

Detection of antibodies to *Toxoplasma gondii* in domesticated ruminants by recombinant truncated SAG2 enzyme-linked immunosorbent assay

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Abstract An antibody detection recombinant enzyme-linked immunosorbent assay (ELISA) specific for *Toxoplasma gondii* was laboratory standardized using recombinant truncated surface antigen 2 (SAG2) protein of *T. gondii*. A 483-bp sequence coding for truncated tachyzoite stage-specific SAG2 protein was amplified and ligated in pPROExHT-b expression vector to transform *Escherichia coli* DH5 α cells. A high-level expression of the histidine-tagged fusion protein was obtained after 8 h of incubation. The recombinant protein was affinity purified using Ni-NTA agarose column and characterized by SDS-PAGE and Western blot analysis. Subsequently, the diagnostic potential of the recombinant protein was assessed with 168 field sera samples from sheep, goats and cattle. Among the small ruminants, 50 % ($n=60$) sheep sera samples and 41.26 % ($n=63$) goat samples were detected positive for *T. gondii*-specific

antibodies. As far as seroprevalence of toxoplasmosis in cattle is concerned, 64.44 % ($n=45$) of sera samples assayed were found to be positive. When compared to indirect fluorescent antibody test (IFAT), the sensitivity of the recombinant truncated SAG2 antigen-based ELISA (rec-SAG2-ELISA) ranged from 81.25 to 87.10 % while the specificity was 85.71 to 91.43 % with substantial agreement between the tests.

Keywords Cattle · Goats · SAG2 · Rec-SAG2-ELISA · Sheep · Serodiagnosis · *Toxoplasma gondii*

Introduction

Toxoplasmosis, caused by a cyst-forming apicomplexan coccidian protozoan parasite, *Toxoplasma gondii*, is a worldwide disease, affecting almost all warm-blooded animals. It has been estimated that about one third of the world's population is at risk to toxoplasmosis with serological evidence of infection (Montoya and Liesenfeld 2004). The disease causes abortions, stillbirth, congenital malformations and neonatal losses, especially in sheep, goats and swine besides humans, leading to significant economic loss to the small and marginal farmers (Haumont et al. 2000; Lind and Buxton 2000; Tenter et al. 2000; Hill and Dubey 2002; Dubey 2004).

The economic impact of disease in developing countries like India is difficult to assess, as there is limited information available on the prevalence in various reared livestock species (Dubey 1987, 1993; Velmurugan et al. 2008). Diagnosis of infection relies on serological detection of *Toxoplasma*-specific IgG and IgM molecules in immunocompetent subjects. Though the parasite can be isolated from placenta and aborted foetus, isolation of the organism from biological samples is difficult. Development of polymerase chain

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reaction (PCR) assays for detection of *T. gondii* DNA in the acute phase of infection has been a remarkable achievement, though the technique has limitations for the detection of infection in chronic reservoirs due to non-availability of *T. gondii* DNA in blood (Savva et al. 1990). Notwithstanding this limitation, PCR is an important application for the determination of the strain of parasite involved (Khan et al. 2006).

Serological tests play a crucial role in the diagnosis of toxoplasmosis especially when a specific clinical sign is absent in immunocompetent subjects. Several serological tests using either particulate tachyzoites or native soluble antigens have been developed; however, these tests, though sensitive, suffer from limitations of specificity. For a high throughput serosurveillance of toxoplasmosis, a purified recombinant protein-based test is more relevant. Several gene sequences from *T. gondii*, including surface antigens, dense granule proteins, microneme proteins, rhoptry-associated proteins, etc. have been heterologously expressed. Among the array of proteins expressed and screened, surface antigen 2 (SAG2), together with SAG1, have added diagnostic relevance. The surface of *T. gondii* is covered with glycosylphosphatidylinositol (GPI)-anchored proteins which are closely related to SAG1. However, lately, existence of a second family of genes defined by homology to SAG2 was reported (Lekutis et al. 2000). One of these SAG2-related proteins, SAG2B, is expressed in tachyzoites with an apparent size of 23 kDa while, SAG2B, SAG2C and SAG2D appear to express on the surface of bradyzoites. These proteins of SAG2 family show weak but significant homology to the SAG1 family. Thus, the large superfamily contains all of the sequenced surface antigens of tachyzoites and many of those of bradyzoites, which is further divided into two subgroups defined by the prototypic and highly immunogenic SAG1 and SAG2, respectively (Lekutis et al. 2000).

