Frequency of Toxoplasmosis among Zagazig University Students in 2017

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Background and study aim: Toxoplasmosis caused by *Toxoplasma gondii*, is a major health problem in which about 30% of people are born with anti Toxoplasma antibodies worldwide. Toxoplasmosis causes congenital infection, neurological and psychatric disorders. The present study aimed to determine prevalence and factors associated with *T. gondii* infection in Zagazig university students in 2017-2018.

Subjects and Methods: Through a cross-sectional study, This study was done in Tropical Medicine Outpatient Clinic and Clinical Pathology Department of Zagazig University Hospitals, and including 159 healthy students coming to do clinical and laboratory evaluation before university attendance at student year 2017-2018. Serum samples were collected and tested for IgG antibodies against *T. gondii* using ELISA method.

Results: In this study 159 healthy students are examined for anti-toxoplasma IgG, 68 males (42.8%) and 91 females (57.2%).65 students have Positive IgG (40.9%), 25.2% (N: 40) of them showing high titre more than 300 and 15.7% (N: 25) of them showing titre low than 300. The percentage of students have no infection is 59.1% (N: 94). There was a significant association between *T.gondii* seropositivity and age (p=0.007), dealing with animals (p=0.001) eating fast food (p=0.002) and drinking non boiled milk (p=0.001).

Conclusion: Toxoplasmosis is present in healthy students. Infection is more prevalent in rural areas due to lack of health education and more dealing with animals. Dealing with animals, eating fast foods and drinking non boiled milk are considered a major risk factor for *T.gondii* infection.

INTRODUCTION

Toxoplasma gondii (T.gondii) is a crucial intracellular protozoan parasite widely prevalent in humans and animals, throughout the planet **[1]** .It may be a specific parasite of the host (usually cats and other members of the Felidae family) but features a broad range of intermediate hosts, including humans and a number of other wild and livestock. The life cycle of *T.gondii* consists of three infective stages: oocysts which are present only in cat feces, tachyzoites present within the host during the acute stage of infection, and bradyzoites that are found in tissue cysts **[2]**.

T.gondii is transmitted to humans either congenitally, or via ingestion of under cooked or raw meat from infected animals, or ingestion of food or water contaminated with oocysts excreted by infected fields. It has been found that about 50% of human cases are meat born. **[3].**

The importance of domestic chickens (Gallus domesticus) in transmission of *T. gondii* was studied only in a few studies in Egypt. Poultry meat is a part of food, consumed widely all over the world; therefore, eating uncooked or not properly cooked poultry meat may have a risk factor for *T. gondii* infection in humans or animals **[4,5].** If we consider the most common food borne pathogens, human toxoplasmosis is considered second cause of death in Western countries.**[6].**

SUBJECTS AND METHOD

This observational cross sectional study was done in Tropical Medicine Outpatient Clinic and Clinical Pathology Department of Zagazig University Hospital, and including 159 healthy students coming to do clinical and laboratory evaluation before university attendance at student year 2017-2018.Informed consent was taken from all participants in the study and IRP approval was taken. We exclude students with history of chronic disease as diabetes, chronic renal or liver failure or malignancy.

All participants were subjected to the following**:** Full medical history with stress on socio demographic characteristics, eating habits; questionnaire is fulfilled by the students including (name, age, residence, marriage, dealing with animals and type of animals (including cats ,dogs, dogs& cats, poultry and cows),eating fast food and type of food (such as : burger, beef, lunchon, uncooked meat),drinking non boiled milk, dealing with soil and blood transfusion),thorough clinical examination (general and local) especially lymph node examination, laboratory investigation: CBC, HCV Ab and (Anti toxoplasma IgG) using ELIZA technique.

3 ml venous blood was taken from every student in plain vacuum tube to clot for 20 minutes at 37c then centrifuged for 5 minutes on 3000 rpm, sera were separated and stored at -20c till used and sandwich principle was used in technique. Results are determined via a calibration curve which is instrument specifically generated by 2 point calibration and a master curve provided via the reagent barcode.

