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# Serodiagnosis of animal trypanosomosis using a recombinant invariant surface glycoprotein of *Trypanosoma evansi*

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Trypanosomosis is endemic in many parts of the world in animals and humans. The diagnosis of asymptomatic carrier animals and a followup treatment is essential for control of the disease. In the present study, the extracellular domain (ED) region of invariant surface glycoprotein (ISG-75) gene of *Trypanosoma evansi* consisting of 1320 nucleotides (nt), encoding a polypeptide of 440 amino acids (aa) has been cloned and expressed in eukaryotic system *Pichia pastoris* (X-33). Immunoreactivity of the expressed protein (~70 kDa) was checked using a panel of standard serum samples. Furthermore, the diagnostic potential of the protein was evaluated using bovine, camel and horse serum samples from the field. Statistical analysis of the bovine data showed optimum combination of sensitivity and specificity at 98.6 and 99.7, respectively (>0.547 cut off OD value). The present finding revealed that the recombinant protein can be exploited as a potential diagnostic antigen in the serodiagnosis of trypanosomosis.

**Keywords**: Immunoreactivity, rISG-75ED, ROC analysis, Surra

Trypanosomosis is an endemic disease in many parts of the world in animals and humans. *Trypanosoma evansi* causes a progressive wasting disease called "surra" and is considered as an important disease of animals including domestic and wild herbivores and carnivores in tropical and sub-tropical countries. *T. evansi* is mainly transmitted mechanically by tabanid flies and the clinical symptoms of trypanosomosis include severe anemia, intermittent fever, weight loss, reduced productivity and occasional abortion, etc. Cattle, buffaloes, camels and horses are the most susceptible hosts of surra in southeast Asia[1]. The carrier status of the infection can be diagnosed by nucleic acid detection by polymerase chain reaction (PCR) or sensitive and specific serological tests. A number of genes of trypanosomes have been heterologously expressed, for instance, variable surface glycoprotein (VSG) gene from *T. evansi*[2,3], invariant surface glycoprotein (ISG) gene[4,5] and actin gene of *T. evansi*[6] in *E. coli*, acid α-mannosidase and trans-sialidase from *T. cruzi*[7,8], congopain from *T. congolense*[9] and ISG and VSG genes[10] in *P. pastoris*.

The ISGs are uniformly distributed over the entire surface of the trypanosomes and are blood stream stage specific. An estimated, $5\times10^4$ ISG-75 molecules are found on the surface of bloodstream forms trypanosomes[11] and do not exhibit antigenic variation like VSG. Many blood stream forms of ISGs such as, ISG-65 or ISG-70[12] and ISG- 75[13], etc., have been identified and characterized. Besides, ISG-75 is conserved among all the species and subspecies of the *Trypanozoon* sub-genus including *T. evansi, T. b. gambiense, T. b. rhodesiense and T. equiperdum*[11]. Post-translational modification systems such as, glycosylation, correct folding, disulphide bond formation, O and N-linked glycosylation exist in *P. pastoris*[14]. These modifications especially the glycosylation play a crucial role in correct protein folding, stability and biological activity of the proteins[15]. Earlier study[16] reported the presence of three potential N-glycosylation sites on ISG. Hence, in the present study, ED region of ISG-75 gene of *T. evansi* was expressed in *P. pastoris* to maximize the interaction of recombinant antigen with antibodies produced against native ISG-75. The expressed protein was evaluated for its potential as a diagnostic antigen in the serodiagnosis of trypanosomosis.

## Materials and Methods

### *Trypanosoma evansi* stabilates, antigens and serum samples

Four different isolates of *T. evansi* available in the parasitology laboratory of NIVEDI were propagated

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and purified[17], and then the whole cell lysate (WCL) antigens were prepared as mentioned earlier[2]. The VSG RoTat 1.2 antigen was imported from the Koning Leopold Institute of Tropical Medicine, Antwerp, Belgium (OIE reference laboratory of surra) and used as per manufacturer's instruction as a standard antigen.