Though a divergence of approximately 20 % has been reported in the open reading frames (ORF) for proteins of SAG2 family, it is considered to be more conserved across the other tissue dwelling coccidian parasites (Jung et al. 2004; Crawford et al. 2010). Simultaneous expression of multiple proteins of the SAG family might have permitted *T. gondii* to have extremely broad intermediate host range. The SAG2 protein serves as an attachment ligand to host cell (Grimwood and Smith 1996) and is immunologically relevant (Prince et al. 1990; Parmley et al. 1992; Aubert et al. 2000; Li et al. 2000). There are reports on heterologous expression of recombinant form of this protein (Prince et al. 1990; Parmley et al. 1992; Aubert et al. 2000; Li et al. 2000; Sawicka et al. 2005; Fong et al. 2008). However, no attempt has so far been made to harness the diagnostic potential of recombinant SAG2 protein for toxoplasmosis in domesticated ruminants (cattle, sheep and goat) from India. We report the development of a recombinant SAG2-based enzyme-linked immunosorbent assay (ELISA) for the serodetection of *T. gondii* in domestic

ruminants. Further, sensitivity and specificity of the recombinant truncated SAG2 antigen-based ELISA (rec-SAG2-ELISA) was determined against indirect fluorescent antibody test (IFAT).

Materials and methods

Parasite

Tachyzoites of *T. gondii* RH strain maintained as cryostock in the Protozoology Laboratory, Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India, was propagated in Swiss albino mice and used in the study. The animal experimentations were conducted in compliance with the ethical considerations and guidelines issued by CPCSEA/Institutional Animal Ethics Committee (IAEC) on laboratory animals

Bacterial strains and growth conditions

Escherichia coli DH5 α was used as prokaryotic bacterial host for experiments pertaining to plasmid DNA manipulation. It was grown in either Luria Bertani (LB) broth or LB agar, supplemented with ampicillin (100 μ g/ml) when required.

PCR amplification of truncated SAG2 gene

For amplification of the truncated SAG2 gene, complementary DNA (cDNA) was synthesized from the isolated total RNA of *T. gondii* using oligo dT primer following a standard protocol (Sambrook et al. 2001) and was used as template. Self-designed PCR primers incorporating suitable restriction sites based on the information generated by molecular cloning and comparative sequence analysis of the entire open reading frame (ORF) of SAG2 gene of the parasite in our earlier study (Singh et al. 2011) were used. The forward primer containing restriction site for *Bam*HI (5'-ACCATGGATCCACCACC GAGACGCCAGCG 3') and a reverse primer containing *Hind*III restriction site (5'-CAGTAAGCTTACACAAACGT GATCAACAAACC 3') at the 5' end were custom-synthesized to facilitate splicing of the amplified PCR fragment into the corresponding enzyme sites of the expression plasmid vector, pPROExHT-b (Invitrogen, USA).

The PCR conditions were optimized in 25- μ l reaction volume using 30 ng cDNA; 20 pmol each for forward and reverse primers; *Pfu* polymerase buffer containing 1.5 mM MgCl₂; 200 μ M dNTP mix and 1 U *Pfu* polymerase. Finally, the PCR assay was performed with the cycling conditions as initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 1 min and elongation at 72 °C for 45 s. Thereafter, 1 cycle of final elongation was given at 72 °C for 10 min. The confirmation of

the PCR product was done based on its size in the 1.5 % ethidium bromide stained agarose gel. Purification of the PCR product was done using commercially available gel extraction kit (Qiagen, Germany), following the manufacturer protocol.

Construction and transformation of recombinant plasmid

The purified DNA fragment (483 bp) was double digested with *Bam*HI and *Hind*III at 37 °C for 4 h and ligated to the corresponding cloning sites of pPROExHT-b vector (Invitrogen, USA) following standard protocol (Sambrook et al. 2001). The *E. coli* DH5 α cells were transformed with recombinant plasmid and plated on LB agar containing ampicillin. Confirmation of the recombinant clones was done by colony PCR, as well as, restriction enzyme analysis for the release of insert following standard protocols (Sambrook et al. 2001).

