

***Toxoplasma gondii* infection in native village chickens (*Gallus domesticus*) in Selangor and Melaka, Malaysia**

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Abstract. Toxoplasmosis is a worldwide zoonosis caused by the protozoa *Toxoplasma gondii* which affects human and animals. Village chickens (*Gallus domesticus*) most commonly known as *Ayam Kampung* or free-range chickens, have been suggested to play a role in the epidemiology of toxoplasmosis. This study determines the presence of *T. gondii* in the village chicken populations in two states of Malaysia. A total of 50 serum samples from the chickens from Selangor (n=20) and Melaka (n=30) were collected and analysed using commercial serological kits. *T. gondii* antigen was detected in 20% (Selangor 30%; Melaka 13%) samples using ELISA test and anti-*T. gondii* antibody was detected in all positive ELISA samples using the indirect haemagglutination test (IHAT). Histopathological examination revealed tissue changes such as inflammation and degeneration in brain and liver of seropositive chickens. This is the first report of *T. gondii* infection in the village chickens in Malaysia.

INTRODUCTION

Toxoplasmosis is a common infection in animals and humans caused by obligate intracellular protozoa *Toxoplasma gondii* (Blader and Saeij, 2009). It is a coccidian parasite with cats as the definitive host, and warm-blooded animals as intermediate hosts (Dubey, 2010). The life cycle of *T. gondii* includes asexual multiplication in the intermediate host and both sexual and asexual reproduction in the definitive host. Many species of warm-blooded animals can act as asymptomatic (showing no symptoms) intermediate hosts which may be able to carry tissue cysts of this parasite (Halonen and Weiss, 2013). Cats and wild felids are the only definitive hosts that may pass oocysts through their feces which will sporulate in the environment before becoming infective. All hosts, including humans, can be infected by three different life cycle stages of the parasite namely tachyzoites and bradyzoites contained in tissue cysts, and sporozoites

contained in sporulated oocysts. Seropositivity to *T. gondii* in humans had recently been associated with various mental disorders (Lovetta *et al.*, 2013; Markovitz *et al.*, 2015).

Native village chickens (*Gallus domesticus*) or *Ayam Kampung* have been considered as one of the best indicators of environmental contamination with *T. gondii* oocysts because of the chickens feeding habit and their resistance toward developing clinical signs (Liu *et al.*, 2017). The works of Dubey *et al.* (2003a; 2003b) reported that *T. gondii* was prevalent in free-range chickens in the United States and Brazil. Reports from India, Nigeria and China had also documented the presence of this protozoa among their free-range chickens (Devada *et al.*, 1998; Ayinmode and Dubey, 2012; Feng *et al.*, 2016). In Southeast Asia, serological evidence of toxoplasmosis had been reported in free-range chickens in Indonesia and several research including that from Malaysia found the organism

to be prevalent among cats in various countries in this region (Dubey *et al.*, 2008; Chandrawathani *et al.*, 2008; Dubey, 2010).

Chicken meat is consumed widely all over the world, and consumption of uncooked or improperly cooked chicken meat is a risk for *T. gondii* infection in humans and other animals (Dubey, 2010). The increasing demand for organically grown chickens may increase the prevalence of *T. gondii* in humans due to the improperly handled or cooked meat. Malaysian Agricultural Research and Development Institute (MARDI) (2012) reported that village chickens constitute 5% of the local poultry industry. The increasing demand for these chickens is due to the belief that their meat is healthier, tastier and leaner as compared to commercial broiler chicken meat.

No information is available on the occurrence of *T. gondii* in village chickens in Malaysia, therefore the risks of infection to humans is unknown. This study was carried out to determine the presence of *T. gondii* infection among village chickens in the state of Selangor and Melaka in Peninsular Malaysia and to identify the tissue changes associated with *T. gondii* in infected chickens.