Hyper immune/immune sera raised in rabbit/ cattle/buffalo[2] and available in the laboratory were used in the present study. The hyper immune serum against recombinant ISG-75 ED (rISG-75ED) was raised by following the standardized protocol[2]. Feed and drinking water to the experimental animals were given *ad libitum* and dealt as per the standard of animal ethics. The field sera from cattle (n=831), buffalo (n=220), camel (n=314) and goat (n=4), were collected randomly across different states of India representing eastern, western, southern and northern regions (Table 1), while horse (n=98) sera were collected from an organized herd. All the animals were apparently healthy.

**Cloning and transformation into *P. pastoris* (X 33)**

The ED region of ISG-75 has been amplified using a set of expression primer, TeE-ISGF (5'-TGTG*GAATTC* **ATG**GAGGAGCTCTCTGTTGCAC-3') and TeE-ISGR (5'-CAAA*GCGGCCGC*ATGA TGATGATGA TGATGTTTCGTTGTCCCAATCCAG-3') and then cloned into pGEMT/A cloning vector. TeE-ISGF corresponds to 85 to 103 bp and TeE-ISGR corresponding to1404 to 1386 bp were designed from our published sequence JN797772. The cloned ED region of ISG-75 contains 440 aa (29-468 aa), the signal peptide sequence constituting 1-28 aa is not included in the expressed protein. The ED region of ISG-75 released from pGEMT/A cloning vector[5] was cloned into eukaryotic expression vector pPICZα(A). The recombinant plasmid pPICZα(A)ISG-75ED, was then transformed into *E. coli* (top 10) competent cells by following the standard protocol[18] and the recombinant cells were selected with zeocin (100 µg/mL) and confirmed by PCR and restriction enzyme (*Eco*RI and *Not*I) digestion. The recombinant plasmid DNA isolated (Invitrogen) from the positive clone was sequenced using vector specific primers (AOXF/R) and insert specific primers (TeE-ISGF/R) to confirm the orientation of the insert.

The recombinant plasmid pPICZα(A)ISG-75ED, isolated from the positive clone was linearised using *Pme*I and then electroporated (Gene pulser, Xcell; Biorad) into eukaryotic expression host *P. pastoris* (X-33) competent cells (Invitrogen) at 25 µF capacitance, 1499V (voltage) for 3.2 mili seconds. The transformed cells were plated on the yeast extract, peptone, dextrose and sorbitol (YPDS) agar plates containing zeocin (300 µg/mL) and incubated at 30°C for 5 days. The translucent colonies appeared on plate were further streaked on to YPD agar plates containing zeocin (300 µg/mL) and incubated at 30°C for 48 h. The DNA from the colonies was isolated (Qiagen, USA) and subjected to PCR using vector specific (AOX F/R) and gene specific (TeE-ISGF/R) primers to select positive recombinant clones for the expression of protein.

**Expression and purification of rISG-75ED**

The inoculum for the protein expression was prepared by inoculating a single positive colony into 10 mL buffered glycerol complex medium (BMGY, Invitrogen) in a 100 mL baffled flask and incubated in a

Table1—Serodiagnosis of trypanosomosis from different regions of India

| | | Test (Antigen) | | | | | |
|---|---|---|---|---|---|---|---|
| | | ELISA (rISG-75ED) | | ELISA(VSG RoTat 1.2) | | CATT/ *T. evansi* | |
| State | Species | P | N | P | N | P | N |
| | | 55 | 140 | 53 | 142 | 57 | 138 |
| Karnataka | Cattle | 55 | 117 | 51 | 121 | 54 | 118 |
| | Buffalo | 2 | 2 | 2 | 2 | 2 | 2 |
| | Goat | 5 | 93 | 5 | 93 | 5 | 93 |
| | Horse | 24 | 56 | 28 | 52 | 25 | 55 |
| Tamil Nadu | Cattle | 25 | 103 | 29 | 99 | 26 | 102 |
| Maharashtra | Cattle | 10 | 38 | 8 | 40 | 9 | 39 |
| | Buffalo | 8 | 34 | 9 | 33 | 8 | 34 |
| Odisha | Cattle | 12 | 102 | 21 | 93 | 12 | 102 |
| Uttarakhand | Cattle | 102 | 170 | 98 | 174 | 102 | 170 |
| West Bengal | Cattle | 60 | 254 | 58 | 256 | 59 | 255 |
| Rajasthan | Camel | | | | | | |

