

## SEROPREVALENCE OF *TOXOPLASMA GONDII* IN MALAYSIAN CATTLE

RAHMAN W.A.<sup>1</sup>, MANIMEGALAI V.<sup>1</sup>, CHANDRAWATHANI P.<sup>2</sup>, NURULAINI R.<sup>2</sup>, ZAINI C.M.<sup>2</sup> AND PREMAALATHA B.<sup>2</sup>

<sup>1</sup> School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

<sup>2</sup> Veterinary Research Institute, Jalan Sultan Azlan Shah, Ipoh, Perak, Malaysia

Corresponding author: arawahab@usm.my

**ABSTRACT.** One hundred and sixteen cattle sera were randomly selected from 17 farms in five different states of Malaysia (Perak, Terengganu, Johor, Melaka and Sabah). All serum samples were tested by Indirect Fluorescent Antibody Test (IFAT) using specific conjugates (from VMRD). The results showed that only 2.6% were positive for *Toxoplasma gondii*.

*Keywords:* cattle sera, Malaysia, IFAT, *Toxoplasma gondii*

### INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by a sporozoan parasite, *Toxoplasma gondii*. It is an intracellular protozoan widely distributed in nature; infection is also widely prevalent in man and many species of warm blooded animals. According to Dubey and Beattie (1988) the only definitive host for toxoplasmosis is feline, while humans act as an intermediate hosts. The organism has a worldwide distribution and is a general cause of infertility, stillbirth, and abortion in animals and man (Aspinall *et al.* 2002). In small ruminants, infections not only result in significant reproductive losses, but it also has implication for public

health, since consumption of infected meat can facilitate zoonotic transmission (Bisson *et al.*, 2000). The parasite can cause problems to pregnant woman and immunodeficiency individuals (Montoya and Liesenfeld, 2004). Toxoplasmosis also causes abortion in sheep and goats as well as neonatal death (Dubey and Jones, 2008).

There are several tests used to detect toxoplasmosis in animals such as Enzyme-linked immunosorbent assay (ELISA), Indirect Fluorescent Antibody Tests (IFAT) and isolation of organism. The IFAT has been widely used for *T. gondii* as a serological tests for the diagnosis of infection in cattle. Positive IFAT results mean that the cows are exposed to the disease but do not necessarily indicates infection status at the time of the test.

Not many studies have been conducted on the seroprevalence of *T. gondii* in animals in Malaysia and some surveys were done a long time ago (Singh *et al.*, 1967; Chooi, 1989; Rajamanickam *et al.*, 1990) and thus there is a need for the present study to be conducted. The objective of this study is to determine the seroprevalence of *T. gondii* in Malaysian cattle using IFAT.

## MATERIALS AND METHOD

### Serum samples

Bovine serum samples from the Veterinary Research Institute (VRI) Serum Bank consisting of 116 adult cattle sera randomly selected from 17 farms in five different states in Malaysia (Perak, Johor, Melaka, Terengganu and Sabah) were used. All samples were tested by IFAT using species specific conjugates (from VMRD).

### Indirect Fluorescent Antibody Test (IFAT)

Fluorescent antibody substrate slides with 12 wells were used for the detection of antibody against *T. gondii*. Other testing materials used were positive or negative control sera, serum diluting buffer, anti-immunoglobulin conjugate, rinse buffer and mounting fluid.

The slides were warmed under room temperature after being removed from foil pouch. The sera were diluted in a serum dilution plate in a portion 1:20 with a serum diluting buffer solution at pH 7.2.

Then, 10-50  $\mu$ l (depending on well size) diluted sera from each sample were placed on the designated wells. The slides were then incubated in a humid chamber at 37°C for 30 minutes. After that, the slides were tenderly rinsed in fluorescent antibody rinse buffer using a wash bottle and then soaked inside the same buffer solution for 10 minutes. Then the slides were drained and dried around the wells by pressing blotter to remove excessive water from the slides.

