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SEROPREVALENCE OF TOXOPLASMA GONDII AMONG PET DOGS IN THREE VETERINARY HOSPITALS LOCATED IN KLANG VALLEY, SELANGOR, MALAYSIA

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It is hereby certified that we have read this project paper entitled "Seroprevalence of *Toxoplasma gondii* Among Pet Dogs in Three Veterinary Hospitals located in Klang Valley, Selangor, Malaysia", by Siti Amila Hureen binti Azman and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD4999 – Final Year Project.

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DEDICATION

This project paper is dedicated to:

All lecturers, staff and students of FPV.

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LIST OF ABBREVIATIONS

%	Percentage
ELISA	Enzyme-linked immunosorbent assay
MAT	Microscopic agglutination test
IFAT	Indirect immunofluorescent antibody test
CDC	Centers for Disease Control
OD	Optical Density
S/P %	Sample to positive ratio percentage
CDV	Canine Distemper Virus
MUE	Meningioencephalomyelitis of unknown etiology

ABSTRAK

Abstrak daripada kertas projek ini yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901- Projek.

SEROPREVALENSI *TOXOPLASMA GONDII* DI KALANGAN ANJING PELIHARAAN DI TIGA BUAH HOSPITAL VETERINAR YANG TERLETAK DI LEMBAH KLANG, SELANGOR, MALAYSIA

Oleh

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2021

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Penyakit toxoplasmosis adalah disebabkan oleh *Toxoplasma gondii* (*T. gondii*) merupakan penyakit zoonotik yang mampu menyebabkan penyakit pada haiwan berdarah panas dan tidak terkecuali juga manusia, haiwan ternakan dan mamalia marin. Toxoplasmosis kanin adalah penyakit opurtunistik yang menyebabkan masalah otot dan saraf, respirasi dan gastro-usus atau jangkitan menyeluruh. Pelbagai penyakit toxoplasmosis yang melibatkan anjing telah dilaporkan di seluruh dunia. Walaupun begitu, status prevalensi *T. gondii* terhadap anjing peliharaan di Malaysia sangat terhad.Oleh itu, kajian ini dijalankan untuk mengetahui status seroprevalensi *T. gondii* terhadap anjing peliharaan dan faktor risiko yang terlibat. Sebuah kajian keratan rentas telah dibuat melibatkan tiga buah hospital veterinar yang terletak di Kawasan Lembah Klang, Selangor, Malaysia. Sebanyak 43 sampel era dan data bagi setiap pesakit anjing

telah dikumpulkan. Kesemua sera tersebut diuji dengan kit ujian “Indirect ELISA Test Kit (ID Screen® Toxoplasmosis Multi-species)”. Seroprevalensi keseluruhan *T. gondii* terhadap anjing peliharaan adalah sebanyak 19% (8/43). Anjing peliharaan yang dibela dan diletakkan di luar rumah (40%) mempunyai status seroprevalensi lagi tinggi dari anjing peliharaan yang dibela di dalam rumah (12%). Tiada lagi perbezaan yang signifikan yang terlibat dengan faktor risiko seperti jantina, jenis baka, umur, diet atau kehadiran penyakit saraf. Status seroprevalensi *T. gondii* yang tinggi terhadap anjing peliharaan yang telah ditemukan melalui kajian ini menyatakan bahawa *T. gondii* mampu memainkan peranan dalam menyebabkan penyakit kepada anjing dan menjadi indikator “sentinel” terhadap tahap pencemaran persekitaran oleh oosit *T. gondii*.

Kata kunci: toxoplasmosis, *Toxoplasma gondii*, seroprevalensi, anjing peliharaan, ELISA.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901- Project.

SEROPREVALENCE OF *TOXOPLASMA GONDII* AMONG PET DOGS IN THREE VETERINARY HOSPITALS LOCATED IN KLANG VALLEY, SELANGOR, MALAYSIA

By

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2021

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Toxoplasmosis caused by *Toxoplasma gondii* (*T. gondii*) is a zoonotic disease capable of infecting all warm-blooded animals including humans, livestock and marine mammals. Canine toxoplasmosis is an opportunistic disease which could elicit neuromuscular, respiratory, gastrointestinal or generalized infection. *Toxoplasma gondii* infection in dogs have been reported worldwide. However, little is known about the prevalence of *T. gondii* in pet dogs in Malaysia. Thus, this study was conducted to determine the seroprevalence of *T. gondii* in pet dogs and the risk factors involved. A cross-sectional study was conducted involving three veterinary hospitals located in Klang Valley, Selangor, Malaysia. A total of 43 sera sample and data on each dog patient were collected. Sera sample were subjected to a commercially available Indirect Elisa test kit (ID Screen® Toxoplasmosis Multi-species). The overall

seroprevalence of *T. gondii* in pet dogs was 19% (8/43). Dogs kept outdoor (40%) had significantly higher seropositive results, compared to those that were kept indoors (12%). No other significant difference was observed in relation to other risk factors such as gender, breed, age, diet or presence of neurological signs. The high seroprevalence of *T. gondii* found among pet dogs in the current study indicated that *T. gondii* could play a role in causing diseases in dogs as well as a sentinel indicator to the level of environment contamination with *T. gondii* oocysts.

Keywords: toxoplasmosis, *Toxoplasma gondii*, seroprevalence, pet dogs, ELISA.

1.0 INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by a protozoan parasite called *Toxoplasma gondii* (*T. gondii*) capable of infecting warm-blooded animals including humans, livestock and marine mammals (Esch, 2010). *T. gondii* was first discovered and described in the spleen and bone marrow of a rodent from North Africa, by Nicolle and Manceaux in 1908 (Ferguson, 2009). This protozoan parasite eventually known as the agent of a widespread zoonosis. Cats (Only cats?) being the definitive host, shed oocysts through their faeces while dogs and other warm blooded animals are intermediate host for *T. gondii* (Dubey, 2004, 2005; CDC, 2017). Intermediate hosts are able to harbour tissue cyst of *T. gondii* (Dubey, 2005).