P, Positive; and N, Negative

shaker (300 rpm) incubator at 30°C for 18h ($OD_{600}$ =4). The cells were pelleted out at 3000×g for 5 min at room temperature (25°C) and then resuspended in 50 mL buffered methanol complex medium (BMMY, Invitrogen) to an $OD_{600}$ of 1.0. The culture was then placed in 500 mL baffled flask and returned to the incubator for growth. The induction of gene expression was maintained by adding 100% methanol to a final concentration of 0.5% at every 24 h. The optimal post induction time (PIT) was determined by SDS PAGE analysis of the induced culture collected (1 mL) at every 24 h intervals (0-120 h). The protein was expressed in bulk after the determination of optimal PIT. The culture supernatant containing the Histidine (His) tag rISG-75ED was purified using NiNTA agarose column (Qiagen, USA) as per the manufacturer's instructions. The control clones, such as induced X-33, X-33 with pPICZα(A) and uninduced X-33 with pPICZα(A)ISG-75ED were run simultaneously and the protein from each control clone was purified and used as control proteins/ antigens. The purified proteins were dialyzed against phosphate buffered saline (PBS) pH 7.2 and after estimating the protein concentration[19], stored in aliquots at −20°C till further use.

**SDS PAGE, immunoblot and serological tests**

The supernatant (300 µg/well) and purified rISG-75ED (50 µg/well) and 50 µg/well control proteins were loaded into polyacrylamide gel to detect the recombinant protein. The purity of the histidine tag rISG-75ED was authenticated in immunoblot using anti histidine-tag antibodies (Bethyl laboratories, USA). Further, the immunoreactivity of the expressed protein was determined in immunoblot using hyper-immune/immune sera raised in experimental animals.

For ELISA, the microtiter plates (Maxisorp®, Nunc) were coated overnight at 4°C with 100 µL/well of purified rISG-75ED (3 µg/well)/ WCL (500 ng/well)/ VSG RoTat 1.2 (600 ng/well) antigens. After overnight incubation the microtiter plates were washed 4 times with washing buffer [0.25% (v/v) Tween-20 in PBS, pH 7.2] and blocked with 150 µL/well blocking buffer (3% skimmed milk powder (SMP) and 0.05% Tween-20 in PBS) for 1 h at 37°C and washed. The rabbit/cattle hyper-immune sera, buffalo immune serum and field/herd serum samples (cattle, buffalo, camel, horse and goat) were diluted (1:100) with blocking buffer (1:1 diluted with PBS) and added (100 µL/well), followed by incubation for 1 h at 37°C. After washing, the respective secondary antibody such as, antirabbit IgG (for rabbit sera), antibovine IgG (for bovine sera), antihorse IgG (for horse sera), protein G (for camel sera) and antigoat IgG (for goat sera) conjugated with horseradish peroxidase [diluted as per manufacturer's instruction (Sigma)] was added (100 µL/well) and incubated for 1h at 37°C. The microtiter plates were then washed and 100 µL/well enzyme substrate (chromogenic) solution (5 mg of O-phenylenediamine dihydrochloride (Sigma) and 0.03% (v/v) $H_2O_2$) was added to develope the colour. The reaction was then stopped by the addition of 1M $H_2SO_4$ (100 µL/well). Upon completion of ELISA, the plates were read at 492 nm in ELISA reader (Bench mark micro plate reader, Biorad). Further, the specificity of rISG-75ED was determined in ELISA with cattle serum samples clinically infected with *Theileria annulata* and *Babesia bigemina* (diagnosed by blood smear examination).

The CATT/*T.evansi* kit developed with freeze dried trypanosomes of *T. evansi* VAT, RoTat 1.2[20] was procured from the Koning Leopold Institute of Tropical Medicine, Antwerp, Belgium (OIE, reference laboratory of surra) and used as per manufacturer's instruction for comparative evaluation. The field/herd serum samples were subjected to ELISA in duplicate using rISG-75ED, VSG RoTat 1.2 antigens and CATT/*T.evansi*, for comparative evaluation.