Subsequently, 10  $\mu$ l of FITC-labeled anti-IgG or IgM conjugate was added onto the wells. Then the wells again were incubated in humid chamber at 37°C for 30 minutes. The wells were then rinsed as mentioned earlier with fluorescent antibody rinse buffer. The slides were drained and the back and edges of the slides were dried using paper towels. The stained surface was not allowed to rinse in trough water or dried. Before viewing the slides under fluorescent microscope at 400X magnification, the slides more mounted with fluorescent antibody mounting fluid per well and covered with cover slip. This was to have a better image of the slide under

**Table 1.** Seroprevalence of *Toxoplasma gondii* in five states in Malaysia

STATE	NO. EXAMINED	NO. POSITIVE	PERCENTAGE
Johor	20	0	0
Melaka	25	3	2.59
Perak	21	0	0
Sabah	20	0	0
Terengganu	30	0	0
<b>TOTAL</b>	<b>116</b>	<b>3</b>	<b>2.59</b>

fluorescent microscope. The existence of bright fluorescent cytoplasmic bodies under microscope indicated the presence of antibody in the sera.

## RESULTS

Table 1 shows the results of *T. gondii* infection in the cattle; 116 samples were collected and the three samples found positive for *T. gondii* (2.59%) were all from the state of Melaka.

## DISCUSSION

From this study, 3 out of 116 sera (2.59%) of cattle were positive for the presence of IgG antibodies to toxoplasmosis infection. These studies showed lower prevalence than the prevalence studies by Singh *et al.* (1967), Rajamanickam *et al.* (1990), Normaznah *et al.* (2004) and Chandrawathani *et al.* (2008).

In humans, several epidemiological studies found an important association between toxoplasmosis and consumption of cattle meat (Baril *et al.*, 1999). Sources of infection are different in human populations and depended on the differences in culture and eating habits (Garcia *et al.*, 2006; Gilot-Fromont *et al.*, 2009). In Europe, up to 63% of human infections are attributable to the consumption of undercooked or cured meat products (Cook *et al.*, 2000). The current literature presents values ranging from 0 to 92% for the presence of anti-*T. gondii* antibodies in cattle (Tenter *et al.*, 2000). High seroprevalence values were

found in Spain (41%, Moreno *et al.*, 1991), Poland (53.8%, Sroka 2001), Serbia (76.3%, Klun *et al.*, 2006), and Brazil (71%, Santos *et al.*, 2009). Lower prevalence of infection in cattle also reported in some countries such as Tanzania (3.6%, Luuk *et al.*, 2009), France (7.8%, Gilot-Fromont *et al.*, 2009), and Malaysia (7.9% in local cattle and 4% in yellow cattle, Chandrawathani *et al.*, 2008). Transmission occurs following ingestion of sporulated oocysts or bradyzoites within the cysts of parasite in the tissues of numerous food animals (Dubey 2008).

Cats are common pets kept in most families in Asia and often found in public places. An increase in the number of cats, especially stray cats, causes the increase in the number of toxoplasmosis cases in both animals and human. The cats can form a chain in transmitting this disease to humans though cattle when the meat and raw milk are consumed (Gargia *et al.*, 1993). Soil and pasture are contaminated by faeces of infected cats, mainly young kittens which are shedding high numbers of oocysts and symptomatic toxoplasmosis is not frequent in bovines (USDA, 2009). A few cats are probably enough to contaminate a field in a short time, since one animal can eliminate millions of oocysts after ingesting just one infected mouse. In the environment, oocysts are spread through wind, earthworms, coprophagous invertebrates, rain and surface water, or harvested feeds (Tenter, 2009). Infection of livestock occurs by ingestion of forage or fodder contaminated with sporulated oocysts (Bisson *et al.*, 2000).

