Research Article

Knowledge, Attitude and Practice Related to *Toxoplasma gondii* Infection among Rural and Semi-Urban Community in Malaysia

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Abstract

Opportunistic infections such as toxoplasmosis pose an increasingly ominous threat to public health as the prevalence of primary and secondary immunocompromise increases. Hence, a cross-sectional study of knowledge, attitude and practice related to *Toxoplasma gondii* infection among rural and semi-urban community was conducted in three semi-urban areas in Malaysia. Three hundred and twenty one participants were recruited through convenience sampling in this study. A self-administered, modified validated questionnaire was used for data collection. The questionnaire covered participants' demographic profiles, source of water, contact with cats, personal health practices and knowledge about toxoplasmosis. Analysis of the data revealed that poor hand hygiene and poor personal health practices were observed to a significant degree amongst the younger age group, males and individuals with low education level. The general population was also found to have low knowledge and awareness about toxoplasmosis. Such knowledge gap needs to be addressed and further studies need to be conducted to evaluate population awareness and practices which place individuals at risk of infection so that more effective educational programmes and other interventions can be carried out.

INTRODUCTION

Toxoplasmosis is a disease caused by the organism *Toxoplasma gondii*. It is classified by the Centers for Disease Control and Prevention (CDC) as one of five Neglected Parasitic Infections (NPI) in the United States, which were identified as priorities for public health action based on their prevalence, severity, and potential for prevention and treatment. The hot, humid climates and lower altitudes of the tropics are especially favorable for the parasite [1]. However, toxoplasmosis is not as well-known to the public as other diseases endemic to the tropics such as dengue and malaria.

Toxoplasma gondii is an obligate intracellular protozoa which infects warm-blooded animals including humans, in whom the central nervous system can sometimes be invaded. Felids, especially wild and domestic cats are the definite host of *T. gondii* [2,3]. The life cycle of *T. gondii* comprises of three infective stages: sporozoite containing oocysts, bradyzoites and tachyzoites. Oocysts are produced by the infected definite host before being shed in faeces [3]. Once oocysts are ingested by an intermediate host, sporozoites are released, which invade enterocytes and differentiate into tachyzoites [2]. Tachyzoites infect nucleated cells, replicating and disseminating rapidly, and are present in the acute phase of the infection. Bradyzoites which differentiate

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from tachyzoites have a lower replication rate and usually remain dormant in brain and muscle cells as tissue cysts, contributing to latent infection [3,4]. Such cysts would be disintegrated by the antibodies of an immunocompetent individual [4]. Modes of transmission of *T. gondii* includes consumption of raw or undercooked meat containing tissue cyst, vegetables or water contaminated with oocysts, blood transfusion, transplantation of organs harvested from a seropositive donor and vertical transmission [3,5].

Immunocompetent individuals are often asymptomatic [6]. Up to 10% of cases may present with self-limiting, isolated cervical or occipital lymphadenopathy [7]. Symptomatic cases associated with high mortality rates are common in immunocompromised individuals (i.e. HIV patients, patients who have undergone chemotherapy) and fetuses of mothers who acquired toxoplasmosis during pregnancy [6,8].

The prevalence of toxoplasmosis worldwide is approximately 30-90% [9]. In Malaysia, a rise in prevalence of toxoplasmosis from 16.0% to 30.0% among the general healthy population has been observed in 2015 by Brandon-Mong et al. [10]. Highest seroprevalence rates have been observed among Malays as compared to other ethnic groups. A higher rate of seropositivity is commonly found in males and it increases with age for both

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genders, most likely due to cumulative exposure to toxoplasma infection as one ages [11,12]. Those with a lower socioeconomic status are also more prone to acquiring the infection [11].

This study aims to assess the knowledge, awareness and practices of the rural and semi-urban community in relation to acquiring toxoplasma infection, which may have contributed to the significant rise in prevalence of toxoplasmosis in Malaysia by influencing their food consumption and hygiene practices. Identifying the higher risk groups for targeted intervention and future prevention strategies are also a key area of interest.

