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Seroprevalence of *Toxoplasma gondii* Among Schizophrenics at Hospital Kajang

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ABSTRACT

Studies have found a high seroprevalence of *T. gondii* in schizophrenia patients than controls. This case-control study was done to determine the serprevalence of *Toxoplasma gondii* and to identify its risk factors among schizophrenia patients at Hospital Kajang. Eighty-eight (88) schizophrenia patients and 88 non-psychiatric controls were examined for the presence of anti-*Toxoplasma* IgG and IgM antibodies by Enzyme Linked Immunosorbent Assay (ELISA). Questionnaires were used to collect socio-demographic and behavioral data among the respondents. From the results, the seroprevalence of anti-*T. gondii* IgG antibodies was significantly higher (51.0%) in schizophrenia patients than in controls (30.7%); (OR = 2.01; 95% CI: 2.16-3.01; p = 0.023. There was no significant association between the socio-demographic factors and toxoplasmosis. Beef consumption (p = 0.004, OR = 3.852, CI: 1.550-9.569), pork consumption (p<0.001, OR = 13.089, 95%CI: 4.730-36.219) and risky cat contact (p = 0.047, OR = 4.061, 95% CI: 1.985-16.745), were found to be significantly associated with *T. gondii* infection. Our results show that there is a relationship between toxoplasmosis and schizophrenia.

Keywords: *Toxoplasma Gondii*, Schizophrenia, Seroprevalence, Socio-Demographic Risk Factors, Environmental Risk Factors

1. INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite in the phylum Apicomplexa. Cats are the only definitive hosts. *T. gondii* however, also infects varieties of intermediate hosts, including humans (Dubey and Jones, 2008). Infection with *T. gondii* occurs pre- natally or post-natally. After birth, humans are generally infected with *T. gondii* after the ingestion of oocysts in contaminated soil or water, usually with cat feces. Infection also occurs by the ingestion of tissue cysts in undercooked meat (Dubey *et al*., 2008). Congenital infection also occurs and can be very fatal to the fetus (Edelhofer and Prossinger, 2010).

Schizophrenia is a complex chronic neuropsychiatric disease of the central nervous system, believed to have multiple etiologies (Torrey *et al*., 2007). Latent toxoplasmosis occurs when the bradyzoite or cyst form inhabits the brain when in the brain, the parasitecan then institute infection within the Central Nervous System (CNS), manipulate the host behavior and can cause neurological and psychiatric symptoms in some infected individuals (Brown and Derkits, 2010). From a recent research carried out by Hamidinejat *et al.* (2010) in Iran,

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it was concluded that *T. gondii* plays a role in the etiology of schizophrenia.

The aims of this study were to determine the seroprevalence of *Toxoplasma gondii* and associated environmental risk factors in schizophrenia patients and non-psychiatric controls, thereby providing an important preliminary data in Malaysia.

2. MATERIALS AND METHODS

The study was conducted at Hospital Kajang. It is a Government-funded district Hospital, founded in 1889 and it is located in the eastern part of Kajang town in the district of Hulu Langat in Selangor, Malaysia. Its land measures 16 acres and is situated about 30 km northeast of Kuala Lumpur. This matched case-control study was conducted in 2 populations: Eighty-eight (88) schizophrenia patients recruited from the Psychiatry department and 88 non-psychiatric controls from the Medical and orthopedic department. Each case was matched to a control by age gender and race (Alvarado-Esquivel *et al*., 2011a). The inclusion criteria included immunocompetent patients aged 18 to 60 years that were not presented with any neurological diseases. Diagnosis were done by psychiatrists and confirmed by Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (First *et al*., 1977). The mean age for both groups was 39.42 (range 18-60) years (Alvarado-Esquivel *et al*., 2012).

Objectives and procedures of the research were explained to the subjects. Since this study involves invasive procedures, written consent form was filled up by respondents before the blood sample was collected. Approval was granted by the ethical committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia and The Medical Research Ethics Committee, Malaysia.

Socio-demographic and behavioral characteristics of the participants were collected through the aid of a standardized self-administered questionnaire. We categorized Socio-demographic data as age, gender, birthplace, urban or rural dwelling, educational level, socioeconomic status and occupation (Goodwin *et al*., 2011). Putative risk factors comprised cat contacts, contact with cat feces; meat consumption (pork, beef, goat, sheep, mutton, chicken, or other); degree of meat cooking; consumption of untreated water, unwashed raw vegetables, fruits and contact with soil (gardening or agriculture) (Leweke *et al*., 2004).