Expression of recombinant plasmids

Five random positive recombinant clones were selected for induction. The colonies were grown overnight in 5 ml of LB broth containing ampicillin at 37 °C in a shaking water bath. One hundred microlitres of the overnight culture were further grown in 10 ml of fresh LB broth at 37 °C with constant shaking until mid-log phase (approx. 4 h when the OD reached to 0.6), and 1 ml of the culture was collected from each tube and kept as an uninduced control. To the rest of the culture, isopropyl- β -D-thiogalactopyranoside (IPTG) was added at a final concentration of 1 mM and incubated at 37 °C with constant shaking at 140 rpm. One millilitre of the induced culture was collected at hourly intervals starting from 3 h onwards and the cells were pelleted by centrifugation at 13,800 \times g kept at 4 °C.

Protein harvest and purification

Based on the expression profile from different colonies, bulk culture for purification of expressed protein at mass level was carried out. In brief, cells from 1 L of the induced culture were pelleted and the pellet was resuspended in 15 ml of lysis buffer containing 8-M urea (pH 8.0). The cell suspension was kept at room temperature for 2 h on rotatory shaker with intermittent vortexing. Following lysis, the debris was pelleted by centrifugation for 10 min at 7600 \times g and the clear supernatant was transferred to a clean tube. Then, 800 μ l of Ni-NTA agarose slurry (Qiagen, Germany) containing 15 mM imidazole (Amresco, USA) and 20 mM β -mercaptoethanol (Amresco, USA) was mixed thoroughly with the supernatant and kept on a rotatory shaker for 1 h with intermittent mixing. The lysate-resin mixture was then loaded on to an empty 5-ml polypropylene column (Qiagen, Germany) equilibrated with

1X Tris-phosphate buffer (pH 8.0). The flow-through passed through the column was collected in the tube and the column was washed with 15 ml of wash buffer (pH 7.0) to which 5 mM imidazole (pH 7.0) was added and finally eluted as 500- μ l fractions with 4 ml of elution buffer (pH 4.2–4.5).

For renaturation, the eluted fractions were dialysed (SnakeSkin Dialysis Tubing 10 kDa cut-off, Pierce, Thermo Scientific, USA) against decreasing molar concentration of urea against tris-saline-EDTA buffer (pH 7.2) and finally with phosphate-buffered saline (PBS, pH 7.2) at 4 °C. Any debris formed during the renaturation was removed by centrifugation at 8200 \times g for 10 min in refrigerated centrifuge. Concentration of the purified recombinant protein was assayed by modified Lowry protein assay kit (Pierce, USA) according to the manufacturer's protocol. The protein was aliquoted in 0.5-ml volume and stored at -20 °C after addition of antiprotease mix (MBI Fermentas) till use.

SDS-PAGE and Western blotting

The purity and immunoreactivity of the expressed protein was checked by SDS-PAGE using 12 % gel (Atto, Japan) under denaturing conditions at 90 V for 2–3 h (Laemmli 1970) and then electro-transferred (Trans-Blot[®], Bio-Rad, USA) to nitrocellulose membrane (Millipore, USA) following standard protocol (Towbin et al. 1979). The protein (500 ng) was probed for immunoreactivity using hyperimmune serum raised in rabbit and known reference positive and negative goat sera (kindly provided by Dr. J.P. Dubey, USDA, Beltsville) at 1:100 dilution at 37 °C for 1 h after overnight blocking of the unsaturated sites on nitrocellulose membrane (Millipore, USA) with a reconstituted 3 % w/v solution of non-fat milk powder at 4 °C. Following stringent washing, with PBS-Tween 20 (PBS-T) the membrane was incubated with anti-rabbit and anti-goat horseradish peroxidase (HRPO) conjugate (Sigma) at 1:1000 dilution at 37 °C for 1 h and then developed with diaminobenzidine tetrahydrochloride (DAB) solution (Biomatik, USA).

Field sera samples

Sera samples, were collected from goats ($n=63$; West Bengal, India), sheep ($n=60$; Makhdoom, Uttar Pradesh, India) and cattle ($n=45$; Bareilly, Uttar Pradesh, India) under aseptic precautions.

