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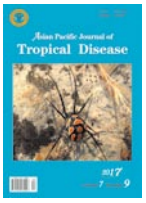

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Sero-prevalence of *Toxoplasma gondii* infection in backyard poultry birds of Tarai Zone of West Bengal, India

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ABSTRACT

Objective: To investigate the sero-prevalence of *Toxoplasma gondii* (*T. gondii*) infection in backyard poultry birds in three blocks of Tarai Zone of West Bengal during the period from January to June, 2015.
Methods: A total of 600 serum samples from Rhode Island Red poultry birds maintained by backyard system were collected and tested for anti-*T. gondii* antibodies using indirect ELISA standardized in the present study.
Results: The overall sero-prevalence of anti-*T. gondii* antibodies in backyard poultry birds of Tarai Zone was 20.33%. The prevalence of anti-*T. gondii* circulating antibodies ranged from 15% to 27.5% in backyard poultry birds in selected places of Tarai Zone of West Bengal.
Conclusions: Presence of such high level of anti-*T. gondii* antibodies in serum of backyard poultry birds maintained for egg and meat production poses a potential threat to the human population of getting infected with *T. gondii* and also suggests for general awareness about this disease to the local people of Tarai Zone of West Bengal, India.

1. Introduction

Toxoplasma gondii (*T. gondii*) is an intracellular zoonotic protozoan parasite of human and animals including poultry birds, and toxoplasmosis is prevalent throughout the world[1,2]. Clinical toxoplasmosis is rare in experimentally and naturally infected chicken and only a few of exposed poultry develop clinical symptoms, such as encephalitis, chorioretinitis and neuritis[1]. Free-range poultry birds with subclinical infection are an important source of *T. gondii* infection for cats which shed millions of oocysts after eating *T. gondii* infected tissues, and these oocysts then contaminate the food and water. Infected poultry meat is also an important source of human infection. Sero-prevalence of *T. gondii* infection has been studied in chickens, ducks and pigeons in various parts of the world[3-5]. Several surveys in different countries have shown that sero-prevalence of *T. gondii* infection in poultry birds varied from moderate to high. Sero-positivity for *T. gondii* and isolation of the parasite have been reported in free-range chickens reared in Egypt[4], Pakistan[5], Mexico[6], Ghana, Indonesia, Italy, Poland, Vietnam[7], Brazil[8] and China[2,9].

Epidemiological survey is the most important method for determining the significance of different sources of *T. gondii* infection in human and animals. Since contaminated meat is an important source of human infection, it will be useful to ensure regular surveillance of *T. gondii* infection in animal and bird species intended for human consumption. Reports on sero-epidemiology of toxoplasmosis are scanty from India. Close co-habitation of human, animals and poultry birds particularly in rural India including West Bengal ideally favours the perpetuation and spread of this important zoonosis. Nevertheless, there has been a disparity between the gravity of this problem and the research attention for this disease. In West Bengal, no work on toxoplasmosis so far has been reported and hence it is imperative to uncover the hidden facts of this

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The experiments involving the laboratory animal (mice) and poultry birds were conducted in accordance to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Govt. of India) and the experimental design was approved by the Institutional Animal Ethics Committee, West Bengal University of Animal and Fishery Sciences.

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problem. Hence the present study was designed to determine the sero-prevalence of *T. gondii* infection in chickens raised in backyard system for producing eggs or meat.

2. Materials and methods

2.1. Sampling area

Sampling for the present study was carried out in the Tarai region, one of the six agro-climatic zones of West Bengal in India. Tarai Zone is the plain land region of West Bengal, India that lies in south of the outer foothills of the Himalaya. Tarai Zone is a belt of marshy grass lands and composed of alternate layers of clay and sand with high water table. Tarai Zone consists mainly of Jalpaiguri District. Three blocks (Malbazar, Meteali, Nagrakata) under Jalpaiguri District and five villages under each block were selected for the present study. In those villages Rhode Island Red (RIR) chickens were maintained by backyard poultry farming system and the chickens were kept free during the day time and in night they were housed in cages at the backyard of owner's house.

2.2. Collection of sera samples

Blood samples were collected with the prior consent of the owner at the farmer's house by wing vein puncture from 600 RIR chickens aged 10–12 weeks old of either sex. Blood samples were collected at the ratio of 40 chickens per village. About 1 mL of blood was collected in 2-mL disposable plastic syringe (Dispovan) and sera were separated from the clotted blood following the standard procedure and dispatched to the laboratory with cold packs. Sera samples were stored at –20 ˚C till further use. The study was continued for six months from January to June, 2015.