MATERIALS AND METHODS

Samples collection

Fifty village chickens of market age (≥ 2 months) were purchased from various locations in two states of Malaysia – Melaka and Selangor. Thirty chickens were purchased from six different farms in Melaka while another 20 chickens were purchased from four different farms in Selangor. The chickens were handled and slaughtered by the farmers and carcasses were purchased by the researcher. All chickens appeared healthy at the time of slaughter.

Serology

Blood samples were collected during slaughter using plain blood tube (BD Vacutainer[®], USA). The blood was allowed to clot overnight at 4°C then centrifuged for 15 minutes at 1000rpm to separate the

serum from whole blood. Serum was collected and stored at -20°C for enzyme-linked immunosorbent assay (ELISA) and indirect haemagglutination test (IHAT) analysis.

Fifty serum samples were analysed using a commercial ELISA kit. The chicken toxoplasma circulating antigen (TCA) ELISA commercial kit (CUSABIO[®], USA) was used for detection of *T. gondii* antigen. The procedure was performed following the protocol provided by the manufacturer. Briefly, serum samples were pipetted into the wells containing horseradish peroxidase (HRP) conjugated antibody. Following a wash to remove any unbound reagent, a substrate solution was added to the wells. Colour will develop in proportion to the amount of chicken TCA bounded. The colour development was stopped and intensity of the color was measured at 405 nm using ELISA microplate reader (Sunrise Tecan, Switzerland).

Ten positive ELISA serum samples, and 10 negative ELISA serum samples (randomly chosen from 40 negative ELISA serum samples) were re-analysed using an IHAT kit. The ELI.H.A Toxo commercial kit (ELITech[®], USA) was used for quantitative determination of anti-*T. gondii* serum antibodies by indirect haemagglutination. The sensitised red blood cells consisting sheep red blood cells covered with *Toxoplasma* antigen were provided in the kit. The presence of serum antibodies against *T. gondii* causes agglutination of the sensitised red blood cells resulting in a cloudy red or brown deposit coating the well. In the absence of specific antibodies, the red blood cells form a ring-like deposit at the bottom of the well.

Histopathology

Brain and liver samples were removed from the slaughtered chickens and fixed in 10% neutral buffered formalin. Then the seropositive samples were processed, sectioned and stained with haematoxylin and eosin (H&E). The H&E stain was used to examine overall condition on tissue sections such as inflammation and degeneration of tissues and cells (Fitzgerald, 2011).

Data analysis

The prevalence of toxoplasmosis was calculated as the number of samples positive for the *T. gondii* antigen from all those tested. Confidence interval (CI) of 95% was calculated using IBM SPSS Statistics Version 23.

RESULTS

Ten out of 50 serum samples were positive for *T. gondii* antigen by ELISA. The overall prevalence of *T. gondii* in the village chickens was 20% (10/20; 95% CI: 10.03% – 33.72%). The prevalence in Selangor was 30% (6/20; 95% CI: 9.90% – 50.10%) and Melaka was 13% (4/30; 95% CI: 1.20% – 25.50%). All positive ELISA samples were also seropositive using IHAT. The positive titers observed were 1:320 (n=4), 1:640 (n=5) and 1:1280 (n=1) as shown in Table 1, which indicate active infection (as specified in the kit). The most common tissue changes observed in brain and liver in seropositive chickens were inflammation (Figures 1 and 2) and degeneration (Figure 3).

DISCUSSION

In this study, ELISA and IHAT were used to determine *Toxoplasma gondii* infection in chickens. The ELISA kit gave qualitative results (positive or negative) indicating whether the *T. gondii* antigen is present in a sample. ELISA is a sensitive test and is a recommended method by researchers (Casartelli-Alves *et al.*, 2014) and has been used in previous studies to determine the prevalence of *T. gondii* in chickens (Dubey, 2010; Fitzgerald, 2011). In addition, IHAT is a specific method (Casartelli-Alves *et al.*, 2014) and most commonly used for research as well as for serological diagnosis and determination of prevalence of anti-*T. gondii*

Table 1. Indirect haemagglutination test (IHAT) antibodies to the *Toxoplasma gondii* in native village chickens