MATERIALS AND METHODS

Study Area

A cross-sectional study was conducted over two weeks from the 9^{th} of April 2017 to the 23^{rd} of April 2017 in the rural and semi-urban areas of Kuala Selangor, Sabak Bernam and Bentong, Malaysia. The total population is approximately 435,184 (as of census 2010) [13,14]. Sabak Bernam is a rural district within the state of Selangor, located in the north-western most region of the state and furthest away from the Federal Territory of Kuala Lumpur. The economy of this district centers around agriculture [15]. Kuala Selangor lies immediately south to Sabak Bernam and it is the second largest district in the state. Fishing and agriculture are the main economic activities in this district [16]. Bentong is located in western Pahang, a state which lies to the east of Selangor. Bentong is rich in light and medium industries, namely timber factories, manufacturing and food industries [17]. The demographic makeup of each study site is displayed in Figure 1 [13,15,16].

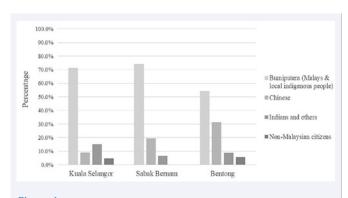
Selection Criteria

The inclusion criteria for this study were those who aged 18 and above, encountered in public and deemed capable of providing informed consent. Exclusion criteria included inability to provide informed consent, \geq 4 empty questionnaire response fields, and nonresidents of the study locales. A total of 321 eligible participants were recruited through convenience sampling, with informed consent. Age group and gender were taken into consideration when recruiting participants to reduce bias. Ethnicity was not taken into consideration due to variable population demographics across the three study sites and to avoid skewing of data collected from minority ethnic groups.

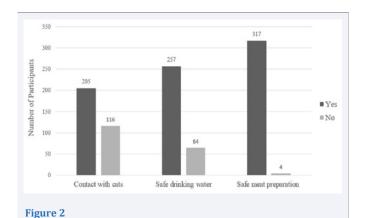
Data Collection

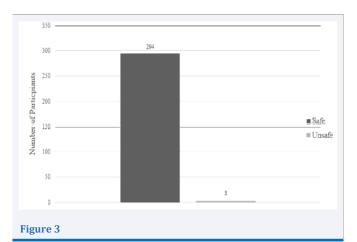
A modified questionnaire validated by University of Malaya (ME CID. NO: 201411-805) with 26 questions was used for this study. The questionnaire was designed to maintain anonymity of participants while important information related to demographic profiles, source of water, contact with cats, personal health practices and knowledge about toxoplasmosis were gathered. Questionnaires were administered by researchers or participants themselves.

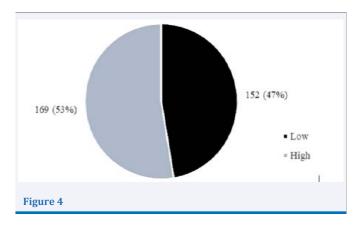
Participants were divided into two age groups: age below 30, and 30 and above. Participants were also segregated into two groups based on their highest education level: Group 1; uneducated or primary level education and Group 2; secondary or tertiary level education. Likewise, the participants' occupations











were also categorized into two categories: Group 1; unemployed or unskilled workers and Group 2; skilled workers. Several risk factors for contracting toxoplasmosis were also assessed through the questionnaire, including participants' primary water source, preparation and consumption of meat and vegetables, contact with cats and/or soil, and hand hygiene. An operational definition was used for the risk factors. Bottled water, water that was boiled or filtered were considered as safe water, otherwise were categorized as unsafe. Vegetables that were thoroughly washed, peeled, boiled or cooked were grouped under safe vegetables preparation, while raw unwashed vegetables were considered unsafe. The definition of safe meat consumption was limited to meat that was well cooked. Any raw or undercooked meat was categorized as unsafe for the purpose of this study. Contact with cats was defined as presence of cats at home or presence of stray cats roaming in the house compound. Contact with soil was defined as person who has direct exposure to soil during gardening, agriculture or construction work.

In the last section of the questionnaire, knowledge regarding toxoplasmosis was assessed. Two multiple response

questions about mode of transmission and prevention of toxoplasmosis were also included. The participants' knowledge of toxoplasmosis prevention was also assessed according to the number of option(s) chosen. Each option was given one score. Participants who scored ≤ 2 were considered to have low knowledge level, whereas those who scored ≥ 3 were deemed to have high knowledge level. Two more questions were included to assess whether or not the participants knew that cat faeces or undercooked meat were sources of infection and that vertical transmission of toxoplasmosis was possible.

