Seroepidemiology of toxoplasmosis among people having close contact with animals

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A cross-sectional study was conducted to determine the seroepidemiology of Toxoplasma infection and its risk association among people having close contact with animals. A total of 312 blood samples were collected from veterinary personnel (veterinarian, technicians, and students) and pet owners from veterinary clinics and hospitals in the area of Klang Valley, Malaysia. About 4 cc of blood samples drawn from agreed participants were processed for measurement of anti-Toxoplasma IgG and IgM antibodies as well as avidity test of Toxoplasma IgG by ELISA I, II, and III kits. Meanwhile, the demographic profiles and possible risk factors of these participants were also recorded in the standardized data collection sheets. Overall seroprevalence of toxoplasmosis was observed in 62 (19.9%) participants being 7 (18.4%) in veterinarians, 15 (33.3%) in veterinary technicians, 29 (14.9%) in veterinary assistants, and 11 (31.4%) in pet owners. Of 19.9% Toxoplasma seropositive samples, 18.3% was positive for IgG antibody, 1.0% for IgM antibody, and 0.6% for both IgG and IgM antibodies. Of three different IgG avidity ELISA kits, ELISA III showed high avidity in all five seropositive samples (lgM and IgG/lgM antibodies) indicating chronic Toxoplasma infection which is consistent with no evidence of clinical toxoplasmosis diagnosed during the time of this study. Univariate analysis showed that age group, gender, study population, gardening, task performance, and working duration were significantly associated with Toxoplasma seropositivity. Further analysis by multivariate analysis using logistic regression showed that age group of ≥30 years old (OR = 0.34, 95% CI = 0.18–0.63, \( p = 0.001 \)) and working or study duration of >10 years having close contact with animals (OR = 5.07, 95% CI = 1.80–14.24, \( p = 0.002 \)) were identified as significant risks for Toxoplasma infection. Based on the results obtained, a comprehensive Toxoplasma screening and health surveillance program on toxoplasmosis should be implemented among people having close contact with animals in general and confirmed Toxoplasma seronegative individuals in particular to prevent seroconversion.

Keywords: anti-Toxoplasma antibodies, IgG avidity, prevalence, risk factors, toxoplasmosis, people with animal-contact

INTRODUCTION

Toxoplasma gondii (T. gondii), an obligate intracellular protozoan parasite (a zoonotic pathogen) is capable of causing both the infection rate that affects approximately one-third of human populations worldwide and the disease burden of clinical toxoplasmosis in human. Toxoplasma infection can be transmitted via several routes in different host species (1). Many species of warm blooded animals can be infected including human and it was recognized by the National Institutes of Health, Bethesda, MD, USA as a category B priority pathogen (2). Consuming undercooked contaminated meat with tissue cysts, ingestion of T. gondii oocysts from water, soil, or cat litter and congenital infection through placenta will lead to toxoplasmosis (3–5). Majority of infected individuals are symptomatic (6). T. gondii poses a greater risk especially found among pregnant women and immunocompromised individuals. Small percentage of infected newborns develop mild to severe clinical manifestations such as lymphadenopathy, fever and malaise in mild infection, ocular disease and mental illness in moderate manifestation, and severe cases among infected pregnant women will lead to stillbirth, abortion, or live birth children with central nervous system impairment or impaired vision (5). Besides, infected newborns with more virulent types of T. gondii may lead to severe and even fatal diseases with pulmonary and multi-visceral involvement (5).

To date, numerous studies have suggested preventive strategies of toxoplasmosis in people having close contact with animals (4, 7, 8), which is due to their high risk behaviors. Unfortunately, scanty data were reported on toxoplasmosis among these people worldwide (9–11). In Malaysia, the seroprevalence of toxoplasmosis in general healthy population increased from 16 to 30% (12). Furthermore, most studies on toxoplasmosis have been mainly conducted in healthy persons, pregnant women, indigenous communities, and HIV-positive patients (12, 13). To the best of our knowledge, this is the first documented data ever reported.
on toxoplasmosis among animal handlers in Malaysia. In addition, a current situation on epidemiology of toxoplasmosis in animal handlers is crucial and timely to be investigated, so that suggested preventive strategies can be achieved pragmatically in implementation. This study was therefore conducted to determine the seroprevalence of *Toxoplasma* infection among people having close contact with animals and their risk factors in acquiring *Toxoplasma* infection.