According to Dubey (2005), there are three infective stages; sporozoites (oocysts), tachyzoites and bradyzoites (tissue cysts). In the intermediate host, upon ingestion of sporulated oocyst, sporozoites will be released and transform into tachyzoites that will be in circulatory and later developed into bradyzoites in tissue cyst mainly embedded in neural and muscle tissues. Zoonotic transmission occurs via consuming meat with tissue cyst or ingestion of feed contaminated with cat faeces. Various methods such as via biologic, serology and molecular or combination have been used to diagnose *T. gondii*. Clinical signs such as neurological signs may associated with toxoplasmosis, however it is not definite as it may appear to other infectious diseases (Dubey, 2005; D. Hill & Dubey, 2002).

Canine toxoplasmosis is an opportunistic disease where infection occurs more often in those dogs with weakened immune system and unvaccinated towards canine

distemper virus (CDV) (Dubey, 2010; Calero-Bernal and Gennari, 2019) in which neuromuscular, respiratory and gastrointestinal signs or generalized infection able to be observed (Da Silva et al., 2005).

Neurological signs in dogs suggest differential diagnosis of canine toxoplasmosis or neosporosis (Mineo et al., 2001) include canine distemper virus , meningioencephalomyelitis of unknown etiology (MUE) and blastomycosis (Levitin et al., 2020). In one study, 74% of confirmed canine toxoplasmosis cases exhibit central nervous system lesion attributed to toxoplasmosis (Kostner & Cole, 1960).

Chandrawathani et al. (2008) reported a seroprevalence of 9.6% towards *T. gondii* among dogs in Peninsular Malaysia. To date, no other studies have been conducted on seroprevalence of *T. gondii* among pet dogs in Malaysia. Furthermore, various recent studies on prevalence of *T. gondii* in dogs have been conducted worldwide. Besides, as mentioned above, the involvement of *T. gondii* in causing disease in dogs that commonly underdiagnosed in Malaysia. Therefore, this study aimed to determine the seroprevalence of *T. gondii* in pet dogs in three participated three veterinary hospitals and to determine the associated risk factors involved.

2.0 LITERATURE REVIEW

2.1 Introduction

According to the Centers for Disease Control (CDC, 2019), toxoplasmosis is an infection caused by a single-celled parasite called *Toxoplasma gondii*. *T. gondii* is an obligate intracellular parasite capable of infecting warm-blooded animals such, as bird and mammals including humans (Robert-Gangneux & Dardé, 2012). Toxoplasmosis is considered endemic worldwide as it is capable of causing life-long chronic infections in all intermediate warm-blooded vertebrae hosts and produces infectious tissue cyst (Grigg & Sundar, 2009). The definitive felid host sheds highly infectious oocysts in its faeces, and transmission to all animals can occur by ingestion of food and water contaminated with oocysts. Dogs and other warm blooded animals are intermediate host for *T. gondii* where they harbour the tissue cyst. Dogs are also capable to play a role in mechanical transmission of the oocyst stage of *T. gondii*. Pet dogs where they are present in the household is attributed as a risk factor for *T. gondii* infection in humans. They may contribute to transmission by spreading oocysts in the environment via shedding after ingestion of oocysts without the replication of the parasite in the intestine, which only occurs in cats. Besides, dogs are able to physically disperse oocysts due to eating and rolling habits on cat faeces , thus contaminating their fur (Frenkel & Parker, 1996). Petting contaminated dog fur with cat faeces that may contain the oocyst, enable the transmission towards human. Thus, dogs are considered as a good indicator of environmental contamination (Da Silva et al., 2010).

2.2 Characteristics of *T. gondii*

T. gondii is an obligate intracellular coccidian parasite. There are 3 infective stages, sporozoites (oocysts), tachyzoites and bradyzoites (tissue cysts). Each oocyst contains two ellipsoidal sporocysts while each sporocyst contains four sporozoites (Dubey et al., 1998). The sporozoite is protected inside the oocyst while tachyzoite is the rapidly dividing stage and bradyzoite is slowly dividing in the cyst. In the intermediate host, upon ingestion of sporulated oocyst, sporozoites will be released and transform into tachyzoites that will be in circulatory and later developed into bradyzoites in tissue cyst mainly in neural and muscle tissues. According to Robert-Gangneux & Dardé (2012), these infective stages are crescent-shaped cells, approximately 5µ long and 2µ wide, with a pointed apical end and a rounded posterior end. According to Howe and Sibley (1995), *T. gondii* can be classified into 3 genotype which are Type I, II and III.

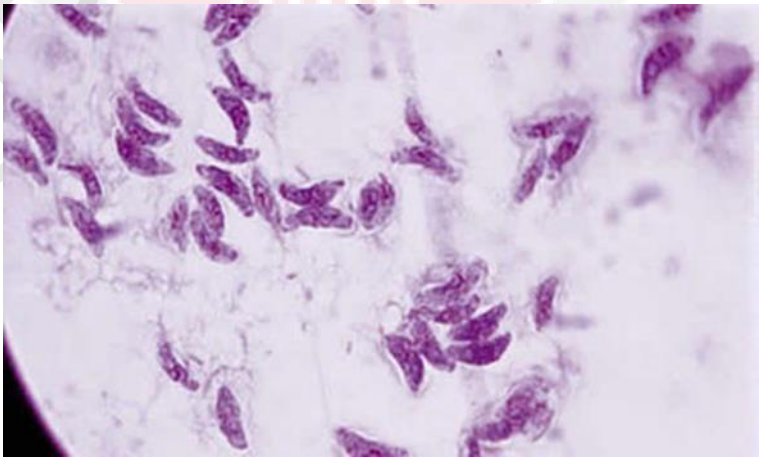

Figure 1: Tachyzoites of *toxoplasma gondii* (Giemsa, x100) (Khalid, 2016)

2.3 Epidemiology and Life cycle of *T. gondii*

Hot and humid climate favours by *T. gondii* (CDC, 2017). This is because of the moist environment enabled the sporulated oocyst to survive for months to years (Dubey and Lindsay, 2006).