Statistical Analysis

IBM-SPSS version 23 was used for statistical analysis. Chisquare test was used for analysis of the association between demographic variables and risk factors as well as the association between demographic variables and knowledge about toxoplasmosis. Statistical significance was determined as $p \le$ 0.05. Participants chose to withhold certain information or did not provide a response to one or more of the questions in the questionnaire (i.e. unwillingness to disclose educational level,

		Personal Hygiene Practices								
Demographic Variables		Do not use soap when washing hands N (%)	P-value	Do not use soap after dealing with soil N (%)	P-value	Do not wash hands after handling cats N (%)	P-value	Finger- nails not cut within past 7 days N (%)	P-value	
Age										
Below 30	N = 183 (57.0%)	73 (39.9%)	0.043	14 (7.7%)	0.319	64 (35.0%)	0.017	39 (23.2%)	0.033	
30 and above	N = 138 (43.0%)	40 (29.0%)		15 (10.9%)		31 (22.6%)		18 (13.5%)		
Gender										
Male	N = 126 (39.3%)	61 (48.4%)	< 0.001	15 (11.9%)	0.149	44 (34.9%)	0.099	24 (20.3%)	0.618	
Female	N = 195 (60.7%)	52 (26.7%)		14 (7.2%)		51 (26.3%)		33 (18.0%)		
Education level *										
Group 1	N = 24 (7.5%)	9 (37.5%)	0.799	6 (25.0%)	0.003	10 (41.7%)	0.190	6 (26.1%)	0.372	
Group 2	N = 295 (92.5%)	103 (34.9%)	0.7 5 5	22 (7.5%)	0.005	85 (28.8%)	0.190	51 (18.5%)	0.372	
Occupation										
Unemployed, unskilled	N = 279 (87.7%)	101 (36.2%)	0.327	27 (9.7%)	0.142	82 (29.5%)	0.624	52 (20.1%)	0.283	
Skilled	N = 39 (12.3%)	11 (26.2%)		1 (2.6%)		13 (33.3%)		5 (12.8%)		
Individual monthly income										
MYR 500 and below	N = 66 (21.3%)	26 (39.4%)	0.381	7 (10.6%)	0.615	22 (33.3%)	0.435	14 (22.2%)	0.541	
Above MYR 500	N = 244 (78.7%)	82 (33.6%)		21 (8.6%)		69 (28.4%)		43 (18.8%)		

The bold value indicates statistical significant for each demographic variable in relation to the personal hygiene practices ($p \le 0.05$ is denoted as statistically significant). Due to empty response fields in returned participant questionnaires (i.e. unwillingness to disclose educational level, occupation or income information, unable to recall if fingernails were cut within the last week etc) the total participants in all demographic categories do not add up to the total sample size of N =321. The statistical analyses of each relevant correlation were adjusted accordingly. Education level*:Group 1: Uneducated / Primary education; Group 2: Secondary / Tertiary education. Individual monthly income: 1 US\$ = 3.95 MYR, 1 MYR = 0.25US\$

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Fable 2: Correlation betwee	en demographic vari	ables and awarene	ess about tox	oplasmosis and key know	vledge		
		Knowledge & Awareness					
Demographic Variables		Toxoplasmosis awareness N (%)	P-value	Transmission of <i>T.</i> gondii via cat faeces / meat N (%)	P-value	Vertical transmission N (%)	P-value
Age					0.574		
Below 30	N = 183 (57.0%)	5 (2.7%)	0.431	99 (54.1%)		99 (54.1%)	P-value 0.638 0.077 0.122 0.010 0.562
30 and above	N = 138 (43.0%)	6 (4.3%)		79 (57.2%)		71 (51.4%)	
Gender							
Male	N = 126 (39.3%)	1 (0.8%)	0.037	72 (57.1%)	0.624	59 (46.8%)	0.077
Female	N = 195 (60.7%)	10 (5.1%)		106 (54.4%)		111 (56.9%)	
Education level *							
Group 1	N = 24 (7.5%)	0 (0.0%)	0.336	9 (37.5%)	0.065	9 (37.5%)	0.122
Group 2	N = 295 (92.5%)	11 (3.7%)		168 (56.9%)		159 (53.9%)	
Occupation							
Unemployed, unskilled	N = 279 (87.7%)	5 (1.8%)	< 0.001	156 (55.9%)	0.953	139 (49.8%)	0.010
Skilled	N = 39 (12.3%)	6 (15.4%)		22 (56.4%)		28 (71.8%)	
Individual monthly income			0.005		0.400		0.540
MYR 500and below	N = 66 (21.3%)	0 (0.0%)	0.095	43 (65.2%)	0.108	37 (56.1%)	0.562
Above MYR 500	N = 244 (78.7%)	10 (4.1%)		132 (54.1%)		127 (52.0%)	1