**MATERIALS AND METHODS**

**STUDY SITE AND POPULATION**

This prospective cross-sectional study was conducted from October 2013 to April 2014. A total of 312 participants were from Faculty of Veterinary Medicine, University Putra Malaysia, Selangor and various private veterinary clinics in the Klang valley (Figure 1) were recruited. The inclusion criteria of this study were (1) immunocompents who have close contacts with animals which include veterinarians (38), veterinary technicians (45), veterinary students (194), and pet owners (35) and (2) age of more than 15 years. All eligible participants gave informed consent before the commencement of this study. All the participants’ information related to socio-demographic such as their age, education level, occupation, and plausible risk-factors exposure associated with toxoplasmosis (presence of own cats at home, presence of stray cats at home, drinking untreated water, and having contact with soils) prior to 3 months before this study were recorded in the formatted questionnaire forms. An operational definition was used for the risk factors. Presence of own cats at home was defined as a person who is the owner of at least one cat or has close contact with cats while feeding and playing in the house. Presence of stray cats at home was defined as a person having a close proximity with stray cats roaming in the house compound. Drinking untreated water was defined as a person who consumes “untreated water,” e.g., water from a pipe, tap, or rain. Contact with soil (gardening) was defined as person who has a direct exposure to soil while gardening or any kind of outdoor activities.

**ETHICAL CONSIDERATION**

This study was approved by the ethical review committee of University of Malaya Medical Centre (UMMC), MEC Ref. No. 1024.6 in accordance with the Helsinki Declaration for the inclusion of human subjects in research. The purpose and procedures of this study were explained to all the participants. Informed consents were obtained from agreed participants prior to samples and data collection.

**SERUM SAMPLE COLLECTION**

Approximately 5 mL venous blood was drawn, sera were processed and were kept at −20°C until further testing.

**DETECTION OF ANTI-TOXOPLASMA ANTIBODIES**

The collected serum was screened primarily for anti-*Toxoplasma* IgG and IgM antibodies by using standard ELISA commercial kit (IgG-NovaLisa, Dietzenbach, Germany) in accordance with the manufacturer’s instruction. A positive sample for the anti-*Toxoplasma* IgG and/or anti-*Toxoplasma* IgM antibody was also tested for its avidity using three standard ELISA commercial kits, namely, ELISA-I, II, and III for comparison and according to its manufacturer instruction. The interpreted results for ELISA-I was that avidity of >40% suggest chronic/past infection and of <40% suggest acute/recent infection, for ELISA-II was that avidity of <15% (low avidity), indicates acute or primary infection and avidity between 15 and 30% (borderline activity) indicates possibility of primary infection during the last 6 months is possible and >30% (high avidity) excludes primary infection within last...
Toxoplasma seropositivity as a dependent variable and possible infection among veterinary personnel (data were not shown). A multivariate logistic regression model (stepwise forward) was used to identify significant predictors of infection found among these subjects. Working duration of more than 10 years (OR = 5.07, 95% CI 1.80–14.25) was identified as significant predictors of infection, as demonstrated by ELISA-II, while ELISA-III showed that age group, gender, and study population were significantly associated with low avidity.

**STATISTICAL ANALYSIS**

The data collected in the questionnaires and the serology results were analyzed by using statistical software SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). The qualitative variables were estimated and presented as frequencies and percentages. Univariate analyses and the χ² test were used to investigate the association between Toxoplasma seropositivity as a dependent variable and possible demographic and risk factors as independent variables; p < 0.05 was regarded as being statistically significant. However, to retain all possible significant association, variables that showed an association with p ≤ 0.20 were used to apply to a multivariate logistic regression model. Each dependent factor was modeled as dichotomous variables.