Recombinant truncated SAG2 antigen-based ELISA

For optimum reactivity, the concentration of antigen and dilution of serum and conjugates were determined by

checker-board titrations The individual wells of ELISA plates (Nunc, MaxiSorp) were coated with 100 μ l of purified recombinant protein (8 μ g/ml) in carbonate-bicarbonate buffer (pH 9.6) at 4 °C overnight. The free binding sites in the well were blocked with a reconstituted 3 % w/v solution of non-fat milk powder (Amresco, USA) prepared in PBS for 2 h at 37 °C. Following stringent washing, field sera samples, diluted 1:100 with PBS containing 1 % non-fat milk powder, were loaded in duplicate wells and incubated for 1 h at 37 °C. Known reference positive and negative sera samples from goat, sheep and cattle (kindly provided by Dr. J.P. Dubey, USDA, Beltsville, USA) were included in the study. Following stringent washing, suitably diluted species-specific HRPO conjugates (Sigma) were added and the plates were incubated further for 1 h at 37 °C. The anti-sheep and anti-goat conjugates were diluted 1:10,000, whereas the anti-bovine conjugate was diluted 1:15,000 in PBS containing 1 % non-fat milk powder. After a final wash with PBS-T, the wells were developed with 100 μ l of o-phenylenediamine dihydrochloride (OPD) substrate (Sigma) dissolved in citrate-phosphate buffer (pH 5.0) with H₂O₂. The reaction was allowed to progress for 15 min in the dark and then stopped by adding 50 μ l of 3 N HCl. The absorbance was read at 492 nm in ELISA reader (Microscan).

Indirect fluorescent antibody test

The antigen (whole tachyzoites of *T. gondii*, RH strain)-coated slides were prepared following the procedure outlined in the USHDEW Manual (1976) and the test was performed following the procedure of Voller and O'Neill (1971) with minor modifications. Briefly, the antigen-coated slides were incubated in the presence of test sera for 45 min at room temperature in a moist chamber and then washed three times for 5 min each in PBS (pH 7.6) and air dried. Ten microlitres of fluorescein isothiocyanate (FITC)-labelled rabbit anti-species (goat, sheep and cattle) and IgG (whole molecule, Bangalore Genei, India), diluted 1:80 in PBS plus 0.01 % Evans blue, was added and incubated for 45 min. The slides were then washed and mounted in buffered glycerol. Reference positive and negative serum samples were also included in the test. A titre of 1:16 or above were considered positive for sheep and goats and 1:128 or above for cattle (Dubey and Beattie 1988).

Comparison of rec-SAG2-ELISA and IFAT

The performance of rec-SAG2-ELISA was compared with IFAT as a standard test for the determination of sensitivity and specificity and kappa value by using online calculation tools, http://www.medcalc.org/calc/diagnostic_test.php for

the calculation of sensitivity and specificity and <http://graphpad.com/quickcalcs/kappa1.cfm> for the calculation of kappa value. The agreement between the two tests was analyzed as per Landis and Koch (1977).

Results

Directional cloning and expression of recombinant protein

The 483-bp SAG2 gene segment coding for C-terminal of the mature protein was PCR amplified (Fig. 1a). The PCR product was purified and double digested with *Bam*HI and *Hind*III at 37 °C for 4 h, further purified and eluted in 30 μ l of elution buffer. Two hundred nanogram of the digested product was used for ligation.

Overnight grown *E. coli* DH5 α cells were transformed with recombinant pPROExHT-b plasmids and plated in presence of ampicillin. The white colonies were selectively isolated. Positive colonies were identified by colony PCR (Fig. 1b) and restriction enzyme analysis (Fig. 1c). A high-level expression of SAG2 protein was achieved after 8 h of induction with IPTG. The expression was confirmed by SDS-PAGE analysis and the recombinant SAG2 was resolved as a distinct band of ~21 kDa (Fig. 2a). Level of expression of a histidine-tagged recombinant fusion protein was measured as 42 % of the total cell protein.

