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Sero-diagnosis of toxoplasmosis by using lateral flow chromatographic assay

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ABSTRACT

The study was carried out to determine the anti-*Toxoplasma gondii* antibody in human, goats, sheep and cat in the selected areas of Savar, Dhaka and Bangladesh Agricultural University (BAU) campus, Mymensingh, Bangladesh. A total of 116 blood samples of which 75 human, 15 goats, 15 sheep and 11 cats samples were collect and tested immediately by using ARIA Toxo IgG/IgM Combo Rapid Test (CTK Biotech. Inc., USA). Information like age, sex, pregnancy status, occupation, availability of cat was gathered from attendants during collection of blood. Obtained data were analyzed by Chi square test as well as p-value was calculated to show the association of risk factors with the disease. Sero-prevalence data revealed that *T. gondii* was 10.66% positive in human, 6.66% in goats and sheep, and 36.36% in cat. 17.39% human of 31-45 years age was seropositive with T. gondii followed by 21-30 years old human. There was significant association of seropositive cases with abortion (66.66%, P=0.0001) in human. Goat butchers were highly affected (18.18%) followed by garments' worker (10.71%), housewife (10.52%) and service holder (8.33%). The results indicate that *T. gondii* is prevalent among the human and animal population which further indicate the zoonotic transmission of this parasite in the study areas.

INTRODUCTION

Toxoplasmosis is a major parasitic zoonotic disease of almost all warm blooded animals including human beings. This disease is caused by an obligate intracellular protozoan parasite *Toxoplasma gondii*. Based on serological investigations, it is estimated that a one third of the world's human population has been exposed to this widespread zoonotic agent (Innes, 2010; Pereira et al., 2010; Montoya and Liesenfeld, 2012). On the other hand, animals may represent a real risk for transmission of the disease to humans, either directly or indirectly (Cenci Goga et al., 2011*). T. gondii* infections are more prevalent in pigs, sheep, and goats than in cattle. Toxoplasmosis causes considerable economic losses to the sheep and goats industry. *T. gondii* induced abortion especially for maiden ewes (Dubey et al., 2013). People usually acquire infection via ingestion of tissue cysts in undercooked meat, consuming food and water that has been contaminated with sporulated oocysts, or by accidentally ingesting oocysts from the environment, or vertically by transplacental transmission of tachyzoites. Congenital toxoplasmosis generally occurs when a woman is newly infected with *T. gondii* during pregnancy. Infection with *T. gondii* during pregnancy can result in fetal death, neonatal death or various congenital defects, such as hydrocephalus, central nervous system abnormalities and chorioretinitis (Zewdu et al., 2013). There are numerous serological methods are available for the detection of IgG and IgM antibodies; these are Sabin-Feldman Dye Test (DT), Indirect Hemagglutination Assay (IHA), Indirect Fluorescent Antibody Assay (IFA), Modified Agglutination Test (MAT), Latex Agglutination Test (LAT), Enzyme-Linked Immunosorbent Assay (ELISA), and Complement Fixation Test (Pal, 2007). Sero-prevalence in different species may vary according to different environments, social customs and habits. Therefore, the present study was designed to determine the sero-prevalence of *T. gondii* in sheep, goats, human and cat in Mymensingh and Dhaka district of Bangladesh.

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MATERIALS AND METHODS

Sample Collection

A total of 75 human blood samples were collected from Prime Hospital and Enam Medical College, Savar. The consent of patient was taken following describing the project aims and objectives before collecting blood. About 2-3 ml of blood sample was collected in a tube aseptically by venipuncture and subjected for examination immediately at the same place. On the other hand, 30 blood samples of sheep and goats were collected and examined after collection at Bangladesh Livestock Research Institute (BLRI), Savar. Blood sample of cats were also collected from Savar, Bangladesh Agricultural University (BAU) campus, Mymensingh and tested at the Department of Parasitology, BAU. Basic relevant information like age, sex, status of body condition-pregnant, aborted, non-pregnant, availability of cats, occupation of human and cooking method of meat were also taken from patient attendants both for human and animals during blood collection in order to analyze the data conveniently.

Rapid diagnostic testing of toxoplasmosis

The blood sample was tested by using a rapid test kit (ARIA Toxo IgG/IgM Combo Rapid Test, CTK Biotech. Inc., USA). The test is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of IgG and IgM anti-*Toxoplasma gondii* (*T. gondii*) in human serum, plasma or whole blood.

Although the kit was developed only for human but it was also used for animal. The kit is a lateral flow chromatographic immunoassay and consists of: 1) A burgundy colored conjugate pad containing recombinant *T. gondii* antigens conjugated with colloidal gold (*T. gondii* conjugates) and rabbit IgG-gold conjugates, 2) A nitrocellulose membrane strip containing two test lines (g and m lines) and a control line (c line).

The m line is pre-coated with monoclonal anti-human IgM for the detection of IgM anti- *T. gondii* antibody, the g line is pre-coated with reagents for the detection of IgG anti- *T. gondii* antibody and the c line are pre-coated with a control line antibody. When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the cassette. IgM anti- *T. gondii,* if present in the specimen, will bind to the *T. gondii* conjugates. The immune complex is then captured on the membrane by the pre-coated anti human IgM antibody forming a burgundy colored m line, indicating a *T. gondii* IgM positive result. IgG anti- *T. gondii* if present in specimen will bind to the *T. gondii* conjugates. The immune complex is then captured by the pre-coated reagents on the membrane forming a burgundy colored g line, indicating a *T. gondii* IgG positive result. Absence of any T lines (m and g) indicates a negative result. If the c line does not develop, the test result is invalid and then repetition of the assay is needed.