**Statistical analysis**

Receiver operating characteristic (ROC) analysis was carried out to determine the cut off OD value and optimum combination of diagnostic specificity and sensitivity of rISG-75ED and VSG RoTat 1.2 antigens in ELISA using bovine sera by keeping CATT/*T. evansi* as a gold standard test. An agreement of rISG-75ED and VSG RoTat 1.2 antigens in relation to CATT/*T. evansi* was determined for diagnosing antibody against *T. evansi*. Software MedCalc (Version 12.7.2, USA) was used to generate ROC curves and area under curves (AUC).

## Results and Discussion

**Expression and purification of rISG-75ED**

The sequencing results revealed that the insert in the recombinant plasmid [pPICZα(A)ISG-75ED] is in correct orientation with respect to promoter sequence and in frame with the ATG codon. The ED of the cloned ISG-75 sequence is of 1320 nt encoding a polypeptide of 440 aa, with an apparent molecular weight of 49kDa. The four recombinant *P. pastoris* (X-33) clones, identified and confirmed by PCR

(Fig. 1 A & B), were preserved at −80°C till further use. The optimum PIT was found to be 92 h (Fig. 2A), the concentration of recombinant protein decreased after 120 h PIT. The yield of the purified rISG-75ED ranged from 20-30 mg/L of culture. SDS-PAGE analysis of the induced supernatant and purified products revealed the presence of rISG-75ED (~70 kDa). However, the protein band corresponding to ~70 kDa was not found in controls (Fig. 2B). The increase in molecular weight of the expressed protein might be due to the glycosylation of the expressed protein and addition of 11 kDa polypeptide from the vector pPICZα(A) to the insert [rISG-75ED (49 kDa) + vector fusion (11 kDa) + glycosylation = ~70 kDa] as a fusion. Earlier study[21] also reported the

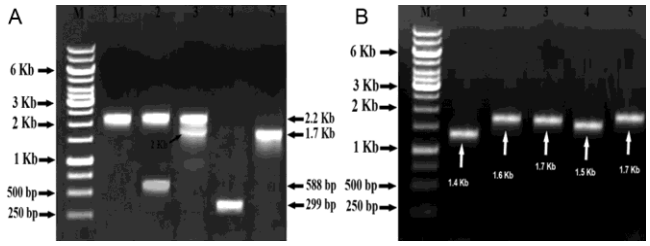


Fig. 1—PCR with DNA from *P. pastoris* (X-33) clones using different combination of primers (A) AOX F/R and α factor primers; (B) TeEISG F/R, AOX F/R and α factor primers with pPICZα(A)+ ISG-75ED. [A: lane 1 = X-33 DNA with AOXF/R, lane 2= X-33 with pPICZα(A) DNA using AOXF/R, lane 3= X-33 with pPICZα(A)+ ISG-75ED DNA using AOXF/R, lane 4= X-33 with pPICZα(A) DNA using α factor/AOXR, lane 5= X-33 with pPICZα(A)+ ISG-75ED DNA using α factor/AOXR; (B): lane 1 =TeEISGF/R, lane 2= TeEISGF/AOXR, lane 3= α factor /AOXR, lane 4= α factor / TeEISGR, lane 5= AOXF/TeEISGR, and lane M= 1Kb DNA ladder]

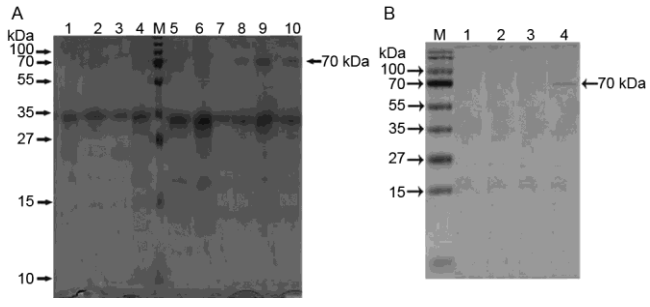


Fig. —2—SDS PAGE analysis of purified and non purified protein samples. [Lane M= prestained protein ladder; (A) Non purified supernatant samples: lanes1 and 2= 72 h and 96 h induced X-33 clones respectively, lane 3 and 4= 72 h and 96 h induced X-33 with pPICZα(A) clones respectively, lane 5-10 = 0, 24, 48, 72, 96 and 120 h induced X-33 with pPICZα(A) +ISG-75ED clones, respectively; (B) Purified protein samples: lanes 1, 2 and 4=purified protein samples from induced X-33, X-33 with pPICZα(A) and X-33 with pPICZα(A) +ISG-75ED, respectively; lane 3= uninduced X-33 with pPICZα(A) +ISG-75ED]

increase in molecular weight of *Aspergillus awamori* glucoamylase protein by 10 kDa when expressed in *P. pastoris* and this could be due to glycosylation[22].