Since direct observation of cysts in tissues is not a suitable diagnostic method to be carried out on live animals and the fact that symptomatic toxoplasmosis is rare in cattle, the serological techniques appear to be the methods of choice (Dubey and Jones, 2008; USDA, 2009). Studies in Malaysia have shown that specific antibodies to *T. gondii* are common among Malaysians. A study by Sinniah *et al.* (1984) reported a positive rate of 30.2% by the immunofluorescent technique (IFAT). Hakim *et al.* (1994) reported a prevalence of 10.6% among Aborigines in Peninsular Malaysia. The antibody prevalence rates were different between various occupational groups, thus signifying nature of work may have determined the different risk levels to being infected. Tan & Zaman (1973) explained that the high prevalence rate of antibodies among paddy planters was due to their close association with cats and other domestic animals like cattle. Veterinarians, because of regular contact with animals, also had high positive antibody rates. A similar explanation may be true for the high prevalence rate among zoo workers (Zahedi *et al.*, 1985).

Reports for toxoplasmosis in other livestock species in Africa and other tropical regions revealed higher prevalence in sheep and goats, as exemplified by 31% in goats in Uganda (Bisson *et al.*, 2000), 29.4% sheep in Brazil (Clementino *et al.*, 2007), 23.4% in sheep and 19.3% in goats in Saudi Arabia (Sanad and Al-Ghabban, 2007), 43% in sheep in Egypt (Shaapan *et al.*, 2008), 52.6% in sheep

and 74% in goats in Ethiopia (Teshale *et al.*, 2007), but also in pigs, where Hove *et al.* (2005) reported a prevalence of 23% in Zimbabwe. Prevalence seems to be higher in sheep and goats in countries with a wet climate than in countries with a hot and dry climate (Samra *et al.*, 2007; Shaapan *et al.*, 2008). The primary host of the *Toxoplasma* parasite is the domestic cat and some wild felines (Dubey and Jones, 2008). These differences may be due to prevalence of *Toxoplasma* infection in different populations (Azab *et al.*, 1992; Zamani *et al.*, 2007).

According to Dubey and Jones (2008), sheep are anatomically able to forage closer to the ground than cattle; therefore they could have more possibilities to ingest oocysts of *T. gondii* contaminating the soil. In this study cattle showed a low percentage of parasitism by *Toxoplasma* which is consistent with the results of Dubey and Jones (2008), who affirmed that cattle are relatively resistant to *T. gondii* infection; while it is still unclear whether this is associated with fast eradication of cysts from cattle tissues, or with inconsistent cyst formation following disease. Klun *et al.* (2006) found similar prevalences in cattle (76%) and sheep (85%) from Serbia, but with relatively low antibody levels in cattle.

In conclusion, although the overall animal prevalence of toxoplasmosis was low, the parasite is normally found to be ubiquitous in warm moist environments and is consistently considered to be a health hazard to human particularly to

those exposed because of their occupation. However, this study is a preliminary study on *T. gondii* infections in cattle so further studies are needed to understand the dynamics of transmission cycles and the genetic diversity of *T. gondii* on the farms, and to identify and if possible, alter management practices that are risk factors for human infections.

