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Original Article

Seroprevalence of *Toxoplasma gondii* infection in ruminants in selected districts in Bangladesh

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ABSTRACT

To estimate the seroprevalence of toxoplasmosis and risk factors for seropositivity in sheep, goats and cattle in Dhaka, Mymensingh, Sirajganj and Chittagong districts in Bangladesh, 1104 sera samples (552 sheep, 300 goats and 252 cattle) were randomly selected and tested by an indirect enzyme linked immunosorbent assay (iELISA). The overall seroprevalence was 12.2% (135/1104), 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 sheep, herd type, district and pregnancy status were significant risk factors.

The odds of seropositivity was 2.1 (95% CI: 1.00–4.84), 7.29 (95% CI: 12.71–423.41) and 10.88 (95% CI: 5.42–23.41 times higher in sheep-only flocks, in Mymensingh district and in pregnant sheep than in mixed flocks, Chittagong district and non-pregnant sheep, respectively. In cattle, breeds and district were significant risk factors. The odds of seropositivity was 5.79 (95% CI: 1.13–24.62) and 4.29 (95% CI: 1.38–16.34) times higher in Holstein Friesian cross cattle and in Mymensingh district than in indigenous cattle and Chittagong district, respectively. This study indicates that exposure of sheep, goats and cattle to oocysts of *T. gondii* is widespread, suggesting that the consumption of raw and undercooked meat of these animals might be a source of human toxoplasmosis. Risk factor information can be used to design control programs to reduce exposure.

1. Introduction

Toxoplasma gondii is a multi-host obligate intracellular protozoan parasite, causing zoonotic infections throughout the world. It infects humans and a wide range of mammalian and avian species (Smith and Reduck, 2000). Toxoplasmosis causes congenital disease and abortion both in humans and livestock (Dubey and Beattie, 1988; Remington and Desmonts, 1990). Almost one third of the human population has been infected with this parasite worldwide (Montoya and Liesenfeld, 2004). Among food animals, sheep and goats are well-known sources of human infection (Dubey, 2010). In many countries, *T. gondii* is a major cause of reproductive disorders, miscarriages and abortions in the sheep industry, and therefore is responsible for substantial economic losses (Buxton et al., 2007; EFSA, 2007). Maternal toxoplasmosis during early pregnancy in humans can lead to death of the fetus or can cause chorioretinitis, hydrocephaly, microcephaly and jaundice in neonates (Joynson and Wreghitt, 2001; James, 2003).

Definitive hosts of this coccidian parasite are felids – both domestic and wild – whilst intermediate hosts are mammals and birds (Nematollahi and Moghddam, 2008; Dubey and Jones, 2008). The intermediate hosts are 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 is the major cause of abortion and perinatal mortality in sheep and goats worldwide (Buxton and Brebner, 1998). Abortion and neonatal mortality occur when sheep and goats suffer a primary infection during pregnancy. Sheep are considered important in the epidemiology of *T. gondii* infection worldwide, in general and Europe, in particular (Cook et al., 2000; Buxton et al., 2007). Ingestion of infected lamb serves as a direct source of infection for humans.

Currently there are several diagnostic procedures for determining *T. gondii* infection. The ELISA is well suited to laboratories in which large numbers of samples need to be analyzed (OIE, 2008). Numerous modifications of the ELISA have been reported to enhance specificity and to simplify the protocol of the conventional ELISA (Dubey and

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Beattie, 1988). The dye test is considered as the "gold standard" serological test at least in humans (OIE, 2008).

Cattle, goats and sheep are widely used as food animals in Bangladesh. Infection of herbivores occurs mostly via ingestion of *T. gondii* oocysts excreted by cats. In Bangladesh most cats are stray; they can be found in association with farm animals, and contaminate the environment by shedding oocysts. One week after a first infection, cats can shed over 100 million oocysts in their faeces for a period of 7–14 days depending on the infection dose, the infection stage and the immune status of the cat. Sporulated oocysts can remain infectious in the environment for > 1 year and contaminate pastures, feed and also drinking water (Dubey and Beattie, 1988; Innes et al., 2009). The reported seroprevalence of toxoplasmosis in cats varied from 33.33–36.36% (Samad et al., 1997; Hossain, 2016), whereas the seroprevalence of human toxoplasmosis varied from 10.66–38.5% (Samad et al., 1993, 1997; Ashrafunnessa et al., 1998; Margia, 2015; Hossain, 2016).

There have been a few studies reporting seroprevalence of *T. gondii* in sheep, goats and cattle in parts of Bangladesh. The range of reported seroprevalence of toxoplasmosis varied from 12–27% in cattle, 12–61% in goats and 18–69% in sheep (Samad et al., 1993; Shahiduzzaman et al., 2011; Rahman et al., 2014). However, sample sizes in these studies were small and the areas included were confined to only Mymensingh and Rajshahi districts. Moreover, the risk factors for toxoplasmosis in sheep, goats and cattle in Bangladesh have not been reported. Hence the objectives of this study were to estimate the seroprevalence of *Toxoplasma gondii* infection in sheep, goats and cattle and to investigate risk factors for seropositivity.