Total samples	Positive	Antibody titre		
		1:320	1:640	1:1280
20	10	4	5	1

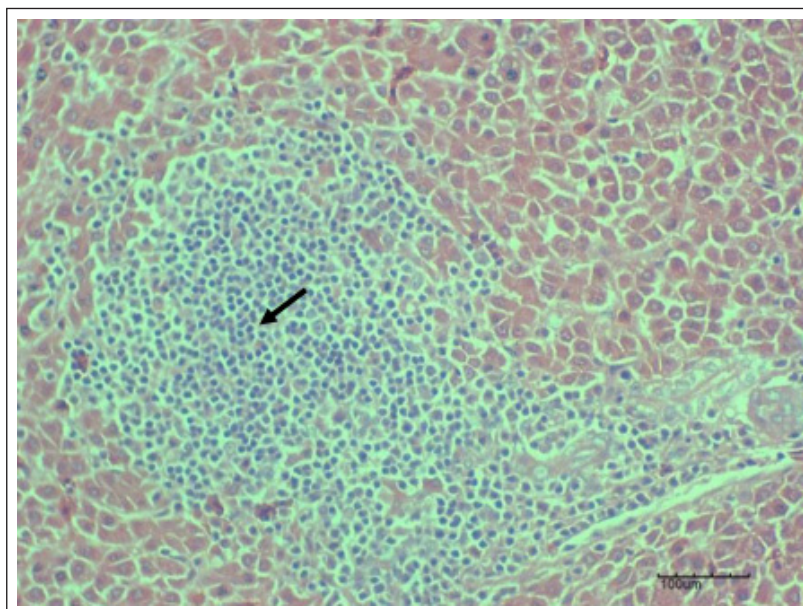


Figure 1. Inflammatory cells infiltration in the liver, stained by H&E.

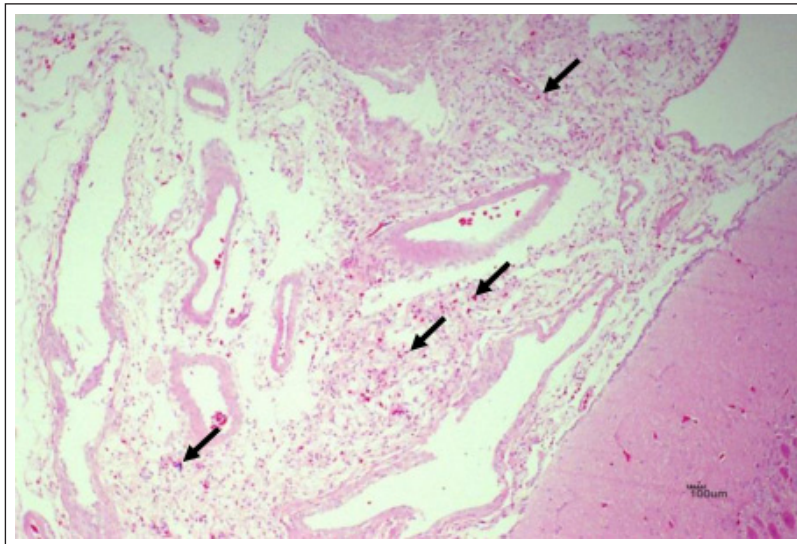


Figure 2. Inflammatory cells infiltration in meninges, stained by H&E.

