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SEROPREVALENCE OF ZONOTIC PROTOZOA *Toxoplasma gondii* INFECTION IN FOOD ANIMALS OF CHATTOGRAM DIVISION

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ABSTRACT

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Toxoplasmosis is a zoonotic protozoan parasitic disease caused by *Toxoplasma gondii*. The aim of the present study was to determine the prevalence of *T. gondii* infection in sheep, goats and cattle in Chattogram division. In his study a total of 220 sera samples from different animals were collected from different herds of Chattogram division. Among the 220 samples, 184 sera were examined for *T. gondii* antibody by indirect Enzyme-Linked Immunosorbent Assay (iELISA) (ID Screen® indirect ELISA kit, IDvet Laboratories, Inc., France) according to the manufacturer's instructions. Samples with more than 50% S/P were considered as positive for *T. gondii*. The overall prevalence of *T. gondii* was 13.59%. The highest prevalence of *T. gondii* was found in goat 16%, sheep 13.04% and cattle 11.90%. The highest prevalence (36.36%) of *T. gondii* infection was observed in sheep aged >5 years compared to other age groups. Similarly, the highest seroprevalence was found in >18 months old sheep (16.067% compared to <6 months group (5.88%) and pregnant (19.63%) and non-pregnant sheep (11.76%). In contrast to cattle and sheep, the seroprevalence of toxoplasmosis in goats were highest in 6 months to 18 months age group (20.83%) compared to >18 months age group (9.09%). Results indicate that *T. gondii* infection in food animals in Chattogram division is widespread. Further investigation on the isolation and characterization of *T. gondii* from the aborted fetus and its zoonotic potential on human population is imperative. The undercooked meat and raw milk of these food animals may serve as a potential source of *T. gondii* infection for humans.

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INTRODUCTION

Toxoplasmosis is one of the most important zoonotic diseases in human and animals caused by the protozoan *T. gondii*. A broad spectrum of animals can be infected by ingestion of raw or undercooked meat containing viable tissue cysts or by ingesting food or water contaminated with oocysts from the feces of infected cats (Dubey, 2004). It affects central nervous system, reproductive system and visceral organs (Dubey, 2004). The parasite infects one third of the human population worldwide and among food animals sheep and goats are well known sources of human infection (Dubey, 2010). Definitive hosts for this coccidian parasite are felids (both domestic and wild); and the intermediate hosts are mammals and birds (Dubey and Jones, 2008). An estimated 500 million humans have been infected with the protozoa (Bob, 2011). The intermediate hosts can be infected by ingesting food or water contaminated with oocysts, eating undercooked meat with tissue cysts or by transplacental infection with tachyzoites (Dubey and Jones, 2008; Dubey, 2010). *T. gondii* infection, however, is the major cause of abortion and perinatal mortality in sheep and goats (Buxton and Brebner, 1998). Sheep are considered important in the epidemiology of *T. gondii* infection worldwide, but especially in Europe (Buxton *et al.*, 2007). *T. gondii* causes subclinical infection in cattle. The use of serological test ELISA to detect the presence of these specific antibodies (IgM, IgA, IgE and IgG) to *T. gondii* antigens in the sera of infected mammals.

There have been a few studies reporting seroprevalence of *T. gondii* in sheep, goats and cattle in some parts of Bangladesh. According to a report, cattle, goats and sheep showed seroprevalence of *T. gondii* were 12%, 32% and 40%, respectively in Bangladesh. Seroprevalence in different populations may vary according to different environments, social customs and habits. Analysis of global sero-survey reports revealed that about 32.9% cats, 38.5% man, 29.0% sheep, 24.2% goats, 18.6% cattle, 20.7% swine, 16.9% horse, 39.3% dogs, 17.7% buffaloes and 18.7% camels had *T. gondii* antibodies (Samad and Begum, 1993). The fecal examination of 4232 cats from 22 reports of 10 countries showed that about 2.7% of the cat population would have been shedding *T. gondii* oocysts (Kamani *et al.*, 2010). Sero-surveillance studies on toxoplasmosis on man and animals showed 16 to 37% cattle, 17.65 to 53.6% sheep, 12.09 to 36.4% goats and 15.89% women had *T. gondii* antibodies in Bangladesh (Hossain *et al.*, 2018; Samad *et al.*, 1997). Number of few recent works reported the overall seroprevalence of toxoplasmosis was 12.2% and was significantly ($P=0.008$) higher in goats (16.0%) than cattle (8.3%). The odds of seropositivity was 2.09 times (95% confidence interval [CI]: 1.23–3.67) higher in goats than cattle in Bangladesh and Nepal. In sheep, herd type, district and pregnancy status were significant risk factors ((Sah *et al.*, 2019; Sah *et al.*, 2018a; Sah *et al.*, 2018b; Sah *et al.*, 2017). Therefore, investigation on the prevalence of toxoplasmosis in ruminants, its distribution and associated risk factors are important to know in Bangladesh. By considering these points, this research work has been carried out for serological screening of toxoplasmosis in sheep, goat and cattle originating from herd by indirect ELISA.