Toxo IgG testing is used as first line screening assay and considered nonreactive if< 1 IU/mL, indeterminate if ≥ 1:< 3 IU/mL and reactive if ≥ 3 IU/mL. A negative test result does not completely rule out infection with *T. gondii*. People may not exhibit any detectable IgG antibodies at the early stage of acute infection. The detection of Toxoplasma specific IgG antibodies in a single sample indicates a previous exposure to *T. gondii* but is not sufficient to distinguish between an acute or latent infection (irrespective of the level of the IgG antibody titer).

Measuring range from 0.13-650 IU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.13 IU/mL. Values above the measuring range are reported as > 650 IU/mL (or up to 13000 IU/mL for 20 fold diluted samples).Samples with values above the measuring range can be diluted with Diluents' Universal.

RESULTS

Table (1) shows: Toxoplasmosis infection among the studied subjects using IgG titre. The mean titre is 138.26+_215.05 with a range of (.13-650).65 students have positive IgG (40.9%), 25.2% (N: 40) of them showing high titre more than 300 and 15.7% (N: 25) of them showing titre lower than 300. The percentage of students have no infection is 59.1% (N: 94).

Table (2) shows: Toxoplasmosis infection among the studied subjects using IgG titre. The mean titre is 138.26+_215.05.65 students have Positive IgG (40.9%), 25.2% (N: 40) of them showing high titre more than 300 and 15.7% (N: 25) of them showing titre low than 300. The percentage of students have no infection is 59.1% (N: 94).Comparison between negative and positive anti- toxoplasma IgG titre (either less than or more than 300) as regarding to demographic data. There is significant difference result as regarding age (P value=.024), but no significant difference as regarding residence and marital status, P value =(0.050) & (0.965) respectively.

Table (3) shows: Comparison between negative anti-Toxoplasmosis IgG, positive anti-Toxoplasmosis IgG titre (less than 300 and more than 300) as regard dealing with animals. There is high significance in students dealing with animals as regarding IgG titre as 92 students (57.9%) are dealing with animals,24 (26.1%) are positive (titre<300) ,and 37 (40.2%) of them are positive (titre>300), P value=(<0.001). There is significant difference result in dealing with cats, but no significant difference in dealing with dogs, cows, poultry and soil.

Table (4) shows: Comparison between negative anti-Toxoplasmosis IgG, positive anti-Toxoplasmosis IgG titre (less than 300 and more than 300) as regard fast food eating. There is significant difference in students eating fast food as regarding titre as there is 142 (89.3%) students are eating fast food,25 (17.6%) of them are positive (titre<300).and 39 (27.5%) of them are positive (titre>300). P value = (0.008).There is high significant difference in students drinking non boiled milk, 13(8.2%) students are drinking non boiled milk, 3(23.1%) of them are positive (titre<300), and 10 (76.9%) of them are positive (titre>300), P value= (<0.001)

Table (1): Toxoplasmosis infection among the studied subjects (N=159).

Toxoplasmosis infection	The studied subjects (N=159)	
	No.	%
Toxoplasmosis IgG Titre		
Mean ± SD	138.26 ± 215.05	
Median (Range)	325.065 (0.13 – 650)	
Negative	94	59.1%
Positive	65	40.9%
<300	25	15.7%
>300	40	25.2%

Table (2): Comparison between negative anti-Toxoplasmosis IgG, positive anti-Toxoplasma IgG titre (either less than 300 and more than 300) as regard demographic data.

Demographic Data	All (N=159)		Anti-Toxoplasmosis IgG (mean titre138.26±215.05						Test	p-value (Sig.)
			Negative (N=94)(59.1%)		Positive <300 (N=25)(15.7%)		Positive >300 (N=40)(25.2%)			
	No.	%	No.	%	No.	%	No.	%		
Sex										
Male	68	42.8%	45	66.2%	9	13.2%	14	20.6%	2.455	0.293 (NS)
Female	91	57.2%	49	53.8%	16	17.6%	26	28.6%		
Age (days)										
Mean ± SD	19.81 ± 0.40		19.73 ± 0.44		19.90 ± 0.27		19.92 ± 0.34		**8.112**	**0.017 (S)**
Median (Range)	20 (18 – 21)		20 (19 – 21)		20 (19 – 21)		20 (18 – 21)			
≥20 years	31	19.5%	25	80.6%	2	6.5%	4	12.9%	**7.422**	**0.024 (S)**
<20 years	128	80.5%	69	53.9%	23	18%	36	28.1%		
Residence										
Urban	80	50.3%	52	65%	7	8.8%	21	26.2%	5.998	0.050 (NS)
Rural	79	49.7%	42	53.2%	18	22.8%	19	24.1%		
Marital status										
Unmarried	151	95%	89	58.9%	24	15.9%	38	25.2%	0.072	0.965 (NS)
Married	8	5%	5	62.5%	1	12.5%	2	25%		