Serum samples were obtained by the centrifugation (at 3000 rpm for 10 min) of blood collected from the participants and kept at-20°C pending ELISA tests. (Bouhamdan *et al*., 2010) Serum samples were assayed for anti *T. gondii* IgG antibodies with a commercially available enzyme immunoassay; Anti-*Toxoplasma* IgG EIA well (RADIM, Italy) and microtiter plate reader (450- 630 nm, DYNEX, MRX) was used to read the optical density of the plates. The samples considered positive were further tested for anti–*T. gondii* IgM antibodies by a commercially available enzyme immunoassay; Anti-*Toxoplasma* IgM EIA well (RADIM, Italy). All tests were performed following the instructions of the manufacturer.

2.1. Statistical Analysis

SPSS version 20.0 was used in analysis. McNemar chi-square analysis was performed to compare the seroprevalence of anti-*Toxoplasma* IgG and anti-*Toxoplasma* IgM antibodies among schizophrenia patients and controls. Odds Ratio (OR) and 95% confidence interval were calculated by multivariate analysis using conditional logistic regression, thus determining the level of risk involved in the study putative environmental risk factors and the subsequent association with *T. gondii* seropositivity. A value of $p<0.05$ was considered statistically significant.

3. RESULTS

Individuals with schizophrenia had increased levels of serum IgG antibodies to *T. gondii* (51%), compared with controls (30.7%). The difference in the seroprevalences was significant (OR = 2.01; 95% CI: 2.16-3.01; p = 0.023) as shown in **Table 1**. IgM antibodies to *T. gondii*, both cases and controls had the same level of seropositivity of 1.1% (p =1.00). For the sociodemographic characteristics, no significant relationship was found among the study variables ($p>0.05$), as shown in **Table 2**.

From the results of the logistic regression conducted, (**Table 3**) *T. gondii* infection was positively associated with several variables; beef consumption (OR = 3.852; 95%CI: 1.550-9.569; p = 0.004); pork consumption (OR = 13.089; 95%CI: 4.730-36.219; $p<0.001$); and risky cat contact (OR 4.061; 95%CI: 1.985-16.745; p = 0.047). Other characteristics in the population did not have any association with anti *T. gondii* IgG seropositivity.

Table 1. Anti-*Toxoplasma* IgG and IgM anti-bodies Analyses in Schizophrenia Patients and Controls

Variable	Cases (schizophrenia patients)	Controls	Chi-square	p-value
IgG positive (%)	45 (51)	27 (30.7)	0.709	
IgG negative (%)	43 (49)	61 (69.3)		0.023*
IgM positive (%)	1 (1.1)	1 (1.1)	0.914	
IgM negative (%)	87 (98.9)	87 (98.9)		1.00

OR: 2.01, 95%CI: (2.16- 3.01); McNemar chi-square

Table 2. Socio-demographic Characteristics and seroprevalence of *T. gondii* IgG antibodies in the study Populations

		Cases			Controls			
Variable	No. (n = 88)	No	(%)	No. (n = 88)	No	(%)	Chi-Square	p-value
Age groups								
18- 30	25	14	(56.0)	25	6	(24.0)		1.00
31- 50	45	21	(46.7)	45	16	(35.6)	3.43	
51-60	18	10	(55.6)	18	5	(27.8)		
Gender								
Male	55	26	(47.3)	55	14	(25.5)		
1.00								
Female	33	19	(57.6)	33	13	(39.4)	5.39	
Ethnicity								
Malay	29	13	(44.8)	29	12	(41.4)		1.00
Chinese	45	25	(55.6)	45	19	(42.0)	4.89	
Indian	13	7	(53.8)	13	4	(30.7)		
Others	1	0	0.0	1	0	0.0		
Education level								
No formal Education	5	1	(20.0)		1	(50.0)		0.05
Primary	15	12	(80.0)		4	(28.6)		
Secondary	59	29	(49.2)		18	(39.1)	8.18	
College	9	3	(33.3)		3	(27.3)		
University	0	0	0.0		1	(6.7)		
Residence								
Rural	20	7	(35.0)	3	0	0.0		0.66
Urban	68	38	(55.9)	85	27	(31.8)	2.69	
Income								
None	66	37	(56.1)	23	3	(13.0)		0.86
Low	15	5	(33.3)	36	15	(41.7)		
Medium	6	2	(33.3)	25	9	(36.0)	4.26	
High	1	0	0.0	4	0	0.0		
Marital status								
Single	61	34	(55.7)	28	4	(14.3)		0.09
Married	19	8	(42.1)	59	22	(37.3)		
Divorced	4	0	0.0	1	1	(100)	2.37	
Widow/Widower	4	2	(50.0)	0	0	0.0		
Occupation								
Employed	25	9	(36)	62	12	(19.4)	3.20	0.08
Non-employed	63	36	(57.1)	26	6	(23.0)		

*: Significant at p<0.05; **: Significant at p<0.01; McNemar chi-square

Table 3. Logistic regression analysis of environmental factors associated with *T. gondii* infection among study respondents