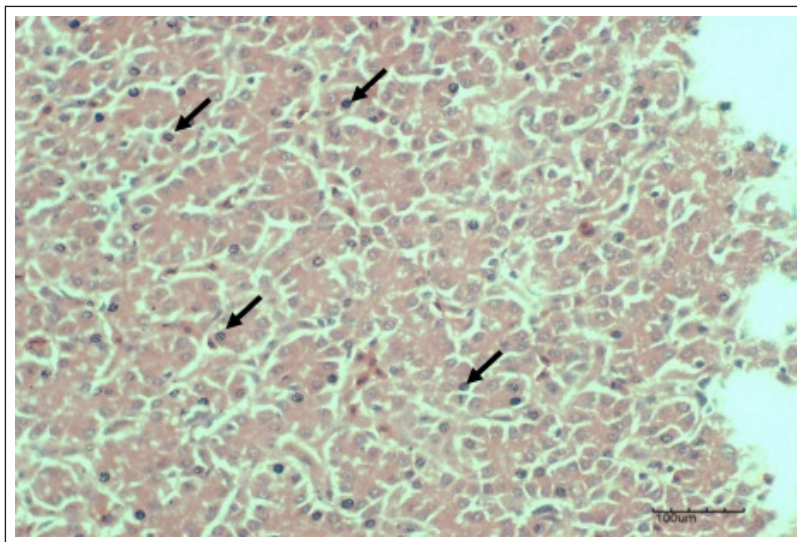


Figure 3. Hepatocyte degeneration in the liver, stained by H&E.

antibody in chickens (Dubey, 2010). We found high prevalence (20.00%) of *Toxoplasma* antigen in the sera of sampled village chickens using the ELISA kit. The finding is similar to that found in Indonesia (26.60%), Peru (26.00%), Portugal (27.10%) and Mumbai (17.90%) but higher than those reported in Kenya (13.30%) and Mexico (6.20%). The finding was expected given the prevalence of infection among Malaysian local cats and the abundance of stray cats in Malaysia. Chandrawathani *et al.* (2008)

found 14.50% of local cats were infected with *T. gondii*. Chickens may become infected during feeding on the ground contaminated with oocysts (Zia-Ali *et al.*, 2005) from infected cat faeces. Oocysts survive in soil and the environment from months to years (Hill and Dubey, 2002), therefore chickens were likely to get infected from exposure to contaminated soil.

Histopathological examination revealed that the most common tissue changes were inflammation and degeneration. Inflamma-

tion was observed in many types of infection including toxoplasmosis, while degeneration was associated with the disease chronicity (Hunter, 2012). The detection of tissue cyst or tachyzoite using H&E was difficult however it may be present in various tissues. Liver is usually consumed undercooked or half-cooked by some population; therefore, tissue cysts of the parasite may survive, hence posing a serious public health risk to humans especially those immunocompromised or pregnant (Mohammed and Abdullah, 2013). The organotropism of tissue cysts varies in different intermediate host species. In many hosts such as pigs, sheep and goats, tissue cysts have a high affinity for neural and muscular tissues. In humans, cysts are located predominantly in the central nervous system, the eye as well as skeletal and cardiac muscles (Tenter *et al.*, 2000). However, to a lesser extent they may also be found in visceral organs, such as lungs, liver, and kidneys (Dubey, 2010). It is difficult to explain the tolerance of chickens towards to *T. gondii* infection even though they seemingly do suffer the pathological changes related to the infection. There were only a few reports of clinical toxoplasmosis in chickens worldwide (Dubey, 2010). Wang *et al.* (2015) reported that, the infection dose was an important factor to the pathogenicity of the parasite. Studies have showed that infection with more than 1×10^7 tachyzoites resulted in death and produced clinical signs as well as pathological changes of the organs in chickens. However chickens were not clinically affected when infected with 1×10^6 tachyzoites (Wang *et al.*, 2015).

CONCLUSION

Toxoplasma gondii infection is detected in 20% of native village chickens from the states of Selangor and Melaka. This study suggests the potential risk of native village chickens as a source of *T. gondii* infection in humans and other animals and need to be further studied. This study did not collect any information on the management of the farms which can elucidate factors that influences the occurrence of the organism. Native

village chickens may play a potential role in the transmission of toxoplasmosis and can be sentinel for monitoring environmental contamination with *T. gondii* oocysts. With the increasing popularity of free-range chickens, this risk needs to be acknowledged and addressed appropriately.

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Conflict of interest

All authors report no conflict of interest relevant to this article.

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