**RESULTS**

During this study period, a total of 312 people were recruited as studied subjects. The age range was 17–64 with a mean ± 27 ± 9.08 years. Majority of the subjects were in the age group of 21 and 30 years (228; 73.1%), female (234; 75%), Malay (139; 44.6%), veterinary students (194; 62.2%), and city dwellers (300; 96.2%).

The overall seroprevalence of toxoplasmosis in this study was 62 (19.9%) in which 57 (18.3%) samples were positive for IgG, 3 (1.0%) samples were positive for IgM, and 2 (0.7%) samples were positive for both IgG and IgM antibodies (Table 1). The positive IgM antibodies and samples with both positive for IgM and IgG antibodies were further tested for IgG avidity measurement using three standard commercial ELISA kits (I, II, and III) for comparison to differentiate between recent and past infections. Of five Toxoplasma seropositive samples, one sample was recently acquired and four other samples were past infections as detected from ELISA-I, all five samples were regarded as recently acquired infection, as demonstrated by ELISA-II, while ELISA-III showed past infection from all five seropositive samples (data were not shown). The end of this study, there was, however, no clinical evidence of toxoplasmosis diagnosed in the Toxoplasma seropositive with low avidity.

Univariate analysis in relation to socio-demographic profiles showed that age group, gender, and study population were significantly associated with Toxoplasma seropositivity (p < 0.05) (Table 2). The results of this study further showed that the highest prevalence of Toxoplasma infection was found among veterinary technicians (33%) followed by pet owners (31.4%), veterinarians (18.4%), and veterinary students (14.9%). Interestingly, gardening (33; 26.6%) was significantly associated with Toxoplasma infection found among these subjects (Table 3). In addition, working duration and task performance were significantly associated with Toxoplasma seropositivity found among veterinary personnel (Table 4).

Further analysis by multivariate logistic regression showed that age group ≥30 years (OR = 0.34, 95% CI 0.18–0.63) contributes to high Toxoplasma seropositivity in the study population and working duration of more than 10 years (OR = 5.07, 95% CI 1.80–14.25) was identified as significant predictors of Toxoplasma infection among veterinary personnel (data were not shown).

**DISCUSSION**

WHY TOXOPLASMOSIS IS IMPORTANT

The present study showed the overall seroprevalence of toxoplasmosis among people having close contact with animals was 19.9% and this infection rate did not appear to be very high. Since this is the first study of its kind conducted in Malaysia, it is therefore no previous study can be compared. However, the numbers of respondents provided us substantial interpretation about the current prevalence of toxoplasmosis among these people in Malaysia to signify this conclusive remark. In the literature, the first report on Toxoplasma infection among general healthy population in

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**Table 1 | Seroprevalence of toxoplasmosis among survey population as assessed by the ELISA test.**

| ELISA test | Toxoplasma seropositivity (62, 19.9%) |
|------------|-------------------------------------|
|            | IgG+ve | IgM+ve | IgG+ve and IgM+ve |
| Positive   | 57 (18.3%) | 3 (1.0%) | 2 (0.6%) |
| Negative   | 255 (81.7%) | 307 (99.0%) | 310 (99.4%) |
| Total      | 312    | 310    | 312    |