Felines are the only definitive hosts and sexual parasitic reproduction happens exclusively in this animal group, resulting in contamination of the environment after excretion of infective *T. gondii* oocysts in feline faeces (Hutchison et al., 1969). *T. gondii* is sexually reproduce in the definitive host while muscle and tissue encystment occur in intermediate hosts such as birds and rodents. Amount of the oocysts that the cat are able to shed is abundant (Dubey & Lindsay, 2006). These oocyst are resistant to disinfecting agent (Dubey, 2004).

Definitive host sheds unsporulated oocyst for 1 to 3 weeks. About 1 to 5 days, the oocyst will sporulated in the environment and become infective. Water, soil or plant material contaminated with oocyst, once ingested by the intermediate host, they will become infected. Upon ingestion of sporulated oocyst, sporozoites will be released and transform into tachyzoites that will be in circulatory and later developed into bradyzoites in tissue cyst mainly in neural and muscle tissues. Thus, this intermediate host will be harbouring tissue cyst. Cat become infected by consuming this intermediate host or directly ingesting the sporulated oocyst.

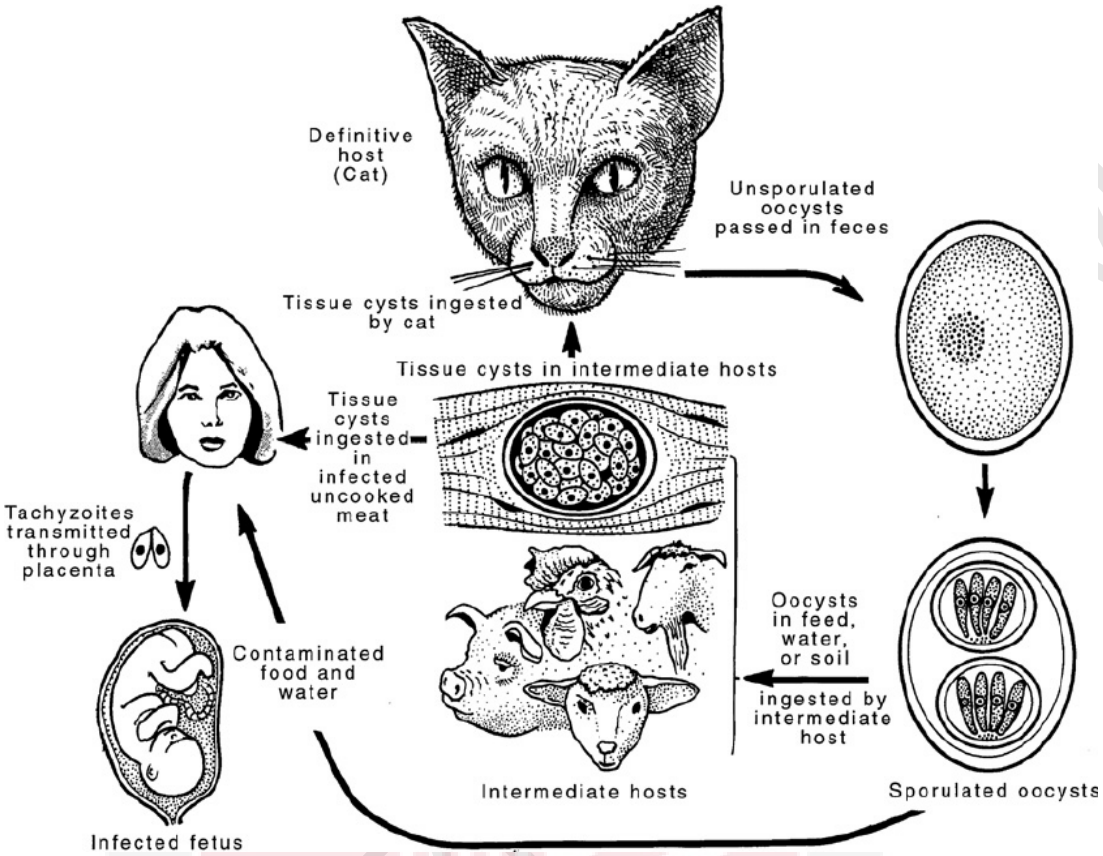

Figure 2: Life cycle of *T. gondii* (Dubey, 2004)

According to Centers for Disease Control (CDC, 2018), below are the possible ways of zoonotic transmission. Consumption raw animal meat that encysts with tissue cyst, consume food and drink contaminated with cat faeces, or vertical transmission (from mother to foetus) or during organ transplantation and blood transfusion. *T. gondii* tissue cyst in human are mostly found in skeletal muscle, myocardium, brain and eyes and prolong remained in immunocompetent individual where it stays unreactive.

2.4 Toxoplasmosis in Dog

Dog served as intermediate host or reservoir for *T. gondii*. Canine toxoplasmosis is an opportunistic disease where the infection occurs more often in

those with weakened immune system. Canine toxoplasmosis as a primary disease is uncommon. According to Calero-Berna & Gennari (2019) , immunosuppressed and unvaccinated dogs towards canine distemper virus are often related to the case of canine toxoplasmosis. Besides, canine toxoplasmosis elicit neuromuscular, respiratory and gastrointestinal signs or generalized infection (A. V. Da Silva et al., 2005). Greene (1998) characterized the neurological signs by ataxia, circling, behavioural changes, seizures, twitching and tremors.