MATERIALS AND METHODS

Study area

The serum samples used in this study were collected from a serum bank in the department of Medicine. The samples were originated from Cumilla, Brahmanbaria and Feni districts of Chattogram division.

Study population

A total of 220 serum samples of sheep, goats and cattle were collected from the study area with the history of abortion in their herds. Among 220 serum samples; 110, 60 and 50 were randomly collected from sheep, goats and cattle, respectively.

Serological test

Antibodies against *T. gondii* were detected using an indirect Enzyme-Linked Immunosorbent Assay (ELISA) by employing a commercially available kit (ID Screen® indirect ELISA kit, IDvet Laboratories, Inc., France) according to the manufacturer's instructions and cut-off recommendations. Wells of ELISA plates were coated with P30 antigen provided by the commercial kits. Optical densities of the samples were detected using an ELISA reader at 450 nm. The positive and negative control sera used in these assays were provided by the commercial kits.

Test Procedure

All reagents were allowed to come to room temperature ($21^{\circ}\text{C}\pm 5^{\circ}\text{C}$) before use. Ninety microlitre of dilution buffer 2 to each microwell was added. Then following things were added- 10 μl of the Negative Control to wells A1 and B1, 10 μl of the Positive Control to wells C1 and D1, 10 μl of each sample to be tested to the remaining wells. Then it was incubated 45 min \pm 4 min at 21°C ($\pm 5^{\circ}\text{C}$). After that the wells were emptied and washed each well 3 times with approximately 300 μl of the wash solution. The Conjugate 1X was prepared by diluting the Concentrated Conjugate 10X to 1/10 in Dilution Buffer 3. Then 100 μl of the Conjugate 1X was added to each well and Incubated 30 min \pm 3min at 21°C ($\pm 5^{\circ}\text{C}$). The wells were emptied and washed each well 3 times with approximately 300 μl of the Wash Solution. Then 100 μl of the substrate solution was added to each well and incubated 15 min \pm 2min at 21°C ($\pm 5^{\circ}\text{C}$) in the dark. Then 100 μl of the Stop Solution was added to each well in order to stop the reaction and the optical densities at 450 nm was read and recorded.

Interpretation

For each sample, the S/P percentage (S/P%) was calculated:

$$\text{S/P}\% = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{NC}}}{\text{OD}_{\text{PC}} - \text{OD}_{\text{NC}}} \times 100$$

Serum/Plasma

Result	Status
S/P % \leq 40%	Negative
40% < S/P % < 50%	Doubtful
S/P % \geq 50%	Positive

Statistical analysis

Obtained data were compiled and analyzed by using MS Excel. The S/P percentage (S/P%) for each sample was calculated from optical density values of sample, positive control and negative control taken in 450 nm by using formula.

RESULTS AND DISCUSSION

Overall prevalence of *T. gondii* infection among food animals

Out of 184 samples, 13.59% samples were found positive. The Prevalence of *T. gondii* infection was 11.90% for cattle, 13.04% for sheep and 16.0% for goats (Figure 1).