**Immunoreactivity of rISG-75ED**

The immunoblot analysis showed that the expressed rISG-75ED is highly immunogenic. The rISG-75ED and control antigens remained respectively, reactive and non reactive with different hyper-immune/ immune serum/anti-His tag antibodies in immunoblot (Fig. 3A). The comparative reactivity of different antigens with immune/hyper-immune sera in ELISA is shown in Fig. 4. Moreover, rISG-75ED remained non reactive with control sera. Hyper-immune serum raised against rISG-75ED showed immunoreactivity in CATT/*T. evansi* and with homologous and WCL antigens, but remained non-reactive with VSG RoTat 1.2 antigen. Moreover, rISG-75ED remained non reactive with serum

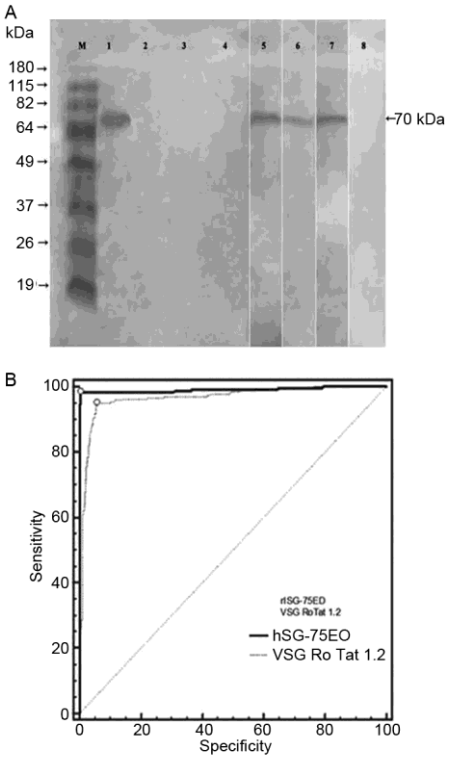


Fig. 3—(A) Immunoblot analysis: Lane M= prestained protein ladder, lanes 1 and 5= rISG-75ED v/s rabbit hyper-immune sera raised against rISG-75ED and different isolates of *T. evansi* respectively, lane 6= rISG-75ED v/s hyper-immune/immune serum from bovine/ buffalo, lane 7= rISG-75ED v/s anti His tag antibodies, lane 8= rISG-75ED v/s healthy sera, lanes 2, 3 and 4= purified proteins from induced X-33 and X-33 with pPICZα(A) and uninduced X-33 with pPICZα(A)+ISG-75ED respectively v/s hyperimmune/immune sera; and (B) Comparative ROC graph depicting diagnostic sensitivity and specificity.

samples clinically infected with *Theileria annulata* and *Babesia bigemina* indicating that ISG-75 is highly specific to trypanosomes.

**Diagnostic potential of rISG-75ED**

Statistical analysis of ELISA using ROC showed optimum combination of diagnostic sensitivity and specificity respectively, 98.6% [95% confidence interval (CI), 97.3-100] and 99.70% (95% CI, 99.4-100.0), at >0.547 cut off OD value with rISG-75ED. While, the sensitivity and specificity of VSG RoTat 1.2 was found to be 95.2 (95% CI, 92.8-97.7) and 94.3 (95% CI, 92.7-96.0), respectively, at >0.508 cut off OD value. The comparative analysis is shown in Table 2 and Fig. 3B. Furthermore, the comparison of AUCs by chi-square revealed no significant differences between results from ELISA ($P$ <0.0001). The AUC indicates that rISG-75ED has a classification accuracy up to 99.3%. The Cohen's kappa coefficient of agreement was found to be 0.98 and 0.86, respectively with rISG-75ED and VSG RoTat 1.2 antigens. Hence, rISG-75ED proved to be a good diagnostic antigen for the serodiagnosis of trypanosomosis using ELISA.