2.3. Preparation of T. gondii tachyzoite antigen

Mouse adopted RH strain of *T. gondii* tachyzoites obtained from All India Institute of Medical Sciences, New Delhi, India was used for the present study. The infection was maintained in Swiss Albino mice by serial passage of tachyzoites through intra-peritoneal route. The mice (n = 10) selected for collection of tachyzoites were euthanized using chloroform and then 5–7 mL of phosphate buffer saline (PBS, pH 7.4) was injected intraperitoneally with a 10-mL syringe fitted with 21 gauge needle, and the peritoneal fluid was drawn back into the same syringe carefully to avoid injury to other internal organs. The peritoneal fluid, containing free tachyzoites as well as macrophages infected with tachyzoites, was washed thrice in PBS by centrifugation at 8 000 r/min for 10 min. After discarding the supernatant the pellet was resuspended in PBS. The intracellular tachyzoites were extracted after rupturing the intact parasitized macrophages by repeated passing of the suspension through a 27-gauge needle fitted to a 10-mL syringe. The tachyzoites suspension was resuspended in PBS and the debris was allowed to settle in a centrifuge tube for 10 min. The supernatant was collected and filtered through a syringe filter (3.0 μm pore size), and filtrate was centrifuged at 8 000 r/min for 10 min. After discarding the supernatant the pellet containing the tachyzoites was resuspended in 9 times of its volume in PBS. The purified tachyzoites were used for antigen preparation. The tachyzoites were lysed by rapid freezing in liquid nitrogen and subsequent thawing, and this process was repeated twice. The ruptured tachyzoites were disintegrated in an ultrasonicator (Nissei, Model–US50, Japan) and then the sonicated extract was centrifuged at 8 000 r/min for 30 min at 4 ˚C. The supernatant was carefully collected as tachyzoite antigen and the protein concentration was estimated following the method of Lowry *et al*.[10].

The experiments involving the laboratory animal (mice) and poultry birds were conducted in accordance to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Govt. of India) and the experimental design was approved by the Institutional Animal Ethics Committee, West Bengal University of Animal and Fishery Sciences.

2.4. ELISA for detection of anti-T. gondii IgG in chickens

An indirect ELISA was standardized to detect anti-*T. gondii* IgG following the standard protocol[11]. Human sera with positive and negative reactivity to the *T. gondii* (RH strain) antigen, confirmed with the commercial kit (ToxoEIAgen IgG kit, Adaltis, Italy), were used as controls[12]. The optimal concentration of the coating antigen (3 μg/well), dilution of the positive and negative sera (1:400) as well as rabbit anti-human IgG-horseradish peroxidase (1:1 000) and the optimal test conditions were determined by checkerboard dilution assay. The cut-off value for the standardized ELISA was considered as the mean optical density (OD) plus three times the standard deviation of the negative control sera[13]. The collected sera samples were tested by the standardized indirect ELISA for the detection of anti-*T. gondii* antibodies in backyard poultry birds.

Flat-bottom 96-well micro-ELISA plates (Nunc, Maxisorp) were coated with 100 μL of *T. gondii* tachyzoite whole antigen diluted in carbonate-bicarbonate buffer and incubated overnight at 4 ˚C. The wells were washed with PBS containing 0.05% Tween 20 (PBST) for five times and blocking was done with 200 μL of 3% bovine serum albumin in PBST for 3 h at 37 ˚C. After washing 100 μL of diluted chicken sera (1:400) in PBST was added to test wells (n = 92) in duplicate keeping positive and negative controls and incubated at 37 ˚C for 2 h. Then 100 μL of rabbit anti-chicken IgG-horseradish peroxidase (Sigma) diluted in PBST was added to test wells, and rabbit anti-human IgG-horseradish peroxidase added to the control wells and incubated at 37 ˚C for 1 h. Finally 200 μL of substrate solution (OPD; Sigma) after washing was added to detect antigen-antibody reaction and incubated at 37 ˚C in dark for 15 min and then 2% H_2SO_4 was added to each well to stop the reaction. The OD of the wells was recorded at 492 nm by the ELISA reader (Multiskan Ex ELISA Reader, Thermo, Japan). A test sample having mean OD value higher than the cut-off value was considered as positive.