Variable	B	Odds Ratio	95% C.I. Lower	95% C.I. Upper	p-value
Wash hands before food					
Cases	1.450	1.206	0.311	58.497	0.278
Controls	0.712	0.140	0.032	1.182	
Wash hands after toilet-					
Cases	3.374	0.425	0.090	2.090	0.343
Controls	2.069	1.002	0.110	1.707	
Work in garden					
Cases	2.001	0.115	1.417	8.662	0.433
Controls	2.033	1.902	1.068	2.454	
Cigarette smoking					
Cases	2.440	0.870	0.017	0.451	2.230
Controls	2.921	0.560	2.273	6.618	
Beef consumption					
Cases	-1.650	3.852	1.550	9.569	0.004*
Controls	1.616	1.785	0.970	6.091	
Mutton consumption					
Cases	0.005	1.902	0.178	5.676	0.145
Controls	-1.269	2.092	0.051	1.550	
Pork consumption					
Cases	-3.458	13.089	4.730	6.210	0.000**
Controls	1.801	10.225	0.902	4.700	
Chicken consumption					
Cases	6.386	1.030	0.095	9.056	0.099
Controls	-2.904	2.371	0.002	1.727	
Drink untreated water					
Cases	2.619	3.243	0.035	1.282	0.551
Controls	0.416	0.613	0.302	7.601	
Barbeque /satay consumption					
Cases	-1.562	0.260	0.381	9.507	0.281
Controls	-1.562	1.209	0.012	3.597	
Cat contact					
Cases	-1.392	4.061	1.985	16.745	0.047*
Controls	-0.176	0.152	0.126	5.593	

Conditional logistic regression; *: Significant at $p<0.05$; **: Significant at $p<0.01$

4. DISCUSSION

Our results showed a higher seroprevalence of anti*T.gondii* IgG antibodies in schizophrenia patients than in controls matched by age, gender and race, thus confirming results by Alvarado-Esquivel *et al*. (2011b); Alipour *et al*. (2011) and Hamidinejat *et al*. (2010). IgG antibodies were not significantly different between the groups due to the fact that IgM antibodies become negative within 4-12 weeks and are indicators of a recent infection.

The positive association between *T. gondii* infection and meat consumption from this study result confirms results from studies done in Mexico by Alvarado-Esquivel *et al.* (2011a); In China by Wang *et al*. (2006) and in the United States by Torrey *et al.* (2012). The prevalence of meat-related factors highlights the significance of cooking meat well (>67°C). Chickens bred for commercial purposes are possibly not included in the transmission of *T. gondii* because methods applied in the modern breeding process decrease soil contact, the chickens are usually kept in its frozen state before consumption(Cavalcante *et al*., 2006; Dubey *et al*., 2007).

From our results, there is a positive association between cat contact and *T. gondii* infection. This suggests that infection may be acquired by cleaning cat excrement containing the parasites. Association between *T. gondii* seropositivity and cat contact was also found in studies done by Alvarado-Esquivel *et al*. (2011b) among low *Toxoplasma* seroprevalence population of Mexico, in which from the bivariate analysis of identified behavioral characteristics,

cleaning cat excrement was significantly associated with *T. gondii* infection (p = 0.001) and other study behavioral characteristics including meat consumption (raw or undercooked meat), consumption of unpasteurized milk, fruits, vegetables, contact with soil and types of floors at home did not show any association with *T. gondii* infection. Our results also confirms those done in Malaysia by Sinniah *et al*. (1984) and Nissapatorn *et al.* (2003).

Toxoplasma gondii infection was also found to be associated with pork consumption (p<0.001). From studies done by Alvarado-Esquivel *et al*. (2012) on a subset of Mexican patients with work accidents and low socioeconomic background, multivariate analysis showed a significant association of *T.gondii* to ham consumption (OR = 0.16; 95%CI: 0.05-0.51; p = 0.002). As food safety has become a focal issue in food production and marketing worldwide, consumers demand not only high quality foods, but also safe foods.

5. CONCLUSION

Our results confirm recent findings that *Toxoplasma gondii* infection is significantly associated with schizophrenia. Our result indicates that toxoplasmosis might be a very serious but ignored public health problem. This study advanced further weight on the hypothesis that *T. gondii* is a risk factor for schizophrenia. The results also show that *T. gondii* is significantly associated with beef consumption, pork consumption and risky cat contact. Our limitation included a relatively lower sample size, which was as a result of limited logistics. Nonetheless, this finding is essential as a preliminary data in Malaysia in establishing an association between *T. gondii* and schizophrenia.

We recommend multicenter studies to outline a significant association and establish a more clearer relationship between *T. gondii* infection and schizophrenia patients.

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6.1. Conflict of Interest

No conflict of Interest to declare for this study.

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