## REFERENCES

1. Aspinall, T.V., Marlee, D., Hyde, J.E., Sims, P.F. (2002). Prevalence of *Toxoplasma gondii* in commercial meat products as monitored by polymerase chain reaction—food for thought? *International Journal of Parasitology* **32**:1193–1199.
2. Azab, M.E., Kamel, K.M., Makled, H., Khatib and El-Zayyat (1992). Naturally occurring *Toxoplasma* antibodies in serum and milk of lactating women. *Journal of Egyptian Society of Parasitology* **22**: 561-258.
3. Baril, L., Ancelle, T., Goulet, V., Thulliez, P., Tirard-Fleury, V., Carme B. (1999). Risk factors for *Toxoplasma* infection in pregnancy: a case control study in France. *Scandinavian Journal of Infectious Diseases* **31** 305–309.
4. Bisson, A., Maley, S., Rubaire-Akiiki, C.M., Watling J.M. (2000). The seroprevalence of antibodies to *Toxoplasma gondii* in domestic goats in Uganda. *Acta Tropica* **76**:33–38.
5. Burg, J.L., Grover, C.M., Pouletty, P., Boothroyd, J.C. (1989). Direct and sensitive detection of a pathogenic protozoan. *Toxoplasma gondii* by polymerase chain reaction. *Journal of Clinical Microbiology* **27**: 1787–1792.
6. Chandrawathani, P., Nurulaini, R., Zanin, C.M., Premaalatha, B., Adnan, M., Jamnah, O., Khor, S.K., Khadijah, S., Lai, S.Z., Shaik, M.A.B., Seah, T.C., Zatil, S.A. (2008). Seroprevalence of *Toxoplasma gondii* antibodies in pigs, goats, cattle, dogs and cats in peninsular Malaysia. *Tropical Biomedicine*, **25**: 257–258.
7. Chooi, K.F. (1989). Serological prevalence of toxoplasmosis in pigs. *Tropical Biomedicine* **6**:137-138.
8. Clementino, M.M., Souza, M.F., and Andrade Neto, V.F. (2007). Seroprevalence and *Toxoplasma gondii*-IgG activity in sheep from Lajez, Brazil. *Journal of Veterinary Parasitology* **146**:199-203.
9. Cook, A.J., Gilbert, R.E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P.A., Foulon, W., Semprini, A.E., Dunn, D.T. (2000). Sources of toxoplasma infection in pregnant women: European multicentre case-control study. *European Research Network on Congenital Toxoplasmosis. British Medical Journal*. **321**: 142–147.
10. Dubey, J.P. and Beattie, C.P. (1988). *Toxoplasmosis of animals and man*, 1st ed. (CRC Press, Boca Raton, FL).
11. Dubey, J. P. & Jones, J. L., (2008). *Toxoplasma gondii* infection in humans and animals in the United States. *International Journal for Parasitology* **38**: 1257–1278.
12. Garcia, J.L., Navarro, I.T., Ogawa, L. and Oliveira, R.C. (1993) *Seroprevalência do Toxoplasmas gondii, em Suínos, Bovinos, Ovinos e Equinos, e sua Correlação com Humanos, Felinos e Caninos, Oriundos de Propriedades Rurais do Norte do Paraná – Brasil, Ciência Rural* **29** : 91–97.
13. Garcia, J.L., Navarro, I.T., Vidotto, O., Gennari, S.M., Machado, R.Z., Pereira, A.B.L., Sinhorini, I.L. (2006). *Toxoplasma gondii*: comparison of a rhoptry- ELISA with IFAT and MAT for antibody detection in sera of experimentally infected pigs. *Experimental Parasitology* **113**:100–105.
14. Gilot-Fromont, E., Aubert, D., Belkilani, S., Hermitte, P., Gibout, O., Geers, R., Villena, I. (2009). Landscape, herd management and within-herd seroprevalence of *Toxoplasma gondii* in beef cattle herds from Champagne-Ardenne, France. *Veterinary Parasitology* **161**:36–40.
15. Hakim, S.L., Radzan, T & Nazma, M. (1994). Distribution of anti- *Toxoplasma gondii* antibodies among Orang Asli (Aborigines) in Peninsular Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health*. **25**:485-489.
16. Hove, T., Lind, P., and Mukaratirwa, S. (2005). Seroprevalence of *Toxoplasma gondii* infection in domestic pigs reared under different management systems in Zimbabwe. *Onderstepoort Journal of Veterinary research* **72**: 231-237
17. Klun, I., Djurkovic-Djakovic, O., Katic-Radivojevic, S., Nikolic, A. (2006). Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: seroprevalence and risk factors. *Veterinary Parasitology* **135**: 121–131.
18. Luuk, B., Schoonman, T., Wilsmore E, S. Swai. (2009) Seroepidemiological investigation of bovine toxoplasmosis in traditional and smallholder cattle production systems of Tanga Region, Tanzania. *Tropical Animal Health and Production* **4**: 579-587.
19. Moreno, T., Martinez-Gomez, F., Becerra, C. (1991). The seroprevalence of bovine toxoplasmosis in Cordoba, Spain. *Annals of Tropical Medicine And Parasitology* **85**:285–286.
20. Normaznah, Y., Saniah, K., Fuzina, N., Nseem, M. and Khatijah, M. (2004). Prevalence of antibodies to *Toxoplasma gondii* among farmers and cattle in Gombak District, Selangor, Malaysia-A preliminary report. *Tropical Biomedicine*. **21**:157-159.
21. Rajamanickam, C., Cheah, T.S. & Paramasvaran, S. (1990). Antibody to *Toxoplasma gondii* from domestic animals in Malaysia. *Tropical Animal Health Production* **22**:61-62.