**Table 2 | Seroprevalence of Toxoplasma infection by the demographic characteristics.**

| Characteristics | Total N = 312 | Toxoplasma seropositivity n (%) |
|-----------------|--------------|---------------------------------|
| Age             |              |                                 |
| Range 17–64 years with a mean of 27 ± 9.08 years | | |
| Age group       |              |                                 |
| ≤ 20            | 22 (71) | 3 (13.6) |
| 21–30           | 228 (73.1) | 36 (15.8) |
| 31–40           | 33 (10.6) | 9 (27.3) |
| ≥ 41            | 29 (9.29) | 14 (48.3) |
| Gender          |              |                                 |
| Male            | 78 (25) | 23 (29.5) |
| Female          | 234 (75) | 39 (16.7) |
| Race            |              |                                 |
| Malay           | 139 (44.6) | 32 (23.0) |
| Chinese         | 115 (38.9) | 14 (12.2) |
| Indian          | 33 (10.6) | 10 (30.3) |
| Aborigine       | 4 (1.3) | 1 (25) |
| Foreigner       | 21 (6.7) | 5 (23.8) |
| Study population|              |                                 |
| Veterinarian    | 38 (12.2) | 7 (18.4) |
| Veterinary technician | 45 (14.4) | 15 (33.3) |
| Veterinary student | 194 (62.2) | 29 (14.9) |
| Pet owner       | 35 (11.2) | 11 (31.4) |
| Primary residency|          | 0.233                           |
| Village         | 12 (3.8) | 4 (33.3) |
| City            | 300 (96.2) | 58 (19.3) |

p < 0.05, significant association as potential risk factors; N, number examined; n, number of positive sample.
Malaysia was quite low (13.9%) and it has been increasing over the years ranging from 16 to 30% (14), 28.1% (15), and 40.8% (16). However, higher prevalence in the healthy population could be normally explained by the fact that males have a higher tendency to be involved in sports activities or other activities at work or outdoor alike that expose them to soil and also they are not inclined to be involved in sports activities or other activities at work or outdoor alike that expose them to soil and also they are not inclined to be involved in sports activities or other activities at work or outdoor alike that expose them to soil.

**Table 3 | Seroprevalence of Toxoplasma infection by plausible risk factors among people having close contact with animals.**

| Variables                        | Total N = 312 | Toxoplasma seropositivity n (%) | p-value |
|----------------------------------|---------------|---------------------------------|---------|
| Close contacts with cats         |               |                                 | 0.173   |
| Yes                              | 142           | 33 (78.6)                       |         |
| No                               | 170           | 29 (17.1)                       |         |
| Water supply at home             |               |                                 | 0.531   |
| River and mountain pipe          | 27            | 6 (22.2)                        |         |
| Government pipe water            | 283           | 55 (19.4)                       |         |
| Private pipe water               | 2             | 1 (50)                          |         |
| Clean water resources            |               |                                 | 0.144   |
| Yes                              | 207           | 46 (22.2)                       |         |
| No                               | 105           | 16 (15.2)                       |         |
| Eating with bare hands           |               |                                 | 0.379   |
| Yes                              | 233           | 49 (21.0)                       |         |
| No                               | 79            | 13 (16.5)                       |         |
| Tasting foods while cooking or seasoning | 251 | 49 (19.5) | 0.438 |
| No                               | 61            | 13 (21.3)                       |         |
| Cleaning cooking utensils        |               |                                 | 0.054   |
| Yes                              | 300           | 57 (19.0)                       |         |
| No                               | 12            | 5 (41.7)                        |         |
| Always gardening                 |               |                                 | 0.015   |
| Yes                              | 124           | 33 (26.6)                       |         |
| No                               | 188           | 29 (15.4)                       |         |

**Table 4 | Seroprevalence of Toxoplasma infection by other plausible risk factors in working or study area for veterinary personnel.**