A total of 50 µl of plasma was placed into the sample well and a drop of sample diluent added and the results read within 15 min. Specimens were brought at room temperature about 30 minutes before performing the test if refrigerated.

Data analysis

All the collected data were compiled in Microsoft Excel sheet. Sero-prevalence percentage and Chi square test was also performed.

RESULTS AND DISCUSSION

Toxoplasmosis in humans

In human the overall seroprevalence was 10.66%. The result was similar with the finding of Amany and Merward (2012), they found 10% seroprevalence in women in Egypt. Higher rate of prevalence (15.89%) was observed in women with different Gyno-obstetrical diseases by Samad et al. (1997); 22.68% in women by Nurjahan et al. (2015) and very high rate 38.5% was reported by Ashrafunnessa et al. (1998) in Bangladesh.

Table 1
Sero-positivity of toxoplasmosis in human, goats, sheep and cats.

Species	Tested sample	Positive sample	Prevalence (%)
Human	75	8	10.66
Goat	15	1	6.66
Sheep	15	1	6.66
Cat	11	4	36.36

Table 2
Distribution of *T. gondii* in human.

Variables	Tested sample	Positive sample	Prevalence (%)	Chi square	P value
Sex					
Female	55	6	10.90	0.0127	0.91
Male	20	2	10		
Age					
Up to 20 years	13	1	7.69		
21-30 years	39	3	7.69	1.574	0.455
31-45 years	23	4	17.39		
History of individual					
Abortion	6	4	66.66		
Not pregnant	48	2	4.16	21.901	0.00018
Not applicable	21	2	9.52		

The present study demonstrated association between toxoplasmosis and different age groups of human. The highest prevalence 17.39% was found among the age group of 31-45 year and this value was lower than the finding of Jafar et al. (2014) who reported 44% prevalence among 36-40 year age group; Nurjahan et al. (2015) reported 27.9% prevalence in age group 31-45 years. Toxoplasmosis was significantly (p value 0.0001) associated with abortion in human. Antibodies against *T. gondii* in human was found higher in butcher (18.18%) followed by 10.71%, 10.52%, and 8.33%, respectively in garments' worker, housewife, and service holder (Table 3). The results of this study indicated that human become infected to this parasite either by direct contact of oocysts or by ingesting oocysts directly from infected cat or animal or by contaminated food and water. People who are involved in slaughtering of animals and selling of meat in rural areas and there is not maintaining hygienic condition at shop and also no trend to wear gloves during meat cutting and selling. However, Illiteracy or absence of knowledge and lack of awareness may contribute to get the infection from the sources.

Toxoplasmosis in goats and sheep

All the tested goats and sheep were female. Here, toxoplasmosis was found highly significant (0.0001) in above 2 years old goats. Pregnant goats showed 9.09% positive (Table 4). The distributions of toxoplasmosis in sheep are shown in Table 5. High prevalence 16.66% was found in above 2 years old sheep and 25% in aborted sheep. Alessia et al. (2015) conduct a survey study from 474 goats and 502 sheep reared on 42 farms in northern Italy and tested for IgG antibodies to *T. gondii* by IFAT (indirect immunofluorescence antibody test) to evaluate *T. gondii* seroprevalence in small ruminants and possible risk factors associated with the infection and reported that 41.7% goats and 59.3% sheep resulted positive. Seroprevalence was significantly higher in sheep than in goats.

Table 3
Distribution of *T. gondii* in human based on occupation and availability of cat.

Variables	Tested sample	Positive sample	Prevalence (%)	Chi square	P value
Occupation					
Butcher	11	2	18.18		
Housewife	19	2	10.52		
Garments' worker	28	3	10.71	1.318	0.858
Service holder	12	1	8.33		
Student	5	0	0		
Contact with cat					
Yes	60	8	13.33	2.238	0.134
No	15	0	0		

Table 4
Distribution of *T. gondii* in goats.

Variables	Tested sample	Positive sample	Prevalence (%)	Chi square	P value
Sex (All female)	15	1	6.66	-	-
Age					
1.4- 2 years	14	0	0	15.0	0.0001
Above 2 years	1	1	100.0		
History of animal					
Pregnant	11	1	9.09	0.3896	0.532
Abortion	4	0	0		

Table 5
Distribution of *T. gondii* in sheep.

Variables	Tested sample	Positive sample	Prevalence (%)	Chi square	P value
Sex (All female)	15	1	6.66	-	-
Age					
1.4- 2 years	9	0	0	1.607	0.204
Above 2 years	6	1	16.66		
History of animal					
Pregnant	11	0	0	2.946	0.086
Abortion	4	1	25.0		

Nisar et al. (2015) reported that prevalence of *T. gondii* antibodies in sheep and goats present in northern parts of Punjab province, Pakistan was 18.16% and 14.32% respectively. Whereas Lahmar et al. (2015) reported that seroprevalence of T. gondi 40.2% in sheep and 34.5% in goats respectively. However the variation of the results might be due to variation of methods and geographical location of the study areas.

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