2. Materials and methods

2.1. Study area and sampling design

The animal management system in Bangladesh is small scale dairy with traditional subsistence management systems. The small-scale dairy system mainly practices zero grazing ("cut-and-carry system") with occasional tethering systems, mainly for goats. Occasionally when there are no crops in the field, different species of animals with different owners graze together in villages. Sheep are usually reared with zero input and mainly live on roadside grasses where they graze freely. Marginal and landless farmers usually rear small ruminants in Bangladesh. The cattle population in Mymensingh, Chittagong and Sirajganj districts is 1.4, 1.1 and 0.8 million, respectively and for goats and sheep it is 1.1 and 0.1; 0.6 and 0.08; and 0.3 and 0.03 million, respectively (DLS, 2016). Livestock herds in Bangladesh are not identified regionally or centrally in the form of a database. Therefore, a GIS-based herd selection procedure was used to achieve random sampling (Rahman et al., 2013). Initially, 15 unions from each district were randomly selected. One geographical coordinate was randomly chosen from each selected union and reached by a hand-held GPS reader. Livestock farmers within 500 m of the selected point were informed about the survey (Cringoli et al., 2002). All animals in a selected herd were sampled. A total of 1104 serum samples were collected from four districts viz. 287 from Dhaka, 375 from Mymensingh, 275 from Chittagong and 167 from Sirajganj during the period January 2014 to December 2016. Among the 1104 blood samples, 552, 300 and 252 were collected from sheep, goats and cattle, respectively. Basic biometric data on animals including age, sex, number of pregnancies and history of abortions were obtained from the owners by personal interview.

2.2. Processing of blood samples

Oral consent of farm owners was obtained prior to the collection of blood samples from their animals. About 5 ml of blood was collected from each animal by jugular venipuncture with disposable needles and venoject tubes, labeled and transported to the laboratory on ice (after clotting) within 12 h of collection. Blood samples were kept refrigerated (2–8 °C) in the laboratory and one-day later sera were harvested by centrifuging at 3000 × *g* for 30 min. Each serum was labeled to identify the animal and stored at − 20 °C. Blood samples collected from other districts were processed in their respective districts and sera were stored at − 20 °C, prior to being transferred to the laboratory of the Department of Parasitology, Bangladesh Agricultural University (BAU).

2.2.1. Serological examination

All serum samples were tested for the presence of *T. gondii* IgG antibodies using an indirect enzyme linked immunosorbent assay (ELISA) by employing a commercially available kit (ID Screen® Toxoplasmosis Indirect Multi-species, TOXOS-MS ver 1013 GB, Product code: TOXOS-MS, batch: 582, Innovative Diagnostic vet Laboratories, Inc., France) according to the manufacturer's instructions. The wells of ELISA plates were coated with *T. gondii* P30 surface antigen provided with the commercial kits. The positive and negative control sera used in these assays were provided with the commercial kits. Optical densities of the samples were detected using an ELISA reader at 450 nm. For interpretation of the result, the sample to positive ratio (S/P) % was calculated as: (S/P) % = (OD value of sample − mean OD value of negative control) ÷ (mean OD value of positive control − mean OD value of negative control) × 100. Any sample with S/P % ≤ 40%, ≥ 50% and 40–50% were considered negative, positive and doubtful, respectively. The doubtful sera were retested. With this cut-off, the manufacturer claims the test to have > 99% specificity and sensitivity. The test was considered valid if the mean OD value of positive control was > 0.350 and the ratio of the mean OD value of positive and negative controls was > 3.

2.3. Data analysis

The data generated were stored in a Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using R 3.2.2 (Team, 2015). Initially a bivariable analysis between *Toxoplasma* serostatus (positive, negative; doubtful results excluded) and explanatory variables was performed using Pearson's chi-square test. Forward stepwise logistic regression was performed for the multivariable analysis, with an inclusion cutoff criterion of $P \leq 0.2$. Collinearity among explanatory variables was also checked by Pearson's chi-square test. If collinearity was detected, only one of the collinear variables was included in multivariable logistic regression model. Initially, the best univariate model was selected based on the lowest Akaike's information criterion (AIC) value. The remaining variables were then included in turn, based on AIC. The final model selected had the lowest AIC. Confounding was assessed by observing the change in the estimated coefficients of the variables that remained in the final model by inserting a non-selected variable into the model. If the inclusion of this non-significant variable caused a change of > 25% of any parameter estimate, that variable was considered to be a confounder and retained in the model (Dohoo et al., 2009). The two-way interactions of all variables remaining in the final model were assessed for significance based on AIC values (Dohoo et al., 2009).

3. Results

3.1. Seroprevalence of toxoplasmosis in different species

The overall seroprevalence in domestic ruminants tested was 12.2% (95% confidence interval [CI]: 10.4–14.3). Species-specific seroprevalence is shown in Table 1. The seroprevalence of toxoplasmosis was significantly ($P = 0.008$) higher in goats than cattle. The odds of seropositivity was 2.09 times (95% CI: 1.23–3.67) higher in goats than cattle.