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High seroprevalence of *Toxoplasma gondii* in goats in Jharkhand state of India

Madhurendra Bachan[a], Asit Ranjan Deb[a], Biswa Ranjan Maharana[b,c], Sudhakar N.R.[b], Vikrant Sudan[b,d], B.C. Saravanan[b], Anup Kumar Tewari[b,*]

[a] Department of Veterinary Parasitology, Ranchi Veterinary College, Birsa Agricultural University, Kanke, Ranchi, Jharkhand 834006, India
[b] Division of Parasitology, Indian Veterinary Research Institute, Izatnagar 243122, Uttar Pradesh, India
[c] RVDEC, LUVAS, Uchani, Karnal, Haryana, 132001, India
[d] Department of Parasitology, College of Veterinary Sciences and Animal Husbandry, Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura, Uttar Pradesh 281001, INDIA

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ABSTRACT

Toxoplasmosis, caused by *Toxoplasma gondii*, is an important food borne zoonosis worldwide. Although goat meat constitutes an important dietary protein source, improperly cooked meat is a potential source of infection to humans. Data on prevalence of toxoplasma in goat is scanty from India. Serological detection is the practical option for prevalence studies on *T. gondii*, as no biological stage of the parasite is present in the clinical materials from the intermediate hosts. The present study was undertaken in the Jharkhand state of India which is largely inhabited by economically weaker aborigine population, who depend largely on animal husbandry for livelihood. A total of 445 serum samples were collected for testing, which represented goats under intensive and free range system of rearing. *T. gondii* specific IgG antibodies were detected in 42.47% (n = 189) samples by rSAG1 based indirect ELISA. The seroprevalence data were analyzed in respect of age, sex, breed of the goats and altitude of the study area as well as rearing conditions of the animals to establish correlation, if any. Though age and sex of the animals had a direct correlation with infection, the same could not be established with the other factors. The sensitivity and specificity of the diagnostic ELISA were compared with IFAT, as well as with a commercially available ELISA kit. The rSAG1-ELISA had 92.66% sensitivity and 90.67% specificity with a positive predictive value of 86.77% and negative predictive value 94.92% when compared with IFAT, whereas when compared with the commercial ELISA kit, 87.50% sensitivity and 90.91% specificity with a positive predictive value of 91.30% and negative predictive value 86.96% were observed. Inter rater agreement (kappa) was calculated. rSAG1-ELISA showed good agreement with IFAT (kappa = 0.824) and commercially available ELISA Kit (kappa = 0.783). Receiver Operating Characteristics (ROC) curve analysis, revealed a larger area under curve (AUC) of 0.99 (95%CI, 0.97–1.0) when compared with IFAT as gold standard and a highest relative sensitivity 91.30 (95% CI 72–98.3) and specificity 1.0 (95% CI 85.2–100) for the cut off value of 0.6005. The present study revealed high seroprevalence of *T. gondii* in goats from Jharkhand, which has public health significance.

1. Introduction

Toxoplasmosis is a worldwide zoonosis caused by *Toxoplasma gondii*, an obligate coccidian parasite. Virtually any warm-blooded animals, including humans are intermediate hosts for the parasite while felines serve as the definitive host (Dubey and Beattie, 1988; Tenter et al., 2000; Dabritz and Conrad, 2010; Dubey and Jones, 2008). *T. gondii* forms tissue cysts in the omnivores and carnivores, including scavengers rendering the infected animals as potential sources of infection to all (Tenter et al., 2000). Herbivores generally acquire infections while grazing on pastures contaminated with environmentally resistant oocysts shed only by cats (Yilmaz and Hopkins, 1972; Frenkel et al., 1975; Dubey and Beattie, 1988; Lindsay et al., 2002, 2003). Humans become infected typically through ingestion of raw or inadequately cooked meat containing live *T. gondii* tissue cysts (Weinman and Chandler, 1954; Desmonts et al., 1965; Sacks et al., 1983; Choi et al., 1997; Dubey et al., 2002, 2005; Belfort-Neto et al., 2007; Robertson, 2007). Transmission through drinking raw goat milk has

* Corresponding author.
E-mail address: anup_tewari@ivri.res.in (A.K. Tewari).

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been documented (Patton et al., 1990; Riemann et al., 1975; Sacks et al., 1982; Skinner et al., 1990). Though *T. gondii* infection may be innocuous in immunocompetent intermediate hosts, abortion or prenatal complication in the fetus is common during congenitally acquired infections (Buxton, 1998; Esteban-Redondo and Innes, 1997; Pereira-Bueno et al., 2004).

Backyard rearing of goats is practiced throughout India and contributes significantly to the rural economy. Data on seroprevalence of caprine toxoplasmosis from India are scanty; a high level of seroprevalence reported in ruminants from India and neighbouring countries using native protein-based DAT, IFAT, ELISA and MDAT (Sah et al., 2018; Khan et al., 2017; Satbige et al., 2016; Rahman et al., 2014; Shahiduzzaman et al., 2011; Sharma et al., 2008; Malik et al., 2005; Vijaya Bharathi et al., 2003; Mirdha et al., 1999; Dubey et al., 1993) and using recombinant protein-based ELISA (Sudan et al., 2015; Singh et al., 2015; Velmurugan et al., 2008). Diagnosis of toxoplasmosis in animals by visualizing bradyzoites in situ using CT scans, though possible, is both cost-prohibitive and impractical. Currently, serological detection of chronic or latent infections using native tachyzoite derived proteins is practiced widely, but development and standardization of high throughput methodologies using the improved techniques remains essential. The commonly used agglutination tests, viz. modified agglutination test and dye test, require handling live tachyzoites with the associated risk of infection to human handlers. Native protein removes the risk of infections in the finished product but this antigen has its own set of limitations. The difficulties associated with the mass production and isolation of *T. gondii* tachyzoites from peritoneal fluid following experimental infection in mice or from tissue culture and the subsequent harvesting of native antigenic proteins from these tachyzoites affect the degree of sensitivity or specificity achieved in diagnostic assays based on these antigen preparations. This constitutes the major drawbacks with the use of commercially produced native protein used in many diagnostic tests.