2.5 Prevalence rate of canine toxoplasmosis

The seroprevalence of canine toxoplasmosis is quite low in Malaysia and varied if compared to worldwide prevalence. Chandrawathani et al. (2008) reported a seroprevalence of 9.6% towards *T. gondii* among dogs in Peninsular Malaysia. In neighbouring country, Thailand, Jittapalapong et al., (2009) reported a seroprevalence of 10.9% in the stray dogs. Table 1 show the recent prevalence of *T. gondii* in dogs worldwide and in Malaysia.

Table 1: Prevalence of *T. gondii* antibodies in dogs worldwide

Country	Type	% positive	Test & Titer	Reference
Argentina	Pets	55.3 (84/152)	IFA 1:100	(Enriquez et al., 2019)
Brazil	Pets	35.9 (46/128)	IFA 1:16	(Paz et al., 2019)
Brazil	Pets	7.9 (21/264)	IFA 1:16	(Cunha et al., 2020)
China	Pets	9.1 (37/408)	ELISA	(Jiang et al., 2016)
Iran	Pets	46.6 (84/180)	ELISA	(Zarra-Nezhad et al., 2017)
Thailand	Stray	10.9 (25/230)	LAT 1:64	(Jittapalapong et al., 2007)
Spain	Pets, clinics	30.6 (235/769)	MAT 1:25	(Cano-Terriza et al., 2016)
Malaysia	Pets, clinics	9.6 (13/135)	IFAT 1:16	(Chandrawathani et al., 2008)

2.6 Identification of the agent

Identification of *T. gondii* can be done by serologically or histology examination of the tissue. These two are helpful to diagnose toxoplasmosis and not just depending on the clinical signs (Hill & Dubey, 2002). Finding *T. gondii* in tissue cross section is difficult but easier to find in inflamed and necrotized brain or placenta tissue. According to Dubey and Lindsay (2006), microscopic examination of impression smears of the lesions on the tissue sample of the host obtained by performing biopsy or during necropsy is considered as rapid diagnosis. This can be done by performing impression smear on the lesions, dry for 30 minutes, fix with methyl alcohol and stain well the smear with Romanowsky stain and finish with Giemsa stain. Crescent shaped of *T. gondii* is able to observe under the microscope.

2.7 Serological test

According to Dubey (1996), Sabin-Feldman dye test is a gold standard method in detecting *T. gondii* antibodies. *T. gondii* live virulent tachyzoites are used as antigen, complement-like 'accessory factor' and test serum. The dye which is methylene blue dye is prevent the entering the cytoplasm of *Toxoplasma*. Whenever a specific antibody of *T. gondii* act on the tachyzoites, the tachyzoites do not stain uniformly with alkaline methylene blue and appear colourless resulting a positive test while tested negative if blue colour appears. However, past or recent infection could not be differentiated. In addition, the procedure is too technical to carry out in diagnostic laboratories (Dubey and Lappin, 2006).

Modified agglutination test (MAT) able to compare acute and chronic toxoplasmosis in human and cat sensitively and specifically. Moreover, this test

detects only IgG. Formalized killed whole tachyzoite antigen was prepared according to the method described by Desmonts & Remington (1980), and the procedures were carried out according to method proposed by Dubey and Desmonts (1987) at 1:25 dilution.

Indirect immunofluorescent antibody test (IFAT) is commercially used where a whole tachyzoite antigen is used and proceed with proposed by Goldman and Sc (1957).

ELISA method able to measure the amount of IgG antibodies in the serum. This method comprised indirect of and conventional methods. Antigen-antibody complex is recognized by indirect method, while conventional method able to differentiate an acute or chronic infection.

Polymerase chain reaction (PCR) technique able to identify *T. gondii* DNA. Biological sample such as serum, blood sample, cerebral spine fluid can be used. PCR is highly sensitive tool where the several strains of tachyzoites grown in cell culture were added to the biological sample. Then, the protocols for identifying *T. gondii* DNA by use of PCR were developed (Stiles et al., 1996). For early detection of *T. gondii*, nested PCR is very helpful especially in those asymptomatically patient (Lee et al., 2008).

2.8 Treatment, prevention and control

The most common drugs used to treat *T. gondii* infection are combination of clindamycin, trimethoprim and sulfa drugs (Rodrigues Hoffmann et al., 2012). Ponazuril and azithromycin were also included (Dubey et al., 2009).

Meat handling is crucial prior to human or animal consumption. Tissue cyst in the meat can be killed with extreme low and high temperature. The tissue cyst is unable to survive at 67 °C or more for 3.6 minutes (Dubey et al., 1990) or -12.37 °C for 11.2 days (Kotula et al. 1990). Another way is to treat the meat with high pressure processing (HPP). According to Lindsay et al., (2005 & 2006), pressure at 550 MPa or more rendered the tissue cyst non-viable. Moreover, salting the meat able to inactivate the tissue cyst in the meat (D. E. Hill et al., 2004).

Besides, practice self-hygiene can prevent *T. gondii* infection in human. Wash hands after handling cat litters and after contact with dogs (Frenkel & Parker, 1996). According to Dubey and Lindsay (2006), avoid contact with cats, cat litter, soil and raw meat especially for pregnant lady. Fed domestic cats with cooked, canned and dry food and daily emptied the cat litter box.

Furthermore, pet owners are advised to restrict their pet's movement from gaining access towards intermediate hosts or mechanical vectors that usually can be found in resident area. This is to refrain the pet from eating it and have contact with it due to the hunting behaviour (Dubey JP., 2005).

To date, there is no vaccine developed towards canine toxoplasmosis including human toxoplasmosis. However, a commercial attenuated vaccine for sheep was developed by Wilkins and O'Connel in 1983.

2.9 Toxoplasmosis in human in Malaysia

Research on human seroprevalence of *T. gondii* has been conducted in Malaysia, focused in pregnant women, healthy patients, patients with disorder ,

migrant workers and patient with schizophrenia (Wana et al., 2020). Seroprevalence of 35.2% in pregnant women (Emelia et al., 2014), 44.2% in patients that showing clinicals signs suspected with toxoplasmosis (Mohamed & Hajissa, 2016), 34.1% and 57.4% in migrant workers (Chan et al., 2008; Sahimin et al., 2017), 59.7% among Pangkor island people (A. F. Ahmad et al., 2014), 51% and 51.5% in schizophrenic patients (Juanah et al., 2013; Omar et al., 2015).