The bold value indicates statistical significant for each demographic variable in relation to the knowledge and awareness about toxoplasmosis ($p \le 0.05$ is denoted as statistically significant). Due to empty response fields in returned participant questionnaires (i.e. unwillingness to disclose educational level, occupation or income information, unable to recall if fingernails were cut within the last week etc) the total participants in all demographic categories do not add up to the total sample size of N =321. The statistical analyses of each relevant correlation were adjusted accordingly. Education level*:Group 1: Uneducated / Primary education; Group 2: Secondary / Tertiary education Individual monthly income: 1 US\$ = 3.95 MYR, 1 MYR = 0.25US\$

Demographic Var	iables	High knowledge level about prevention N (%)	P-value		
Age					
Below 30	N = 183 (57.0%)	88 (48.1%)	0.059		
30 and above	N = 138 (43.0%)	81 (58.7%)			
Gender					
Male	N = 126 (39.3%)	71 (56.3%)	0.286		
Female	N = 195 (60.7%)	98 (50.3%)	1		
Education level *					
Group 1	N = 24 (7.5%)	9 (37.5%)	0.114		
Group 2	N = 295 (92.5%)	160 (54.2%)	-		
Occupation					
Unemployed, unskilled	N = 279 (87.7%)	141 (50.5%)	0.112		
Skilled	N = 39 (12.3%)	25 (64.1%)			
Individual monthly income					
MYR 500and below	N = 66 (21.3%)	46 (69.7%)	0.003		
Above MYR 500	N = 244 (78.7%)	120 (49.2%)	-		

The bold value indicates statistical significant for each demographic variable in relation to high knowledge level about *T. gondii* prevention ($p \le 0.05$ is denoted as statistically significant). Individuals who scored ≥ 3 out of 6 for that particular question in the questionnaire were selected and identified as those who had high knowledge level about *T. gondii* prevention. Due to empty response fields in returned participant questionnaires (i.e. unwillingness to disclose educational level, occupation or income information, unable to recall if fingernails were cut within the last week etc) the total participants in all demographic categories do not add up to the total sample size of N =321. The statistical analyses of each relevant correlation were adjusted accordingly.

Education level*:Group 1: Uneducated / Primary education; Group 2: Secondary / Tertiary education Individual monthly income: 1 US\$ = 3.95 MYR, 1 MYR = 0.25US\$

occupation or income information, unable to recall if fingernails were cut within the last week etc) were excluded from the total sample space when statistical analysis for those specific correlations were performed.

Ethical Consideration

This study was approved by the Institutional Review Board of Perdana University (PU-IRB), IRB ID: PU IRBHR0133. All participants were fully informed about the aim and procedure of the study. It was emphasized that participation was voluntary and that withdrawal was allowed without further question. Informed consents were gained and dated prior to data collection and confidentiality was reassured. No identifying or sensitive information was collected or recorded.

RESULTS

A total of 321 participants were recruited in this study. The participants' ages ranged from 18-80 years old. Mean age was 32.2 years. Correlation between demographic variables and personal hygiene practice showed that there was significant difference in relation to hand hygiene between the two age groups. Furthermore, male participants were disproportionately less likely to use soap when washing hands (48.4% versus 26.7%, p < 0.001) (Table 1). A statistically significant correlation was also observed between the participants' education level and the likelihood that they do not wash their hands after handling soil. Participants with lower education level were more likely to forgo the practice. Occupation group and individual monthly income were not found to be reliable predictors of the participants personal hygiene practices (Table 1). Figure 2 and Figure 3 show the total number of participants in each risk category (i.e. contact with cats, consumption of unsafe water, meat and vegetables). No statistically significant correlations were found between any of the measured demographic variables and these risk factors.