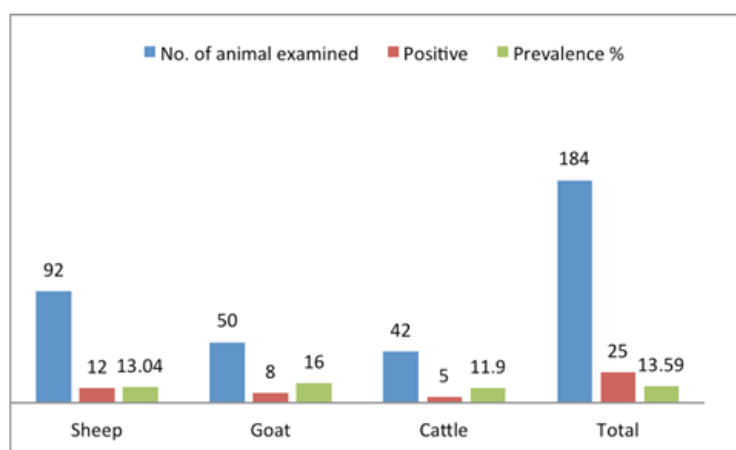


Figure 1. Overall seroprevalence of *T. gondii* in food animals in Chattogram division

Prevalence of *T. gondii* infection in food animals associated with age, sex and pregnancy

The highest prevalence of *T. gondii* infection was observed in cattle aged >5years (36.36%) in comparison to other age groups (Table 1). Similarly the highest prevalence of *T. gondii* infection was estimated 16.07% in > 18 months old sheep than other age groups (Table 2). However, in goats the prevalence of *T. gondii* infection was highest in the age group of 6-18 months (10.52%) compared to other groups (Table 3).

Summary of ELISA test results on the presence of *T. gondii* antibodies (S/P values) in serum samples of food animals

The mean S/P values of positive samples are 97.14%, 86.71% and 78.93% for sheep, goats and cattle respectively (Table 4). From the result of the present study, it was observed that *T. gondii* infection is endemic in herds with the history of abortion in Bangladesh. In this study, the overall seroprevalence of *T. gondii* infection was 13.59%. Similar result (13.91%) was also reported by Onyiche *et al.*, (2013) in Ibadan, Nigeria but higher prevalence (18.3%) were also reported by Jula *et al.*, (2013) in Iran and Ahmed *et al.*, (2014) in Punjab, Pakistan. The seroprevalence *T. gondii* infection was 11.90% for cattle, 13.04% for sheep and 16% for goat. Higher prevalence of *T. gondii* infection than this study result was reported by other authors (Al-Ramahi *et al.*, 2007; Bocanegra *et al.*, 2013) from Iraq and Spain. However, lower prevalence than this study result was also described by Gondim *et al.*, (1999). The sample size was small and the sample does not represent the population of sheep, goats and cattle in Bangladesh. It is a limitation of this study. So, this finding of seroprevalence of *T. gondii* infection will not represent the true status of this disease in ruminants of the study areas. The cattle were divided into three age groups (2.5 years, >2.5- 5 Years and >5 years). The highest prevalence of *T. gondii* infection was found in >5 years old cattle. Similar observations were also reported by other authors (Gebremedhin *et al.*, 2014; Mikail *et al.*, 2014; Nematollahi and Moghddam, 2008). The prevalence of *T. gondii* infection was 9.37% for female and 20% for male cattle. Similar results were obtained by other authors (Nematollahi and Moghddam, 2008; Elfahal *et al.*, 2013). The prevalence of *T. gondii* infection was 7.69% for Pregnant 15.79% for Non pregnant 10% for not applicable cattle samples. Similar results were obtained by other authors in South-West of Iran (Hamidinejat *et al.*, 2010), in Pakistan (Nisar Ahmad *et al.*, 2014) and in Turkey (Akca *et al.*, 2010). In case of sheep, the sheep were divided into three age groups (< 6 months, 6-18 months and >18 months). Among them the highest prevalence was found in more than 18 months old sheep (16.07%). Similar observations were also reported by other authors in Ethiopia (Negash *et al.*, 2004; Tzanidakisa *et al.*, 2012).

Table 1. Seroprevalence of *T. gondii* in cattle associated with age, sex and pregnancy

Parameters	No. of animal examined	Positive	Prevalence (%)
Age	2.5years	17	0
	>2.5-5years	14	7.14
	>5years	11	36.36
Sex	Female	32	9.37
	Male	10	20
Pregnancy	Pregnant	13	7.69
	Non pregnant	19	15.79
	Not applicable	10	10

Sex related samples were 74 (female) and 18 (male) of sheep, among them 11 female and 1 male sample was positive and the prevalence was 14.86% in female and 5.55% in male. Similar results were obtained by other authors in Tabriz, prevalence in females was 19.2% (25.8% for ewes and 10.8% for does) and this for males was 10.5% (12.5% for rams and 9.1% for bucks) (Jula *et al.*, 2013). The prevalence of *T. gondii* infection was 29.63% for pregnant 11.76% for non-pregnant 0% for anoestrus and 8% for not applicable sheep. Similar results were obtained by other authors in South-West of Iran (Hamidinejat *et al.*, 2010), in Pakistan (Nisar *et al.*, 2014) and in Turkey (Akca *et al.*, 2010).