Purification and Western blot analysis of expressed histidine-tagged protein

One litre of bulk culture yielded nearly 5–6.5 g of cell pellet which was lysed in 15 ml of lysis buffer containing 8 M urea. Affinity purification using Ni-NTA agarose slurry containing

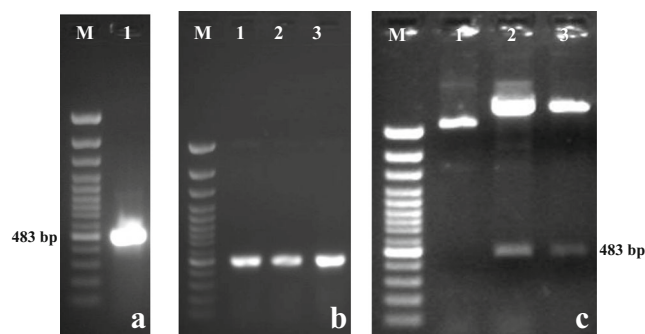


Fig. 1 a PCR amplification of truncated SAG2 gene of *T. gondii*. Lane M Marker 100 bp DNA ladder plus, Lane 1 amplicon of 483 bp size. b Colony PCR of transformed colonies of truncated SAG2 gene of *T. gondii*. Lane M Marker 100 bp DNA ladder plus, Lanes 1 to 3 amplicon from colony PCR. c Restriction digestion of recombinant pPROEXHT-b plasmid. Lane M Marker 100 bp DNA ladder plus, Lane 1 uncut recombinant plasmid, Lanes 2–3 released insert

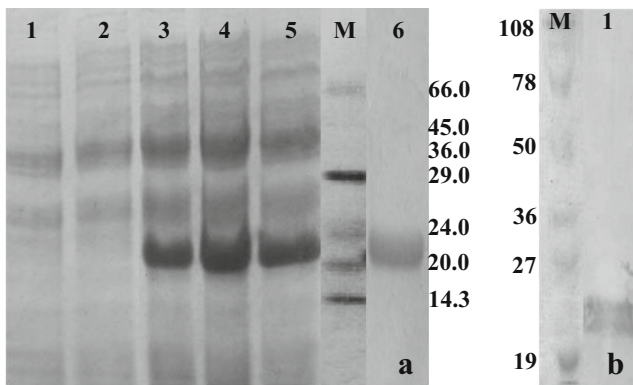


Fig. 2 **a** SDS-PAGE analysis of expression of recombinant truncated SAG2 protein. *Lane M* molecular weight marker, pre-stained (Bangalore Genei), *Lanes 1 to 2* uninduced control, *Lane 3* 4-h post induction, *Lane 4* 6-h post induction, *Lane 5* 8-h post induction, *Lane 6* Eluted protein after dialysis, **b** Western blot analysis of purified recombinant truncated SAG2 protein, *Lane M* Molecular weight marker, pre-stained (Biomatik), *Lane 1* strong reactivity with known positive sera

15 mM imidazole and 20 mM β -mercaptoethanol yielded histidine-tagged recombinant fusion protein. The recombinant protein was washed in buffer containing 5 mM imidazole. The protein was dialysed against decreasing concentration of urea for 3 to 4 h and finally, against PBS for 2–3 h at 4 °C for refolding. SDS-PAGE analysis resolved the recombinant protein as a clear band corresponding to the molecular weight of ~21 kDa (Fig. 2a).

The concentration of the recombinant protein was measured as 0.557 mg/ml by modified Lowry protein assay kit (Pierce, USA). The specific reactivity and purity of the recombinant protein was checked by Western blot analysis using hyperimmune as well as the known positive serum (Fig. 2b).

Evaluation of immunodiagnostic potential of recombinant protein by rec-SAG2-ELISA

The serodiagnostic potential of rec-SAG2-ELISA was studied with sera samples collected from different

ruminants. The cut-off value for positive samples was determined by adding two standard deviations to average OD₄₉₂ nanometer values of six negative controls. The cut-off values for goats, sheep and cattle were determined as 0.107, 0.193 and 0.177, respectively. Based on OD₄₉₂ nanometer value, 41.26 % goat, 50 % sheep and 64.44 % cattle sera samples were positive for *T. gondii*-specific IgG antibodies (Fig. 3a–c).