In general, only 11 (3.4%) participants had heard about toxoplasmosis. Two of them recognized toxoplasmosis as an infection caused by parasite or *T. gondii*, another two respondents indicated that cat was a source of infection while others had only heard about the disease without much understanding. Their sources of information were academic programmes or educational courses, television programme, self-reading and information from family or peers. It was statistically significant that females had higher awareness in comparison to males (5.1% versus 0.8%, p = 0.037). Higher awareness among skilled workers as compared to unemployed or unskilled workers was also statistically significant (15.4% versus 1.8%, p < 0.001) (Table 2).

Although majority of participants had not heard about toxoplasmosis, yet 178 (55.5%) of them still agreed that cat faces and animal meat may contribute to transmission of zoonotic diseases. However, no significant correlation was found between demographic variables and knowledge. In view of key knowledge about vertical transmission of *T. gondii*, 170 (53.0%) participants were mindful that toxoplasmosis could be transmitted from mother to child. It was statistically significant that higher percentage of skilled workers were more aware of such transmission in comparison to those who were unemployed or unskilled (71.8% versus 49.8%, p = 0.010) (Table 2).

The three main preventive measures selected by participants were hand hygiene, drinking safe water and ensuring meat is cooked thoroughly. Other preventive measures proposed by the participants were taking vitamin supplements to boost immune system and cleanliness of surrounding environment. 169 (53.0%) participants were found to be highly knowledgeable about prevention of *T. gondii* (Figure 4). Across the demographic variables, higher knowledge level was observed among those with individual monthly income of \leq MYR 500 (1 US\$ = 3.95 MYR, 1 MYR = 0.25 US\$) as compared to those who earned more than MYR 500 per month (69.7% versus 49.2%, p = 0.003) (Table 3). Further analysis among the females showed that those aged 30 and above were significantly more knowledgeable of *T. gondii* preventive measures than those aged below 30 (62.7% versus 42.5%, p = 0.006).

DISCUSSION

In this study, statistical analysis revealed correlations between several risky behaviours which predispose towards toxoplasmosis infection and certain demographic groups. Younger generations and males were found to be less likely to always use soap when washing hands. This was in line with the results of previous observational studies conducted in Korea and the Unites States of America. The researchers noted that males were less adherent to hand washing significantly [18]. On the other hand, other researchers noticed that males were significantly more likely to simply wet their hands without use of soap than females [19]. Their study also showed that those estimated to be older than college group were significantly more likely to wash their hands with soap as compared to those who were in the college group or younger [19].

Eating with hands among Malaysians is a cultural etiquette, especially for Malays and Indians. Hands could be a source infection if they are not washed thoroughly, especially after contact with soil or cat faces as it poses risk of unintentional ingestion of oocysts [5]. In this study, it was found that higher percentage of individuals aged below 30 were less likely to wash their hands after contact with cats. This was in accordance with a previous study which showed that Malaysian pregnant women aged above 40 significantly reported to wash their hands after changing cat litters. Research done by Torrey and Yolken had found that 7-13 mg of soil could be collected from the nails of a cat after digging in dirt, harboring up to 100 oocysts [20]. This suggests that individuals who have contact with cats are still at risk for contracting toxoplasmosis even in the absence of any handling of soil. However, based on a review study on that aspect, it is surprising that no significant relationship was found between contact with cats and toxoplasmosis in most studies, although such finding in the developing world (e.g. Malaysia) is yet to be evaluated [21]. Nevertheless, CDC advices hand washing immediately after being around pets, with or without contact. Ideally, hands should be washed with soap under running water. Hand sanitizers could be used as an alternative in the absence of soap and running water [22].

Our results have also shown that a higher percentage of the younger age group did not cut their fingernails in the past 7 days, as compared to those aged 30 and above. Further analysis showed that there is no significant difference between genders within