22. Samra, N.A., McCrindle, C.M., Pezhorn, B.L., and cenci-Goga, B. (2007). Seroprevalence of toxoplasmosis in sheep in South Africa. *Journal of South Africa Veterinary Association* **78**: 116-120.
23. Sanad, M.M. and Al-Ghabban, A.J. (2007). Serological survey on toxoplasmosis among slaughtered sheep and goats in Tabouk, Saudia Arabia. *Journa of the Egyptian Society of Parasitology* **37**: 329-340.
24. Santos, T. R., Costa, A. J., Toniollo, G. H., Luvizotto, M. C., Benetti, A. H., Santos, R. R., Matta, D. H., Lopes, W. D., Oliveira, J. A. and Oliveira, G. P. (2009). Seroprevalence of anti-*Toxoplasma gondii* antibodies in dairy cattle, dogs, and human from the Jauru micro-region, Mato Grosso State, Brazil. *Journal of Veterinary Parasitology* **161**: 324–6.
25. Shaapan, R.M., El-Nawawi, F.A., and Tawfik, M.A. (2008). Sensivity and specificity of various serological tests for the detection of *Toxoplasma gondii* infection in naturally infected sheep. *Journal of veterinary Parasitology* **31**: 359-362
26. Singh, M., Zaman, B. & Chang, S. (1967). A survey on the prevalence of toxoplasmosis antibodies in animal sera. *Medical Journal of Malaysia* **22**:115-117.
27. Sinniah, B., Thomas, V. & Yap, P.L. (1984). Toxoplasmosis in West Malaysian population. *Tropical Biomedicine* **1**:81-83.
28. Sroka, J. (2001). Seroepidemiology of toxoplasmosis in the Lublin region. *Annals of Agricultural and Environmental Medicine* **8**: 25–31.
29. Tan, D.S.K. and Zaman, V. (1973). *Toxoplasma* antibody survey in West Malaysia. *Medical Journal of Malaysia* **27**: 188-191
30. Tenter, A.M., Heckerroth, A.R., Weiss, L.M. (2000). *Toxoplasma gondii*: from animals to humans. *International Journal for Parasitology*. **30**: 1217–1258.
31. Tenter, A. M. (2009). *Toxoplasma gondii* in animals used for human consumption. *Memórias do Instituto Oswaldo Cruz* **104**: 364–369.
32. Teshale, S., Dumetre, A., Darde, M.L., Merqa, B. and Dorchies, P. (2007). Serological survey of caprine toxoplasmosis in Ethiopia. *Journal of Parasitology* **14**: 155-159 USDA (2009). Parasites:<http://www.ars.usda.gov/Main/docs.htm?docid=11013>, accessed on 23/03/2009.
33. Zahedi, M., Indrus, H., Osman, S., Salleh, F. & Ghauth, I. (1985). Toxoplasmosis in Malaysia. Some epidemiological consideration. *Journal of Malaysian Society of Health* **5**:91-93.
34. Zamani, A., Malekmadani, M.H., and Daneshjou, K. (2007). *Toxoplasma* chorioretinitis in primary school children in Tehran, Iran 2003-2004. *Journal of Medical Science Monitor* **13**: 201-205.