| Activities/variables | Total N = 277 | Toxoplasma seropositivity n (%) | p-value |
|----------------------|---------------|---------------------------------|---------|
| Working duration     |               |                                 | 0.004   |
| ≤ 1 years            | 43            | 6 (14.0)                        |         |
| 2–10 years           | 218           | 37 (17.0)                       |         |
| 11–20 years          | 6             | 2 (33.3)                        |         |
| ≥ 21 years           | 10            | 6 (60)                          |         |
| Task performance     |               |                                 | 0.019   |
| Working field        | 83            | 22 (26.5)                       |         |
| Study field          | 194           | 29 (14.9)                       |         |
| Cleaning cat excrement |             |                                 | 0.286   |
| Yes                  | 217           | 42 (19.4)                       |         |
| No                   | 60            | 9 (15)                          |         |
| Wearing gloves       |               |                                 | 0.433   |
| Yes                  | 222           | 40 (18.0)                       |         |
| No                   | 55            | 11 (20)                         |         |
| Washing hands        |               |                                 | 0.665   |
| Yes                  | 275           | 51 (18.5)                       |         |
| No                   | 2             | 0 (0)                           |         |

*p < 0.05, significant association as potential risk factors; N, number examined; n, number of positive sample.

A positive result for only anti-Toxoplasma IgG antibodies in this study was 18.3% indicating past or chronic infection. Positive result for both IgG and IgM antibodies in this study was 0.6% indicates either a recent infection or false positive test result (10). Therefore, IgG avidity (brand I, II, and III) measurement, a confirmatory test, was subsequently performed, which is to assist, in determining the time of infection (18, 19). Of this, ELISA-III showed the most accurate result on five seropositive samples indicating chronic or past infection followed by ELISA-II and -I. Supporting to this finding, there was no clinically confirmed case of toxoplasmosis diagnosed during the time of this study. This therefore suggests the following: more than one avidity tests should be performed in a single serum sample, a second blood sample (if no avidity test available) is required to be tested after 2–4 weeks of infection to confirm a recent infection or it can be considered as a false positive with supporting information of whether there is an evidence of clinical toxoplasmosis.

**WHEN IgG AVIDITY DOES ITS ROLE**

In the present study, the infection rate of anti-Toxoplasma IgM antibodies was 1.0% suggesting a recently acquired Toxoplasma infection. Negative results for IgM antibodies strongly exclude the recent infection, while positive result for IgM test is difficult to interpret (10). Hence, the positive result of IgM antibodies was further analyzed using IgG avidity test to help differentiate between past and recent infections (17). Of this, all three seropositive samples for anti-Toxoplasma IgM antibodies showed high avidities indicating past infection.
Toxoplasma infection should be further investigated before any conclusion could be made. Of note, working duration was also significantly associated with Toxoplasma infection. Based on this finding, other unidentified risks associated with Toxoplasma transmission in this study.

HOW RISK FACTOR AFFECTS THE TRANSMISSION OF T. GONDII

Our univariate analysis showed that gardening was identified as one of significant risk factors in this study (p = 0.015). The participants who frequently do gardening were highly infected (26.6%) compared with the ones who spent less time (15.4%). This could be explained due to the fact that the buried sporulated oocysts of cats might be contaminating the soil the soil and sand and the oocysts remain infectious for about several months and can last beyond 1 year (25). Oocysts have a buoyancy characteristic that may become infectious after raining since oocysts will float on the upper layer of the soil (26). Therefore, it is very important to avoid any materials or foods that come into close contacts with unforeseen contaminated soil. The analysis further showed that the task performance in working field was significantly associated with Toxoplasma seropositivity (p = 0.019). Of this, veterinarians and veterinary technicians had the higher Toxoplasma infection (26.5%) compared to veterinary students (14.9%). This finding was not surprising since the daily task like animal surgery and cleaning the cat excrement are most probably increasing the chance of infection. After multivariate logistic regression model was applied, it was not significantly associated with Toxoplasma infection. Future similar study is recommended periodically and also to investigate other unidentified risk factors to eliminate the infection rate and to eradicate this parasite from the region.

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AUTHOR CONTRIBUTIONS

VN and TTC designed the study. NAA, TTC, RSKS, and VN carried out the study. GJBM contributed most on manuscript writing. RSKS, YALL, TTC, and VN provided opinions and suggestions about this manuscript. All authors read and approved the final version of the manuscript.

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