According to Hosseini et al., (2019), the clinical signs of toxoplasmosis such as fever, malaise and lymphadenopathy in an immunocompromised individuals are usually mild and self-limiting. In addition, Tenter et al., (2000) stated that severe infection are seen in immunocompromised and pregnant women associated with complications of encephalitis, retinochoroiditis, foetus abortion, splenomegaly and pneumonitis.

3.0 MATERIAL AND METHOD

3.1 Study design, area and population

A cross sectional study was carried out in three veterinary hospitals. The study was conducted in three veterinary hospitals in Klang Valley over the period of 2 weeks. The required sample size was calculated using Epi Tools website (http://epitools.ausvet.com.au) based on the expected prevalence of 9.6 % from previous study conducted in Malaysia by Chandrawathani et al. at a 95% confidence level (CI), 5% precision and a target population of 118 clinics veterinary clinics in Selangor. A total of 139 sample size was calculated. Pet dogs were selected randomly for this study. The animal identification, history, age, sex, breed, dietary habit and management of the participated pet dogs were recorded.

3.3 Sample collection and storage

Selected dogs were physically restraint and 2ml of blood in plain tube was withdrawn from the cephalic vein by the veterinarians in the participating hospitals. Blood in plain tube was left aside for about 10 to 15 minutes to allow for it to clot. Once blood has clotted, it was centrifuged at 5500 rpm for 5 minutes. The resulting serum was then aliquoted into microcentrifuge tube and labeled. The serum was stored at -20°C until further analysis. Data of the participated pet dogs such as history, age, sex, breed, dietary habit of each dog, clinical signs and other diagnostic work out was recorded.

3.4 ELISA

All sera sample were tested using a commercially available indirect ELISA test kit (ID Screen ® Toxoplasmosis Indirect Multi-species, IDvet, France). The microwells were coated with the P30 antigen of Toxoplasma gondii. 10µl of the sample to be tested and controls were added to the wells. After three times washing 300µl with wash solution, a 100µl of multi-species peroxidase (Po) conjugate was added to the wells. After elimination of the excess conjugate by washing, 100µl of the substrate solution (TMB) is added. In the presence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution. The strip plates were read at 450nm on the ELISA reader (Tecan Infinite M 200 PRO). The results obtain is the optical density (OD) value. Based on the OD value, the S/P % is calculated as follow: S/P % = [(OD $_{sample}$ – OD $_{NC}$) / (OD_{PC} – OD_{NC})] x 100. Prior calculating the S/P%, a validity test WAS done. Mean value of the positive control OD value (OD_{PC}) must be greater than 0.350 and the ratio of the mean OD value of positive and negative controls (OD_{PC}/OD_{NC}) greater than 3 are considered a valid test. Any sample with S/P% less than or equal to 40% are considered negative, between 40% to 50% are considered doubtful and greater than or equal to 50% are considered positive. The kit specificity and sensitivity were both 100% as reported by the manufacturer.

3.5 Data analysis

The result and data are recorded in a Microsoft Excel spreadsheet (Microsoft Corporation) and analysed using IBM SPSS Statistics 25. Chi-Square test was used and the level of significance set at p-value < 0.05. Seroprevalence of *T. gondii* was

calculated by dividing the total number of dogs tested positive by the total number of samples.

3.6 Ethical issues

All the ethical guidelines for using animals for scientific purpose were followed and the study was granted permission by the Institutional Animal Care and Use (IACUC) UPM.

4.0 RESULTS

4.1 Seroprevalence of *T. gondii* in pet dogs

A total of 43 sera sample were able to be collected from the three participating clinics consisted of a total of 27 pet dogs from veterinary hospital A, 12 pet dogs from veterinary hospital B and 4 pet dogs from veterinary hospital C. The sera sample was collected in the span of two weeks' time by the veterinarians involved in respective hospitals and were stored at -20°C until further analysis. These sera sample were stored in an ice box and transported back to the laboratory for testing. Of the 43 sera examined, 19% were tested positive for *T. gondii*.

Table 2 compares seroprevalence of *T. gondii* antibodies in pet dogs in three veterinary hospitals. Based on table 2, only 5 (18.52%) sera collected in veterinary hospital A were tested positive for *T. gondii*. Veterinary hospital B recorded 3 (25%) positive result while none was recorded from veterinary hospital C. There were no significant association of *T. gondii* between these three veterinary hospitals (χ^2 =1.239, p=0.538).

4.2 Seroprevalence of *T. gondii* in pet dogs based on risk factors

Table 3 illustrates the comparison of seroprevalence of *T. gondii* antibodies between male and female pet dogs. From the collected sample, a higher proportion of the sera sample from the three veterinary hospitals were from male (65%;28/43) as compared to female (35%;15/43) pet dogs. Based on the Table 3, 6 of 28 (21.43%) male dogs were tested positive for *T. gondii* antibodies and 2 of 15 (13.33%) female dogs were tested positive for *T. gondii* antibodies. There were no significance

association of *T. gondii* in pet dogs between sexes (χ^2 =0.423, p=0.516, OR=1.773, 95% CI= 0.311-10.110).

Table 3 illustrates the comparison of *T. gondii* positive cases in relation to the breeds of dogs. 21 local and 22 pedigree dogs involved in this study. From this study, 3 of 21 (14.3%) local breed dogs were detected positive for *T. gondii* antibodies while 5 of 22 (22.7%) pedigree dogs were positive for the *T. gondii* antibodies. It was also found that there were no significance association of *T. gondii* between the breed of dogs (χ^2 =0.506, p=0.477, OR=0.567, 95% CI= 0.117-2.744).