Kruskal Wallis H test.
Chi-square test.
p< 0.05 is significant.

Table (3): Comparison between negative anti-Toxoplasmosis IgG, positive anti-Toxoplasma IgG titre (less than 300 and more than 300) as regard dealing with animals.

Dealing with Animals & soil	All (N=159)		Anti-Toxoplasmosis IgG						Test	p-value (Sig.)
			Negative (N=94)		Positive <300 (N=25)		Positive >300 (N=40)			
	No.	%	No.	%	No.	%	No.	%		
Any animals										
No	67	42.1%	46	68.7%	7	10.4%	14	20.9%	**15.721**	**<0.001 (HS)**
Yes	92	57.9%	31	33.7%	24	26.1%	37	40.2%		
Cats	19	11.95%	7	36.8%	5	26.3%	7	36.8%	**26.276**	**0.003 (S)**
Dogs	5	3.15%	4	80%	0	0%	1	20%		
Cats & dogs	26	16.4%	3	11.5%	13	50%	10	38.5%		
Cows	17	10.7%	3	17.6%	4	23.5%	10	58.9%		
Poultry	25	15.7%	5	20%	5	20%	15	60%		
Cats										
No	114	71.7%	74	64.9%	14	12.3%	26	22.8%	**13.023**	**0.001 (S)**
Yes	45	28.3%	13	28.9%	15	33.3%	17	37.8%		
Dogs										
No	128	80.5%	78	60.9%	18	14.1%	32	25%	3.238	0.198 (NS)
Yes	31	19.5%	12	38.7%	9	29%	10	32.3%		
Cows										
No	142	89.3%	86	60.6%	22	15.5%	34	23.9%	3.888	0.143 (NS)
Yes	17	10.7%	3	17.6%	4	23.5%	10	58.9%		
Poultry										
No	134	84.3%	81	60.5%	24	15.6%	37	24%	2.897	0.123 (NS)
Yes	25	15.7%	5	20%	5	20%	15	60%		
Dealing with soil										
No	128	80.5%	80	62.5%	18	14.1%	30	23.4%	3.192	0.203 (NS)
Yes	31	19.5%	14	45.2%	7	22.6%	10	32.3%		

Chi-square test.
p< 0.05 is significant.
Sig.: significance

Table (4): Comparison between negative anti-Toxoplasmosis IgG, positive anti-Toxoplasmosis IgG titre (less than 300 and more than 300) as regard food borne.

