutination (LA) test and IgM-enzyme-linked immunosorbent assay (IgM-ELISA).

Location	No. of serum samples	Titer of LA test ^a		IgM - ELISA ^b	
		No. seropositive	% seropositive	No. seropositive	% seropositive
Taoyuan	288	128	44.44	170	59.02
Taichung	663	183	27.60	356	53.69
Tainan	534	119	22.28	279	52.24
Kaohsiung	500	98	19.60	273	54.60
Pingtung	480	86	17.92	91	18.95
Ilian	480	152	31.46	228	47.50
Hualian	480	163	33.95	205	42.70
Taitung	455	144	31.64	226	49.67
(Total)	3880	1073	27.65	1828	47.11

^a: + (positive reaction) LA titer ≥ 32

^b: + (positive reaction) Absorbance value at OD₄₁₀ > 0.485

Table 2

Prevalence of *Toxoplasma gondii* antibodies in pigs (2 age groups from 3 locations in Taiwan).

Location	Total No. of sera	Younger than 3-months % seropositive			Older than 3-months % seropositive		
		No. of sera	LA test	IgM-ELISA	No. of sera	LA test	IgM-ELISA
Taichung	663	127	6.3	49.6	536	32.6	54.6
Tainan	534	200	7.0	13.5	334	41.4	75.4
Kaohsiung	500	106	2.8	55.6	394	24.1	54.3
(Total)	1697	433	5.7	34.4	1264	29.6	60.0

IgM-ELISA positive reactions in swine may represent recent infections because IgM antibody occurs during acute infection (Wieland *et al*, 1983).

The seroprevalence in our study is higher than those of the reports published prior to 1988 in Taiwan, 9.44% by agar gel immunodiffusion test (AGID) (Chang *et al*, 1988), 8% by AGID (Kuo

et al, 1984), 9% by DT (Weng *et al*, 1977). Using the indirect hemagglutination test Liu *et al* (1973) found 31% (Pingtung), 55% (Kaohsiung), and 59% (Chaochow) seropositive pigs. These differences in seropositivity may be due to different serotests used. Another reason for this variation could be that since 1987, owners of pig farms had been forbidden to feed rations medicated with sulfa drugs to swine raised for export to Japan and other countries. Sulfa drugs had been conventionally used for preventing and treating swine toxoplasmosis in the pig farms in the past decades.

Although toxoplasmosis induced abortion was diagnosed on one farm in this study and has been reported previously, there are no estimates of economic losses due to toxoplasmosis in swine in Taiwan.

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