Table 2. Seroprevalence of *T. gondii* in sheep associated with age sex and pregnancy status.

Parameters		No. of animal examined	Positive	Prevalence (%)
Age	<6 months	17	1	5.88
	6-18 months	19	2	10.52
	>18 months	56	9	16.07
Sex	Female	74	11	14.86
	Male	18	1	5.55
Pregnancy	Pregnant	27	8	29.63
	Non pregnant	17	2	11.76
	Anoestrus	23	0	0
	Not applicable	25	2	8

Table 3. Seroprevalence of *T. gondii* infection in goats associated with age sex and pregnancy status.

Parameters		No. of animal examined	Positive	Prevalence (%)
Age	<6 months	15	2	13.33%
	6-18 months	24	5	20.83%
	>18 months	11	1	9.09%
Sex	Female	36	5	13.89
	Male	14	3	21.43
Pregnancy	Pregnant	16	1	6.25%
	Non pregnant	21	4	19.05
	Not applicable	13	3	23.08

Table 4. Summary of ELISA test results on the presence of *T. gondii* antibodies (S/P values) in serum samples of food animals.

Species	Test result	No. of test result	Percent (%)	Range of S/P values (%)	Mean S/P values (%)
Cattle	Positive	5	11.90	51.45-130.11	78.93
	Negative	37	88.09	0.29-32.51	6.30
Sheep	Positive	12	13.04	62.01-133.21	97.14
	Negative	80	86.96	0.48-28.80	7.70
Goats	Positive	8	16	50.46-119.53	86.71
	Negative	42	84	3.79-30.61	10.88

In goat, samples were categorized into <6 months, 6-18 months and >18 months aged groups. *T. gondii* seroprevalence was significantly higher in adult goats (6-18 months age, 20.83%) than in the young age group (< 6 months, 13.33%) and (> 18 months, 9.09%). Similar results were obtained by other authors in Central Ethiopia. (>1 year age, 22.5%) and (61 year, 11.4%) (Endrias *et al.*, 2013) and in Ethiopia (Negash *et al.*, 2004) and (Tzanidakisa *et al.*, 2012). Therefore, this difference in prevalence among age group can be explained by the cumulative effect of age (Hall *et al.*, 2001; Dubey, 2010) and suggests that most goats in Ethiopia acquire the infection after birth. Our finding is in conformity with other reports on caprine toxoplasmosis from Ethiopia (Teshale *et al.*, 2007) and from other countries (Carneiro *et al.*, 2009; Chikweto *et al.*, 2011; Dorny *et al.*, 1993; Dubey, 1990; Jittapalapong *et al.*, 2005; Opel *et al.*, 1991). The prevalence of *T. gondii* infection was 13.89% for female and 21.43% for male goat. Similar results were obtained by other authors in Central Ethiopia females (22.6%) and males (10.3%) (Endrias *et al.*, 2013; Negash *et al.*, 2004; Teshale *et al.*, 2007), Thailand (Jittapalapong *et al.*, 2005) and Pakistan (Ramzan *et al.*, 2009). The prevalence of *T. gondii* infection was 6.25% for pregnant, 19.05% for non-pregnant and 23.08% for not

applicable goat. Similar results were obtained by other authors in South-West of Iran (Hamidinejat *et al.*, 2010), in Pakistan (Nisar *et al.*, 2014) and in Turkey (Akca *et al.*, 2010). The highest mean S/P value of positive samples was found in sheep then in descending order in goats and cattle. This finding indicates that among the three food animal species, sheep and goats are more prone to *T. gondii* infection than cattle in Chattogram division.

CONCLUSION

Toxoplasmosis is endemic in sheep, goats and cattle in Chattogram division. The undercooked meat and raw milk of these food animals may serve as a potential source of *T. gondi* infection for humans. Further studies are imperative to explore the transmission dynamics of this zoonotic protozoan pathogen.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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