the below 30 age group. It is believed that younger generations, regardless of gender, preferred to keep their fingernails long as they appear to enhance beauty and in some contexts symbolize higher socioeconomic status [23]. In China around 3000 BC, long fingernails were seen as aristocratic, and fingernails were painted in different colours to represent social classes [24,25]. In today's modern society, fingernails have evolved as a beauty standard, especially among young females. According to Patrick & Van Wicklin, nail polish with uneven surface and nail polish worn for more than four days can harbor potentially harmful microorganisms [26]. Therefore, the possibility of T. gondii inoculating fingernails exists if proper hand hygiene is not observed, particularly after handling of cats and soil. This inoculum could subsequently be transferred onto food and ingested if hand hygiene before eating is neglected. As fingernails grow faster during pregnancy, pregnant women should pay closer attention to their hand hygiene by always washing with soap, remembering to scrub their fingernails and trimming their nails more often [27]. With regard to assessing knowledge of participants concerning toxoplasmosis, this is the first study done on the general population of Malaysia. Majority of the participants in this study had relatively low awareness and knowledge about toxoplasmosis (3.4%). Prior research was centered on pregnant women attending antenatal care clinics where 11% were found to have heard of toxoplasmosis in contrast to our results [6]. However comparisons between the two studies should be avoided because pregnant women in the previous study were given a briefing concerning toxoplasmosis prior to assessing their knowledge, they also had access to and was engaged with regular antenatal care which increases the likelihood of them having heard of toxoplasmosis via the TORCH screening [28]. Females were found to be more aware of the disease in this study, likely due to realization that women and children are more vulnerable to zoonotic diseases [29]. This result was in line with majority of studies stating that higher seroprevalence of toxoplasmosis was observed in males than females in Malaysia, as a result of negligence [11]. Skilled workers were found to have higher awareness about toxoplasmosis and that it can be transmitted vertically. This was in accordance with the study conducted by Cediel et al., stating that unskilled workers had lower perception of zoonotic diseases [30]. However our finding was unexpected as studies in Northwestern Italy and United States have found that empowerment of workers with appropriate education and training is essential in promoting awareness about zoonosis [30,31]. No such education or training programs exist in Malaysia beyond specific skilled and unskilled fields such as healthcare and food handling respectively.

This study had also highlighted that 169 (53.0%) participants were highly knowledgeable about prevention of *T. gondii*, exceeding the percentage of participants who had general knowledge about toxoplasmosis. This is possibly due to the fact that despite all lack of knowledge about toxoplasmosis, there was certain level of awareness about other zoonotic infections among the participants. Studies conducted by Pawlowski et al., and Andiappan et al., also showed that most of their participants had limited knowledge about toxoplasmosis, yet they practiced preventive behaviour routinely [6,8]. Similar patterns were found in studies conducted in the United States and North East

Ethiopia [32,33]. In this study, the aforementioned pattern was found to be more evident in the lower monthly income group. This could be because of repeatedly falling sick and recognition over time that they are more vulnerable to diseases. Statistical significance was also found whereby women aged 30 and above had higher level of knowledge in comparison to women aged below 30. It was believed that women from this age group were more likely to be married and had children, thus were previously exposed to the TORCH screening and were taught about related preventive measures.

The study by Pawlowski et al., conducted in Poland to assess the efficacy of health education programs carried out over the early to mid-90s, showed that the combination of educational health promotion campaigns in mass media as well as key demographics such as pregnant women, has been proven effective, whereby there was approximately two times increased in awareness of toxoplasmosis and knowledge about its relevant preventive measures within 4 years [8].

LIMITATIONS

The sample size was relatively small due to time constraint for data collection. Hence, the results were not representative of the whole rural and semi-urban community in Malaysia. It should be noted that as this study relied on participants' self-reporting on habits such as hand hygiene, a socially desirable activity, the data likely underestimates the actual population at risk due to reporting bias [18,34]. Besides that, there was a potential bias due to misconception between toxoplasmosis and non-zoonotic diseases among the participants. Furthermore, this study does not investigate any associations between specific^[2] ethnic groups and risk factors despite previous research indicating variable seroprevalence between different ethnic groups. However, this current study only served as a pioneer study assessing the knowledge, attitude and practices related to toxoplasmosis among rural and semi-urban community in Malaysia.

CONCLUSION

In conclusion, the present findings have drawn attention to poor awareness and knowledge about toxoplasmosis among the rural and semi-urban community in Malaysia. It is important to identify the knowledge gap and provide adequate education in order to halt the rising prevalence and reduce the disease burden on society. Similar improvement in the rural and semi-urban population of Malaysia to that of Poland's, as found by Pawlowski et al., in their aforementioned study, seems possible given the already high prevalence of common preventative measures such as hand washing and the similarity of toxoplasmosis preventive measures to those of other more common infectious diseases.

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