Table 3 illustrates the comparison of seroprevalence of *T. gondii* antibodies in relation to age of dog. Classified into two groups, age of six years old and less and seven to 12 years old. 18 dogs aged from six years old and less and 25 dogs aged between seven to 12 years old participated in this study. From the Table 3, none was positive for *T. gondii* antibodies. For the age group between six years old and less, four of 18 (22.2%) dogs were tested positive for *T. gondii* antibodies. Age group between 7 to 12 years old were found that 4 of 25 (16%) positive for *T. gondii* antibodies. It was also found that there were no significance association of *T. gondii* between the dog age (χ^2 =0.268, p=0.605, OR=1.5, 95% CI=0.321-7.012).

Table 3 illustrates comparison of seroprevalence of *T. gondii* antibodies in relation to management of dogs. Four of 33 (12.1%) dogs managed indoor showed positive for *T. gondii* antibodies while only four of 10 (40%) dogs managed outdoor tested positive for *T. gondii* antibodies. There were a significance association of *T. gondii* in relation to management of dogs (χ^2 =3.939, p=0.047, OR=0.207, 95% CI= 0.04-1.068).

Table 3 illustrates comparison of seroprevalence of *T. gondii* antibodies in relation to feeding of dogs. There were nine dogs fed with homecooked meal such as rice, chicken and meat. 34 feds with commercial feed such as kibble and canned food. Based on the Table 3, eight of 34 (23.5%) dogs fed with commercial feed were positive for *T. gondii* antibodies. None of the nine dogs fed with homecooked meal tested positive for *T. gondii* antibodies. It was also found that there were no significance association of *T. gondii* in relation to feeding of the dogs (χ^2 =2.602, p=0.107).

In this study, the 43 sera sample collected were comprised of nine neurological patients and 34 non-neurological patients. Table 3 illustrates comparison of seroprevalence of *T. gondii* antibodies in relation to neurological status of dogs. Three of nine (33.33%) neurological patients and five of 34 (14.70%) non-neurological patients were tested positive for *T. gondii* antibodies. It was also found that there were no significance association of *T. gondii* between neurological and non-neurological dogs (χ^2 =1.631, p=0.202, OR=0.345, 95% CI= 0.064-1.850).

Table 2: Comparison of seroprevalence of *T. gondii* antibodies in pet dogs in three veterinary hospitals

Veterinary Hospitals	**Numbers of sera sample collected**	**Numbers of positive**	**Percentage of positive**
A	27	5	18.52 (5/37)
B	12	3	25.00 (3/12)
C	4	0	0.00 (0/4)
Total	**43**	**8**	**19 (8/43)**

Table 3: Seropositive samples according to risk factors involved

Risk Factors	Categories	Samples (n)	Positive (n)	Seroprevalence (%)
Gender	Male	28	6	21.4
	Female	15	2	13.3
Breed	Local	21	3	14.3
	Pedigree	22	5	22.7
Age (year)	≤ 6	18	4	22.2
	7 - 12	25	4	16
Management	Indoor	33	4	12.1
	Outdoor	10	4	40
Feeding	Commercial feed	34	8	23.5
	Homecooked meal	9	0	0
Neurological patient	Yes	9	3	33.3
	No	34	5	14.7

5.0 DISCUSSION

From the current study, 19% dog sera sample collected from three different veterinary hospitals were positive for the presence of IgG antibodies to *T. gondii*. The seroprevalence found in this study was higher than previous study conducted by Chandrawathani et al., (2008) which showed that 13 out of 135 dogs were tested positive which giving a seroprevalence of 9.6 %. Sera sample tested using an indirect fluorescence antibody test (IFAT, cut-off titer 1:200).

Studies in Thailand, using latex agglutination test by (Jittapalapong et al., 2007, 2009) showed seroprevalence of about 10% (40 of 427) stray dogs in Bangkok against *T. gondii*. Even though the seroprevalence is high in this current study compared to previous study conducted in Thailand, this is because of the low sample size. However, the high seroprevalence in the current study may be attributed to the low sampling size and diagnostic work used that contributed from the ELISA test that is highly sensitive which would have few false positive results and highly specific in detecting the presence of the antibodies. Garcia et al., (2006) and Zhu et al., (2012) demonstrated high seroprevalence from the ELISA test (different ELISA kit used in this current study) which has higher sensitivity compared to MAT. Furthermore, according to a study conducted by Salehi et al., (2017) , the high seroprevalence of *T. gondii* in Tehran is due to high contamination in the environment. Hence, high environmental contamination may also contribute to the high *T. gondii* seroprevalence of this current study.

Veterinary hospital B recorded the highest seroprevalence of *T. gondii* antibodies compared to veterinary hospital A and C. Small sample size may cause the

high prevalence in veterinary hospital B. No seropositive cases recorded by veterinary hospital C. This is because the sample collected was too small which was only four sera sample. However, there were no significant differences of *T. gondii* between these three veterinary hospitals.

In current study, high seroprevalence of *T. gondii* in male dogs than female dogs. However, there were no significance association of *T. gondii* in pet dogs between sexes. Other research studies were also in agreement with the findings that gender was not significantly associated with the *T. gondii* (Jadoon et al., 2009; Jittapalapong et al., 2007;Wu et al., 2011). These findings may be due to the fact that male and female have similar behaviour as no distinctive behaviour differences were observed. According to Dubey (1987), he suggested that female dogs are more susceptible towards infection compared to male dogs because of the reduced immunity in a pregnant and lactating bitch that is prone to the *T. gondii* infection.