Food borne exposure	All (N=159)		Anti-Toxoplasmosis IgG						Test	p-value (Sig.)
			Negative (N=94)		Positive <300 (N=25)		Positive >300 (N=40)			
	No.	%	No.	%	No.	%	No.	%		
Any fast food										
No	17	10.7%	16	94.1%	0	0%	1	5.9%	**9.748**	**0.008 (S)**
Yes	142	89.3%	78	54.9%	25	17.6%	39	27.5%		
Burger	36	22.6%	29	80.5%	6	16.7%	1	2.8%	**58.550**	**<0.001 (HS)**
Lunchon	28	17.6%	18	64.3%	4	14.3%	6	21.4%		
Beef	10	6.3%	7	70%	2	20%	1	10%		
Uncooked meat	10	6.3%	5	50%	4	40%	1	10%		
Burger & Lunchon	35	22%	13	37.1%	6	17.1%	16	45.7%		
Burger & Beef	17	10.7%	4	23.5%	3	17.6%	10	58.8%		
Lunchon & Beef	6	3.8%	1	16.7%	0	0%	5	83.3%		
Burger										
No	70	44%	48	68.6%	10	14.3%	12	17.1%	5.247	0.073 (NS)
Yes	89	56%	46	51.7%	15	16.8%	28	31.5%		
Lunchon										
No	90	56.6%	61	68.5%	15	16.9%	13	14.6%	**12.143**	**0.002 (S)**
Yes	69	43.4%	32	46.4%	10	14.3%	27	38.6%		
Beef										
No	126	79.2%	82	65.1%	20	15.9%	24	19%	**12.664**	**0.002 (S)**
Yes	33	20.8%	12	36.4%	5	15.2%	16	48.5%		
Uncooked meat										
No	149	93.7%	89	59.7%	21	14.1%	39	26.2%	5.125	0.077 (NS)
Yes	10	6.3%	5	50%	4	40%	1	10%		
Drinking non-boiled milk										
No	146	91.8%	94	64.4%	22	15.1%	30	20.5%	**23.937**	**<0.001 (HS)**
Yes	13	8.2%	0	0%	3	23.1%	10	76.9%		

Chi-square test.
p< 0.05 is significant.
Sig.: significance.

DISCUSSION

Toxoplasma gondii is an intracellular protozoan parasite highly prevalent in humans and animals, including poultry, in the world [**1,4**]. It has been evaluated that about 30 %of population has been infected with *Toxoplasma gondii* **[2]**. *T. gondii* is mostly transmitted to humans either congenitally, or via eating under cooked or raw meat of infected animals, or ingestion of food or water contaminated with oocysts excreted by infected animals **[7]**.

The aim of this study is to evaluate the frequency of toxoplasma IgG among Zagazig University Students and to evaluate some socio-demographic factors, characteristic features and eating habits in toxoplasma cases. In this study 159 healthy students are examined for anti-toxoplasma IgG, 68 males (42.8%) and 91 females (57.2%). Mean age is 19.81 ± 0.40 with a range of (18-21). 80 are urban (50.3%) and 79 are rural (49.7%). (table 2)

The overall seroprevalence of toxoplasma IgG in this study is (40.9%) 65 students.25 students (15.7%) have IgG titre<300 and 40 students (25.2%) have IgG titre>300 (Table 1).In comparison to previous studies conducted on healthy blood donors in Egypt, this rate of seroprevalence was lower than the prevalence in Alexandria Governorates (65.3%) [8] and also lower than the study reported from Mansoura governorate, Egypt, in 2009, in which the prevalence of *Toxoplasma* -specific IgG in blood donors, was 59.6% **[9]**. The incidence in this study was higher than the 19.5% incidence reported much earlier in 1986 from Cairo **[10]**.

When compared with different countries, the

recorded seropositivity was much higher than that reported in blood donors in Mexico (7.4%) **[11]**, Thailand (9%) **[12]**, Chile (21.2%) [13], Malaysia (28.1%) **[14]**, Czech Republic (33.1%) **[15]**. Although, seropositivity was lower than that reported in blood donors in Brazil (79.0%) **[16]** and in Cuba (73.43%), Mali (41.2%) **[17]**, and Saudi Arabia (52.1%) [**18]**.The variability in the prevalence levels of *T. gondii* infection among blood donor population may be attributed to the individual habits and characteristics of the populations.

In this study prevalence is higher in females (46.2%) than in males (33.8%) with no significant difference (Table 2). A similar finding was reported previously **[13,19,20,21]**. Increasing of anti-*T.gondii* antibodies among female donors may be due to more exposure of females to oocysts and tissue cysts during their daily activities. In contrast, *T. gondii* seropositivity was significantly higher among male donors as detected by 22. Sundar et al and Ormazdi et al**[22,23]**.

As regarding age, the prevalence is higher in young age (46.1% in age <20 years and 19.4%) in age >=20 years) with significant difference (Table 2). This may be due to more consumption of this age group to fast food. Such a finding was in contrary with those observed in other studies **[9,11,16]** that showed increase in *T.gondii* prevalence in older age groups (35-45).