Comparison of ELISA and IFAT

The sensitivity and specificity of rec-SAG2-ELISA and kappa values at 95 % confidence interval (95 % CI) were calculated using IFAT as standard test. The sensitivity and specificity of the rec-SAG2-ELISA ranged from 81.25–87.10 and 85.71–91.43 %, respectively, in different domesticated ruminants which is evident by the kappa values obtained for each host species, thus indicating a substantial agreement among the two tests (details in Tables 1, 2 and 3).

Discussion

Toxoplasmosis is widely prevalent among vertebrates in all continents; however, the absence of clinical manifestation in immunocompetent subjects makes any assessment on prevalence difficult. In immunocompetent individuals, diagnosis relies on serological methods (Pfrepper et al. 2005). Among several serodetection tests employed, indirect haemagglutination (IHA) and latex agglutination test (LAT) were shown to be insensitive in their present form (Dubey 1987). Modified agglutination test (MAT), though specific, is considered as time consuming and expensive, while ELISA has been accepted as the most practical test for diagnosis of toxoplasmosis (Dubey et al. 1987). Keeping in view the limitations of specificity of native protein-based ELISAs, application of purified recombinant proteins is

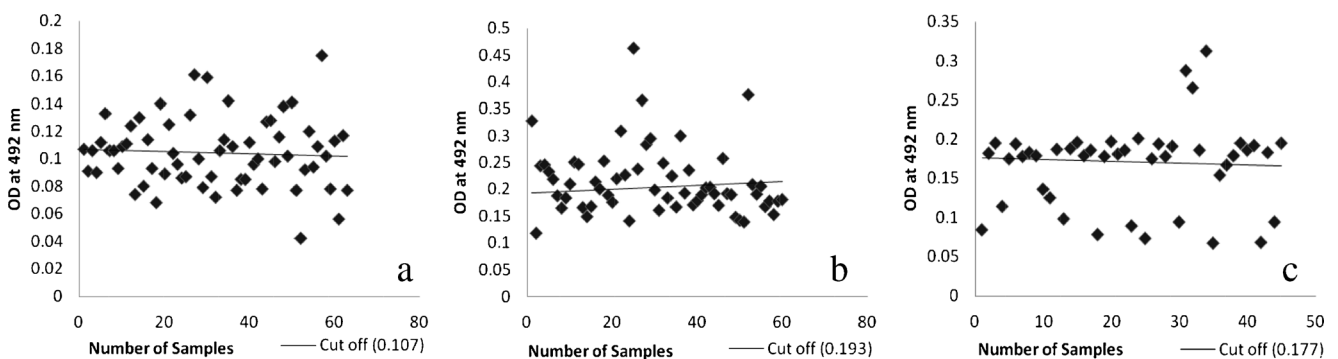


Fig. 3 **a** Antibody response against *T. gondii* in field sera of goats by rec-SAG2-ELISA. **b** Antibody response against *T. gondii* in field sera of sheep by rec-SAG2-ELISA. **c** Antibody response against *T. gondii* in field sera of cattle by rec-SAG2-ELISA

Table 1 Comparison of IFAT and ELISA for detection of antibodies against *Toxoplasma gondii* in goats

| Test | IFAT | | | | | |
|----------|----------|----------|-------|-----------------------|-----------------------|-------------|
| | Positive | Negative | Total | Sensitivity (95 % CI) | Specificity (95 % CI) | Kappa value |
| ELISA | | | | | | |
| Positive | 23 | 3 | 26 | 82.14 % | 91.43 % | 0.741 |
| Negative | 5 | 32 | 37 | (63.09–93.87) | (76.92–98.10) | |
| Total | 28 | 35 | 63 | | | |

considered to be the promising option for high throughput applications.

Numerous attempts have been made worldwide to produce several recombinant *T. gondii* antigens, particularly using the prokaryotic expression systems. Among the various parasitic proteins of diagnostic significance, cell surface proteins have been in focus for their potential as diagnostic markers. Since the surface proteins are easily accessible to the host immune system from the beginning of infection, major surface antigen SAG1 and related proteins like SAG2 have relative advantage over less abundant and immunogenic proteins in detection of infection induced antibodies.