2.5. Statistical analysis

The data on OD values of serum samples from different blocks were analysed separately *i.e.* between blocks and within blocks

by Duncan method (One-way ANOVA). The complete statistical analyses were done with the help of Statistical Package for Social Scientists (SPSS), Windows Version 15.0.

3. Results

A total of 600 serum samples of RIR poultry chicken maintained in backyard system were collected from Tarai Zone of West Bengal. Those sera samples were tested by the standardized indirect (Ab-detection) ELISA for the determination of sero-prevalence of *T. gondii* antibodies in RIR poultry birds of Tarai Zone of West Bengal.

The cut-off value (mean OD plus three times the standard deviation of the negative control serum) of the standardized indirect ELISA was 0.198 (Figure 1). Out of 600 serum samples collected from the different blocks of Tarai Zone of West Bengal, only 122 samples showed OD value higher than the cut-off value (Figures 1–3). The overall sero-prevalence of *T. gondii* antibodies in RIR birds maintained in backyard was 20.33%. Out of the three blocks, Meteali block showed the highest prevalence (21%) of *T. gondii* circulating antibodies in serum samples of backyard poultry birds and the lowest sero-prevalence (19.5%) was recorded in chickens of Malbazar block of Tarai Zone (Table 1). No significant ($P > 0.05$) difference was observed in sero-prevalence of *T. gondii* infection in backyard poultry birds among the three different blocks of Tarai Zone of West Bengal. The prevalence of anti-*T. gondii* IgG in serum samples of backyard RIR poultry birds ranged from 15% to 27.5% in the selected villages under the three blocks of Tarai Zone (Table 1).

Table 1

Sero-prevalence of *T. gondii* antibodies in backyard poultry birds of different places of Tarai Zone of West Bengal, India.

Malbazar block		Meteali block		Nagrakata block	
Places	Prevalence (%)	Places	Prevalence (%)	Places	Prevalence (%)
Village 1	20.00	Village 1	17.50	Village 1	15.00
Village 2	20.50	Village 2	22.50	Village 2	25.00
Village 3	17.50	Village 3	27.50	Village 3	20.00
Village 4	20.00	Village 4	20.00	Village 4	20.00
Village 5	15.00	Village 5	17.50	Village 5	22.50
Overall	19.50	Overall	21.00	Overall	20.50

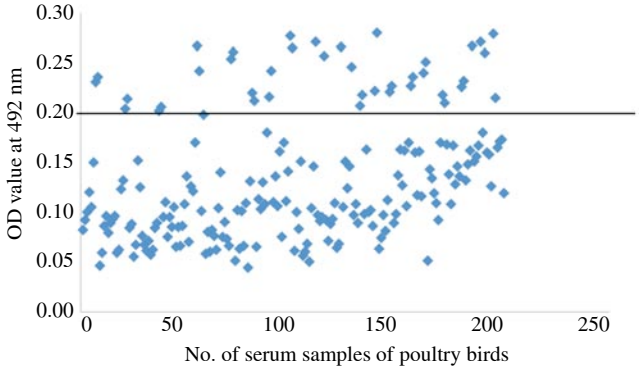

Figure 1. Detection of *T. gondii* antibodies by standardized indirect ELISA in serum samples of 200 RIR birds of Malbazar block of Jalpaiguri District of West Bengal.

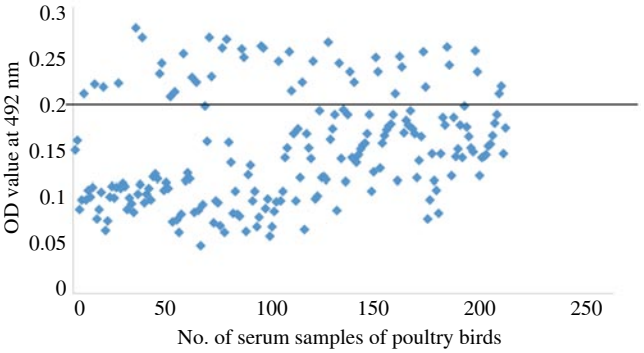

Figure 2. Detection of *T. gondii* antibodies by standardized indirect ELISA in serum samples of 200 RIR birds of Meteali block of Jalpaiguri District of West Bengal.