In this study seropositivity in rural areas (46.8%) is higher than in urban areas (35%).This finding is in agreement with El sheikha [**9]** and Mahmoud [**21**] who showed significant difference between rural and urban areas and may be due to low socioeconomic standards, lack of hand hygiene before meals, eating unwashed vegetables, drinking unfiltered water and frequent exposure to animal excreta.

As regarding dealing with animals, prevalence in students dealing with animals (66.3%) is higher than those not dealing with animals (31.3%) with high significant difference ,especially those dealing with cats, cows and poultry (Table 3). This is in agreement with study done in Benisuef, Egypt which found a relation between toxoplasma infection and contact with chickens **[24]**. Also El sheikha and Alvarado-Esquivel found relation between toxoplasmosis and contact with cats **[11,25]**. However, in contrary to our study El-Deeb **[26]** found no significant correlation between seropositivity to toxoplasma antibodies and contact with domestic cats in Menoufia, Egypt. This may be attributed to prescence of pet cats and stray cats which are more exposed to parasites.

As regarding food-borne, prevalence of toxoplasmosis in students eating fast food (45.1%) is higher than those not eating fast food (5.9%) with high significant difference (Table 4) with high prevalence in eating more than one type of fast food ,this may be attributed to more exposure to cysts in undercooked or raw meat. Our study is in agreement with previous studies done by Mahmoud **[21]** and Excler **[27]**. Similarly, several studies have reported the food-borne transmission as a major infection route in blood donors **[23,29]**.

This study showed high prevalence of toxoplasmosis in students drinking non boiled milk or homemade cheese (100%) with high significant difference (Table 4).The data suggest that drinking raw milk from infected animals might be another possible route for the transmission of *T.gondii* as was reported by Sacks **[30]**. This also in agreement with study done in pregnant women in Sharkia, Egypt that showed a correlation between raw milk and toxoplasmosis **[31]**. Similarly, El-Deeb **[26]** in Menoufia, Egypt, found significant correlation between raw milk consumption and *T.gondii* seroprevalence. In contrary, this finding was in contrast with other studies in USA **[29]**, Ethiopia **[32]**, Brazil **[33]** and Kyrgyz Republic [**34]** that reported no significant relation between raw milk consumption and *T. gondii* seroprevalence. This could be attributed to the habit in using raw milk for making cheese in the current study and hygienic measures and sanitation used in growing animals in developed countries.

As regarding dealing with soil, Prevalence in students dealing (54.8%) is higher than those not dealing with soil (37.5%), with no significant difference (Table 3).This finding corroborates with other studies in USA **[29]**, Ethiopia **[35]** and Brazil **[33,36]**.

As regarding anti-toxoplasmosis IgG titre, (13.2%) of male cases had IgG titre less than 300IU/ml and (20.6%) of males had IgG titre more than 300 IU/ml. Also,(17.6%) of females showed IgG titre less than 300 IU/ml and (28.6%) showed titre more than 300 IU/ml. Also,(28.1%) of subjects <20 years had positive IgG titre>300 IU/ml and 18% had IgG titre<300IU/ml. 26.2% of urban subjects showed

IgG titre>300 IU/ml and 24.1% of rural subjects had IgG titre>300 IU/ml (Table 2).As regarding dealing with animals, 40.2% of the studied subjects had IgG titre>300IU/ml and were dealing with poultry, cows, cats and dogs (Table 3). As regarding fast food ,89.3% of subjects were eating fast food and 27.5% of them showed anti- toxoplasma IgG titre>300 IU/ml (Table 4). More studies are needed to validate various titres as a cut off value for diagnosis.

CONCLUSION

Toxoplasmosis is present in apparently healthy students. It is more prevalent in young age group. Infection is more prevalent in rural areas due to lack of health education and more dealing with animals. Dealing with animals, eating fast foods and drinking non boiled milk are considered a major risk factor for *T.gondii* infection. Eating fast food mainly Burger, Lunchon and uncooked meat are hidden sources for toxoplasma transmission. Increased consumption of fast food between students is a source of toxoplasma infection even without symptoms.

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Ethical consideration: the study design was revised and approved by the institutional review board in the Faculty of Medicine, Zagazig University.

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