In this study, the prevalence rate increased by approximately one percent as the age group increases but no positive result recorded in the youngest age group. Age group 11 to 15 years old recorded the highest prevalence compared to other age groups. This is because older dogs had the longer time of environmental exposure compared to younger dogs (Dubey and Lappin, 2006). However, in this study, there were no significance association of *T. gondii* in pet dogs between age. Compared to other studies, age has been found to be associated with *T. gondii* where these studies shown that seroprevalence increases with the age of dogs due to the longer exposure to *T. gondii* over the time (Ahmad et al., 2014; Sharifdini et al., 2016; Zarra-Nezhad et al., 2017). Other studies also discussed on possible factor of the young dogs

susceptible to the *T.* gondii where Ahmed et al., (1983) stated that transplacental transmission also may contribute to the toxoplasmosis in less than a year-old dog. Furthermore, according to Bresciani et al., (1999) showed that puppies able to congenitally infected with *T. gondii* after the pregnant bitch ingests sporulated oocyst.

In this study, dogs that were managed outdoor was significantly high in seroprevalence towards *T. gondii* compared to those managed indoor. This is because outdoor dogs are exposed to environmental contamination. Other studies have shown that management plays a role because they suggested that outdoor dogs have increase contact time with the stray cats, consume infected intermediate host and easily exposed to contaminated food, soil, or water sources with sporulated oocysts (Liu et al., 2014; Salehi et al., 2017). Furthermore, according to Frenkel and Parker (1996), coprophagy and rolling on *T. gondii* infected cat faeces allow the transmission of *T. gondii* oocyst to the dogs fur. Hence, this behaviour contributes to risk of infection. Transmission of *T. gondii* to human occurs when petting the contaminated fur and accidentally ingested the oocyst. Moreover, pet dogs living together with toxoplasmosis cats also considered as a risk of getting the infection.

In this study, dogs fed with commercial feed have higher seroprevalence of *T. gondii* compared to dogs fed with homecooked meal. However, the difference was not significant. Other study has shown that home cooked food is related to the seropositive. Improper cooked meat or raw meat consumption contribute to the *T. gondii* exposure (Ali et al., 2003). Hence, the owner must exercise cooking the meat at temperature 67°C or more for about 3.6 minutes (Dubey et al., 1990) and store the meat prior cooking at -12.37°C for 11.2 days (Kotula et al. 1990). Although in this

study, high seroprevalence of *T. gondii* in regards with commercial feed, it is unlikely to associated with the exposure of *T. gondii*, hence it may relate with the environmental exposure where pet dogs accidently consume contaminated feed or drinks with oocyst or been exposed to the rodent that harbouring the tissue cyst (Dubey & Beattie 1988, Souza et al. 2003, Langoni et al.2006).

6.0 CONCLUSION

Results from this study showed 8 of 43 (19%) dog sera were tested positive for *T. gondii* antibodies. This finding revealed a high seroprevalence of *T. gondii* compared to the previous study reported in 2008 and dogs that managed outdoor were significantly associated with the seropositive sample. This high seroprevalence also reflect the level of environmental contamination with *T. gondii*. Therefore, it is necessary for pet owner to practise preventive measures in preventing and control *T. gondii* infection in their pet dogs. In multi-pet household such as cat and dog, disposal of cat faeces in time is recommended. Besides, it is advisable for the pet owner to manage their pet dogs indoor as it can reduce exposure towards contaminated environment and contact with intermediate hosts that harbours the tissue cyst. Furthermore, it best to cook meat meant for the pet dogs prior to consumption. As for the pet owner, wash hands with soap and water after petting their pet dogs will reduce transmission of oocyst from contaminated dog fur towards human.

RECOMMENDATIONS

For future studies, it is recommended to have more sample by extending the sampling area. The appropriate sample size is 139 sample, calculated at a 95% confidence level (CI), 5% precision. To achieve these 139 samples, participation of 10 veterinary clinics from the population of 118 veterinary clinics in Selangor are needed since the average collected sample is 14 in the period of 3 weeks of sampling. By doing this, it can increase the accuracy of the findings. Furthermore, ensure the patient details are more detailed such as to include more age group, sex, feeding, management, presence of cats in their environment and previous medical history.

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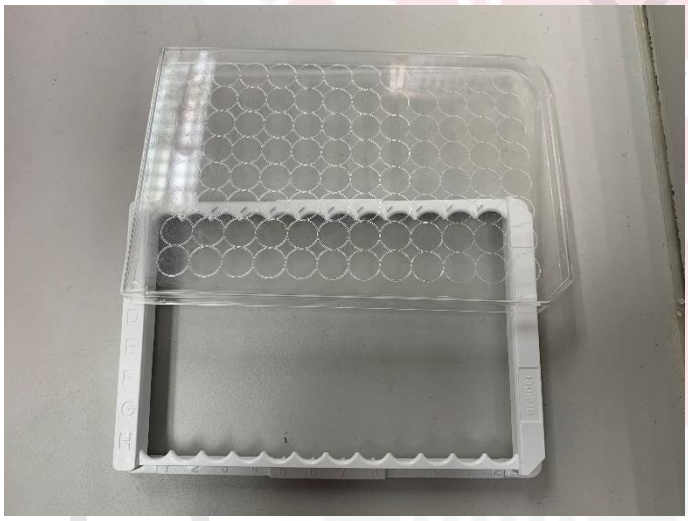
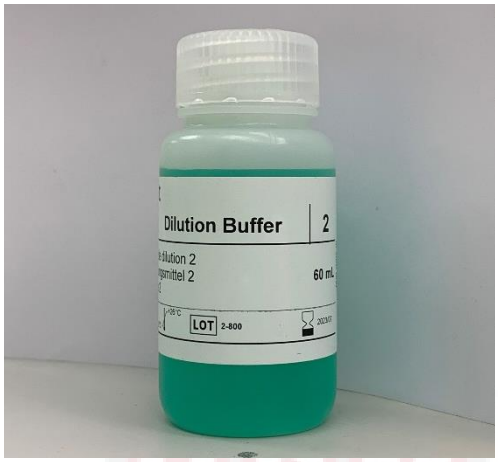
APPENDICES

Appendix 1: ELISA reader machine that been used.