Though recombinant SAG2 antigen has been used for serodetection of toxoplasmosis in humans (Li et al. 2000; Sawicka et al. 2005; Fong et al. 2008), published reports for the same in animals are scanty. It is important to note that SAG2 is one of the most conserved proteins in the SRS superfamily of *T. gondii* (Jung et al. 2004; Crawford et al. 2010). Further, because of its immunodominant nature (Saeij et al. 2008), a SAG2-based ELISA can detect *T. gondii*-specific antibodies with high sensitivity from both acute and chronically infected patients when used either alone or in combination with other proteins (Prince et al. 1990; Parmley et al. 1992; Li et al. 2000; Sawicka et al. 2005; Fong et al. 2008). The recombinant SAG2 protein has adequate diagnostic sensitivity and specificity for *T. gondii*-specific IgG and IgM antibodies without cross reactivity (Lau and Fong 2008). All these attributes have made SAG2 a promising candidate for the serodiagnosis of toxoplasmosis in livestock. We expressed and purified the histidine-tagged truncated recombinant SAG2 fusion protein for the serodiagnosis of

Table 2 Comparison of IFAT and ELISA for detection of antibodies against *Toxoplasma gondii* in sheep

| Test | IFAT | | | | | |
|----------|----------|----------|-------|-----------------------|-----------------------|-------------|
| | Positive | Negative | Total | Sensitivity (95 % CI) | Specificity (95 % CI) | Kappa value |
| ELISA | | | | | | |
| Positive | 26 | 4 | 30 | 81.25 % | 85.71 % | 0.667 |
| Negative | 6 | 24 | 30 | (63.55–92.75) | (67.32–95.88) | |
| Total | 32 | 28 | 60 | | | |

Table 3 Comparison of IFAT and ELISA for detection of antibodies against *Toxoplasma gondii* in cattle

| Test | IFAT | | | | | |
|----------|----------|----------|-------|-----------------------|-----------------------|-------------|
| | Positive | Negative | Total | Sensitivity (95 % CI) | Specificity (95 % CI) | Kappa value |
| ELISA | | | | | | |
| Positive | 27 | 2 | 29 | 87.10 % | 85.71 % | 0.701 |
| Negative | 4 | 12 | 16 | (70.15–96.29) | (57.16–97.80) | |
| Total | 31 | 14 | 45 | | | |

toxoplasmosis in goats, sheep and cattle. There is no published report available on the use of recombinant SAG2 protein-based ELISA for serodetection of *T. gondii*-specific IgG in domesticated and/or pet animals from India and only a few reports exist worldwide (Huang et al. 2002). A recombinant SAG2 protein-based ELISA was reported to be sensitive and specific without cross reactivity for diagnosis of feline toxoplasmosis by Huang et al. (2002).

The specific selection of the C-terminal of SAG2 protein for serodiagnostic application was based on our earlier study where we cloned and sequenced the complete ORF of SAG2 gene (561 bp) from *T. gondii* RH strain as well as from two local isolates (Izatnagar and Chennai). Comparison of nucleotide sequence revealed 100 % homology between the deduced amino acid sequences of these isolates/strains (Singh et al. 2011). In silico analysis of antigenicity index, hydrophilicity plot and surface probability plot by DNA STAR software revealed that the immunogenic regions were located towards the C-terminal region of the protein succeeding the signal sequence. Further, C-terminal region of SAG2 has been used for specific detection of *Toxoplasma*-specific IgG and IgM antibodies in humans (Lau et al. 2007).

IFAT has been an important reference test for serodiagnosis of toxoplasmosis (Dubey et al. 1995; Masala et al. 2003; Hove et al. 2005; Sharif et al. 2007; Langoni et al. 2011; Luciano et al. 2011; Rahman et al. 2011; Cenci-Goga et al. 2013). The sensitivity and specificity of the rec-SAG2-ELISA ranged from 81.25 to 87.10 and 85.71 to 91.43 %, respectively in respect of IFAT. Furthermore, substantial agreement was observed between IFAT and ELISA for all the three different species studied when interpreted by kappa value statistics. Therefore, it may be concluded that ELISA using recombinant truncated SAG2 fusion protein would be a useful field serodiagnostic test for the detection of *T. gondii* infection in goats, sheep and cattle in developing countries like India.

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Conflict of interest The authors declare no conflict of interest.

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