The seroprevalence of *T. gondii* in goats from the Jharkhand state of India, which is largely inhabited by economically weak tribal people, was examined in the present study. Jharkhand extends from 21° 55′ to 25° 35′ North Latitude to 83° 20′ to 88° 02′ East Longitude. The state has a geographical area of 7.970 million hectares, of which about 0.1 million hectares are pastures and other grazing lands, 0.3 million hectares are cultivable wastelands and 1.795 million hectares are the net sown area. The state comprises of a hilly undulating Chotanagpur Plateau with predominantly tropical forests and tribal settlements. The Tropic of Cancer passes through the middle of Ranchi City and the average temperature of the state is 25 °C. The average annual rainfall is 1400 mm and > 80% precipitation occurs during June to September. Jharkhand hosts a goat population of about 6.5 million, of which 6.3 million is reared under rural environments and about 0.18 million goats are reared in the urban areas (19th Livestock Census of India, 2012). A locally developed recombinant *T. gondii* surface antigen 1 (rSAG1) - ELISA (Velmurugan et al., 2008) was used to establish the seroprevalence of *T. gondii* in goats. The association between *T. gondii* seropositivity to the host related variables viz. age, gender and breed, as well as, to the type of production system of that region was also studied. The diagnostic specificity and sensitivity of the rSAG1-based ELISA was established with these caprine serum samples and results were compared with IFAT as well as commercially available CHEKIT-Toxotest enzyme immuno-assay (EIA) kit (IDEXX Lab.).

2. Materials and methods

2.1. Sampling

Stratified random sampling method was applied for the present seroprevalence study. A total of 445 sera samples were collected from goats of two different breeds viz. Black Bengal and Beetal from two different organized government farms located in the Chatra and Ranchi districts of Jharkhand (n = 185) as well as from some selected villages of Ranchi (n = 260) where the goats are reared free-range. The sampling was done during July to November 2010 from animals differing in age, sex and body weight.

2.2. Blood collection

The blood samples (2–3 ml) were collected aseptically from the jugular vein using sterile syringes without anticoagulant for separation of serum in wide mouth test tubes (Borosil). Kids < 6 months of age were excluded from sampling to avoid measuring antibodies passively transferred. The blood was allowed to clot at room temperature; serum separated and then transported on ice to the Protozoology laboratory of Parasitology Division, Indian Veterinary Research Institute, Izatnagar, where they were stored at −20 °C until use.

2.3. Reference sera

The reference positive and negative sera were kindly donated by Dr. J. P. Dubey, USDA, Beltsville, USA.

2.4. Expression of rSAG1

The recombinant *T. gondii* surface antigen protein 1 (rSAG1) was expressed as reported elsewhere (Velmurugan et al., 2008). In brief, the complete 1183 bp ORF of SAG1 coding region was PCR amplified using the primer pair (5′-GGTTGTATGTCGGTTTCGCTGAC-3′ and 5′-GATCA CTCACGCGACACAAGCTGC-3′) for cloning into pGEM-T vector. A truncated 958 bp SAG1 fragment was PCR amplified using the primer pair containing the same forward primer and a new 27 nucleotide long reverse primer 5′-CGTCAAGCTTCAGCCGATTTTGCTGGAC-3′ containing a *Hin*dIII restriction site. The 958 bp PCR product was restriction digested with *Bam*HI and *Hin*dIII to yield an 814 bp product and ligated to pET-32b(+) (Novagen) for expression in BL-21(DE3) pLysS (Novagen) *E. coli.* The overexpression of recombinant SAG1 protein was achieved after 7 h induction with 1 mM isopropylthio-βgalactoside (IPTG) at 37 °C. The purity of fusion protein was checked by SDS-PAGE (Fig. 1).

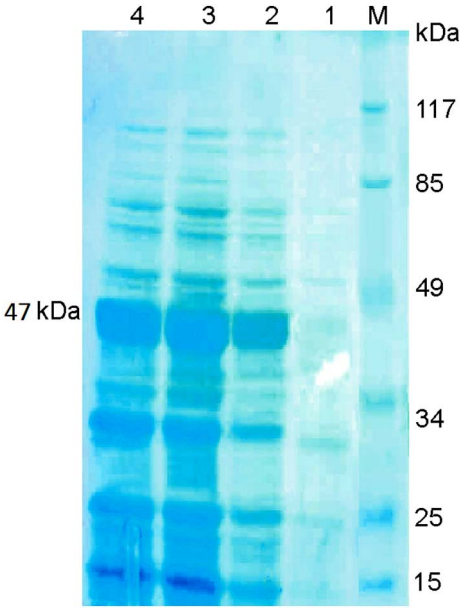

Fig. 1. Confirmation of rSAG1 protein in BL21 (DE3) pLysS *E. coli* cells by SDS-PAGE. The 12% polyacrylamide gel showing high level expression of rSAG1 at 47 kDa region; Lane M: Pre-stained molecular weight marker, Lane 1, 2, 3, 4: Expression of r SAG1 protein 0, 4, 6 and 8 h post induction, respectively.