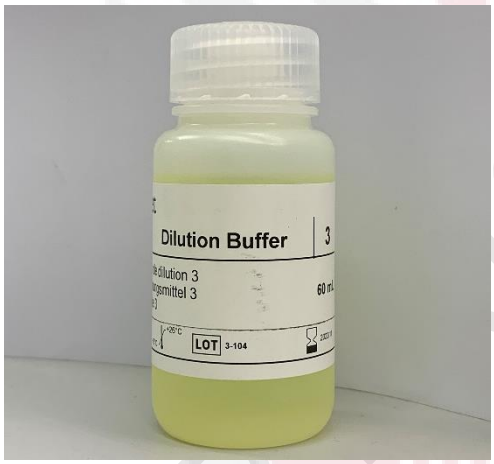


Appendix 2: 96 well plate and plate cover.

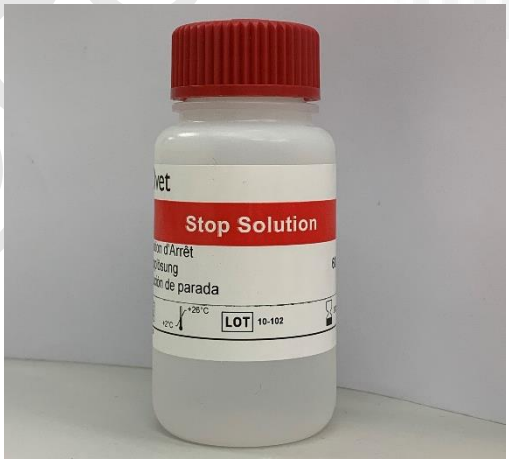
Appendix 3: Conjugate solution, positive and negative control solutions of ID Screen ® Toxoplasmosis Indirect Multi-species indirect ELISA test kit.



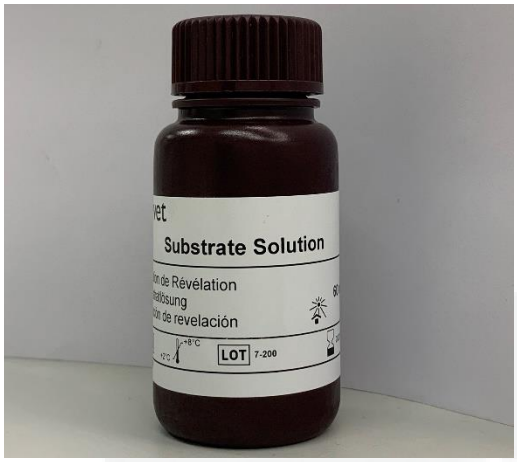
Appendix 4: Number 2 dilution buffer solution of ID Screen ® Toxoplasmosis Indirect Multi-species indirect ELISA test kit.



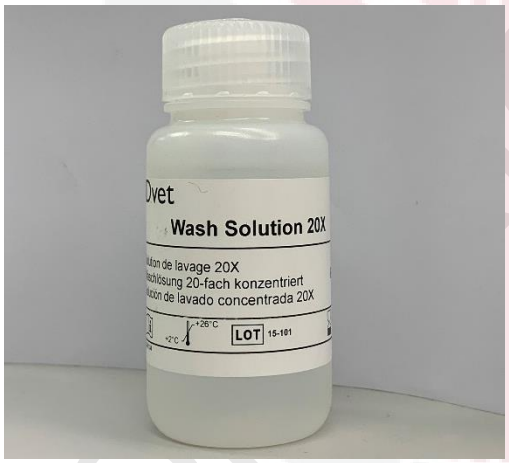
Appendix 5: Number 3 dilution buffer of ID Screen ® Toxoplasmosis Indirect Multi-species indirect ELISA test kit.



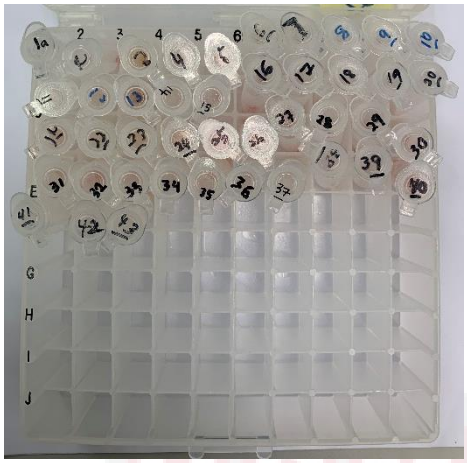
Appendix 6: Stop solution of ID Screen ® Toxoplasmosis Indirect Multi-species indirect ELISA test kit.



Appendix 7: Substrate solution of ID Screen ® Toxoplasmosis Indirect Multi-species indirect ELISA test kit.

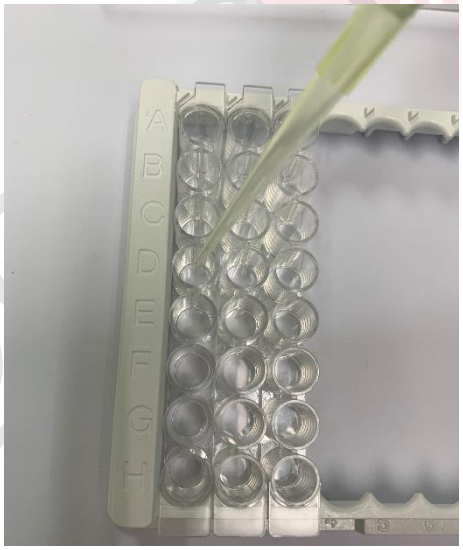


Appendix 8: Wash solution of ID Screen ® Toxoplasmosis Indirect Multi-species indirect ELISA test kit.



Appendix 9: Labeled serum sample stored in microcentrifuge tube.

Appendix 10: Micropippeted out 10µl of serum.



Appendix 11: Micropippeted in the serum sample in a well of the strip plate.