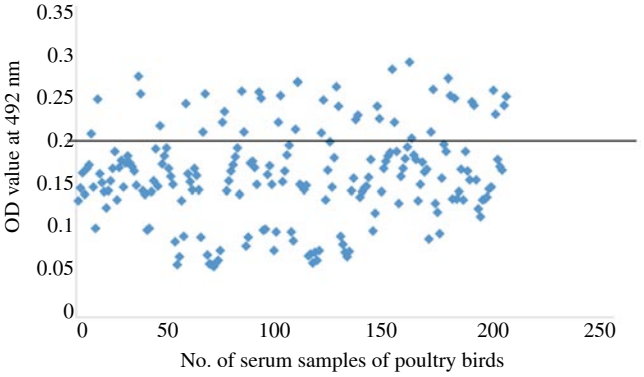

Figure 3. Detection of *T. gondii* antibodies by standardized indirect ELISA in serum samples of 200 RIR birds of Nagrakata block of Jalpaiguri District of West Bengal.

4. Discussion

Several surveys have been conducted in different countries for the detection of *T. gondii* infection in free-range chickens and in most of the cases the prevalence ranged between 36.03% and 85.7%[2,6,8,9,12]. In West Bengal there are six different zones based on soil character and climatic condition, and in our preliminary study we have selected one agro-climatic zone: Tarai Zone. The sero-prevalence of *T. gondii* antibodies was 20.33% in backyard RIR poultry birds in Tarai Zone of West Bengal. There are scanty reports on prevalence of toxoplasmosis in animals including poultry birds in India. Type II and III genotypes of *T. gondii* were isolated from free-range chickens in India[14]. The prevalence of *T. gondii* antibodies was recorded as 21.7% using indirect fluorescent antibody technique[15], and by modified agglutination technique 17.9% sero-prevalence was recorded in free-range chickens in Central and South India by Sreekumar *et al*.[15] and 39.5% sero-prevalence recorded by Devada *et al*.[16] in serum samples of free-range chickens in South India. Garcia *et al*.[17] and Hill *et al*.[18] showed that ELISA method had a higher sensitivity rate and performed better than MAT for detecting serum antibodies for *Toxoplasma*. So in this research indirect ELISA was standardized to detect the antibodies of *T. gondii* in serum samples of backyard poultry birds.

Sero-prevalence (20.33%) recorded in the present study though lower than that of the earlier reports from India[16] and other parts of the world[2,8,12], in some villages more than 25% backyard poultry

birds were found seropositive. The sources of human infection vary all over the world with the culture of the society, ethnicity, geographical location and food habits of the population[19]. *T. gondii* was isolated from brain and hearts of hens from India[14]. Jacobs and Melton[20] reported *T. gondii* in ovaries, oviduct and muscle of chicken by performing bioassay study in mice. All the genetic types (I, II, III) of *T. gondii* that have been isolated from human patients were also reported in free-range chickens[9,14]. Therefore, the poultry meat as an important part of cuisine consumed throughout the world should be considered as a source of infection in human. Poultry egg is not considered as a source of infection[7] although *T. gondii* infection is possible in ovary and oviduct of chickens[20]. High prevalence in free-range birds might be due to their habit of scratching the earth and feeding, which facilitates easy access to the feces of cat[1,8]. Backyard poultry birds in the present study also take their feed from soil during daytime. Backyard poultry birds are important sources of *T. gondii* infection for cats, which shed millions of oocysts after eating infected meat. This also increases the risk of contamination of food and water by cat feces which again increase the risk of toxoplasmosis in human and other animals including free-range chickens. In the present study the RIR birds were reared by backyard system mainly for meat and egg. The traditional cooking habits of West Bengal prevent the transmission of organisms through cooked meat, but due to globalization there is change in culinary habits and practice of consuming half-roasted or half-boiled food is increasing nowadays, increasing the risk of food borne diseases.

The prevalence of anti-*T. gondii* antibodies in the sera of backyard RIR chickens indicates the distribution of *T. gondii* oocysts on the ground surrounding human in the Tarai Zone of West Bengal, India and also poses great threat to the human population consuming improperly cooked poultry meat. Other agro-climatic zones of West Bengal should be covered for sero-prevalence studies in other food animals including poultry birds. Considering the above stated observations, hygienic standard in chicken rearing and general awareness about toxoplasmosis are prerequisites to prevent human infection.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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