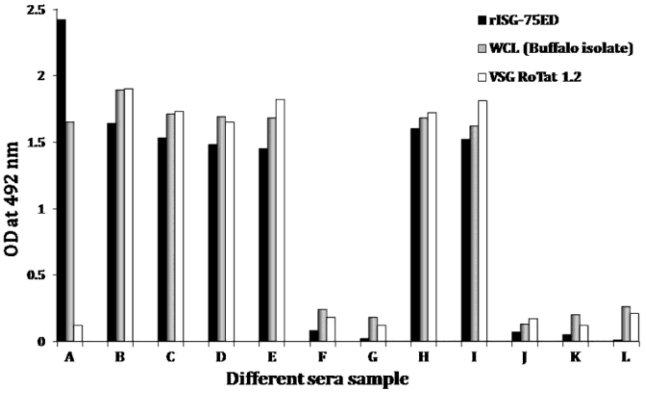


Fig. 4—Comparative immunoreactivity of different antigens in ELISA. (A**)**= hyper immune serum against rISG-75ED; B–F, hyper-immune serum against *T. evansi* buffalo, dog, lion, leopard isolates and healthy rabbit serum, respectively; (G), anti rabbit conjugate control; (H & I), hyper immune and immune serum from cattle and buffalo, respectively; (J & K), healthy sera from cattle and buffalo, respectively; and L, antibovine conjugate control]

Trypanosomosis, an important tropical disease of domestic livestock, is caused by a wide range of trypanosome species, such as *T. evansi, T. equiperdum* and *T. brucei* and causes severe economical losses in animal production. Moreover, the two genetically distinct subspecies *T. brucei gambiense* and *T. brucei rhodesiense,* cause Human African Trypanosomosis. The effective serodiagnosis of trypanosomosis depends on the identification of a protein unique to trypanosomes, lesser chance of cross-reactivity with proteins from other pathogens and the selection of an invariant protein which is present in a wide range of different isolates and/or strains of the pathogen[23]. The whole cell lysates of *T. evansi* leads to strong cross reactions with *T. vivax*, *T. congolense* and even *T. cruzi*[24]. Hence, the proteins that are not subjected to antigenic variation such as, ISG-75 represent a promising alternative in the development of diagnostic tests[23].

The cloned and expressed portion of rISG-75ED is of 1320 nt in length encoding a polypeptide of 440 aa and is highly conserved, but a little variation in the aa sequence at the amino terminal portion of ED has been observed (Fig. 5). The immunoblot analysis revealed that, the expressed protein is highly immunoreactive with hyper-immune sera raised in rabbits and cattle and immune serum raised in buffalo. Further, the immunoreactivity of rISG-75ED with immune and hyper immune sera from the laboratory animals and natural host species in ELISA also revealed that, the rISG-75ED is highly immunogenic. The comparative analysis of the immunoreactivity of rISG-75ED, VSG RoTat 1.2 and WCL antigens of *T. evansi* in ELISA using rabbit/cattle hyperimmune sera and buffalo immune serum showed higher OD values in WCL antigen and VSG RoTat 1.2 antigens than with the rISG-75ED. However, the immunoreactivity of rabbit hyperimmune sera raised against rISG-75ED showed higher OD values with recombinant antigen. This might be due to the generation of homologous antibodies. In CATT/

Table 2—ROC analysis: An agreement of rISG-75ED and VSG RoTat 1.2 in relation to CATT/ *T.evansi* for diagnosing antibody against *T.evansi.*

| Antigen Criteria | Cut-off OD | Sensitivity | Specificity | AUC | SE | P value | PPV | NPV | Cohen's Kappa | +LR | -LR | CA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| rISG-75ED | >0.547 | 98.6 | 99.7 | 0.992 | 0.00434 | <0.0001** | 99.3 | 99.5 | 0.9840 | 332.82 | 0.015 | 99.36 |
| VSG RoTat 1.2 | >0.508 | 95.2 | 94.3 | 0.966 | 0.00694 | <0.0001** | 86.6 | 98.1 | 0.8684 | 16.90 | 0.053 | 94.56 |

[AUC, area under curve; SE, standard error; P value, significance level P (Area=0.5); PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; −LR, negative likelihood ratio; and CA, classification accuracy]

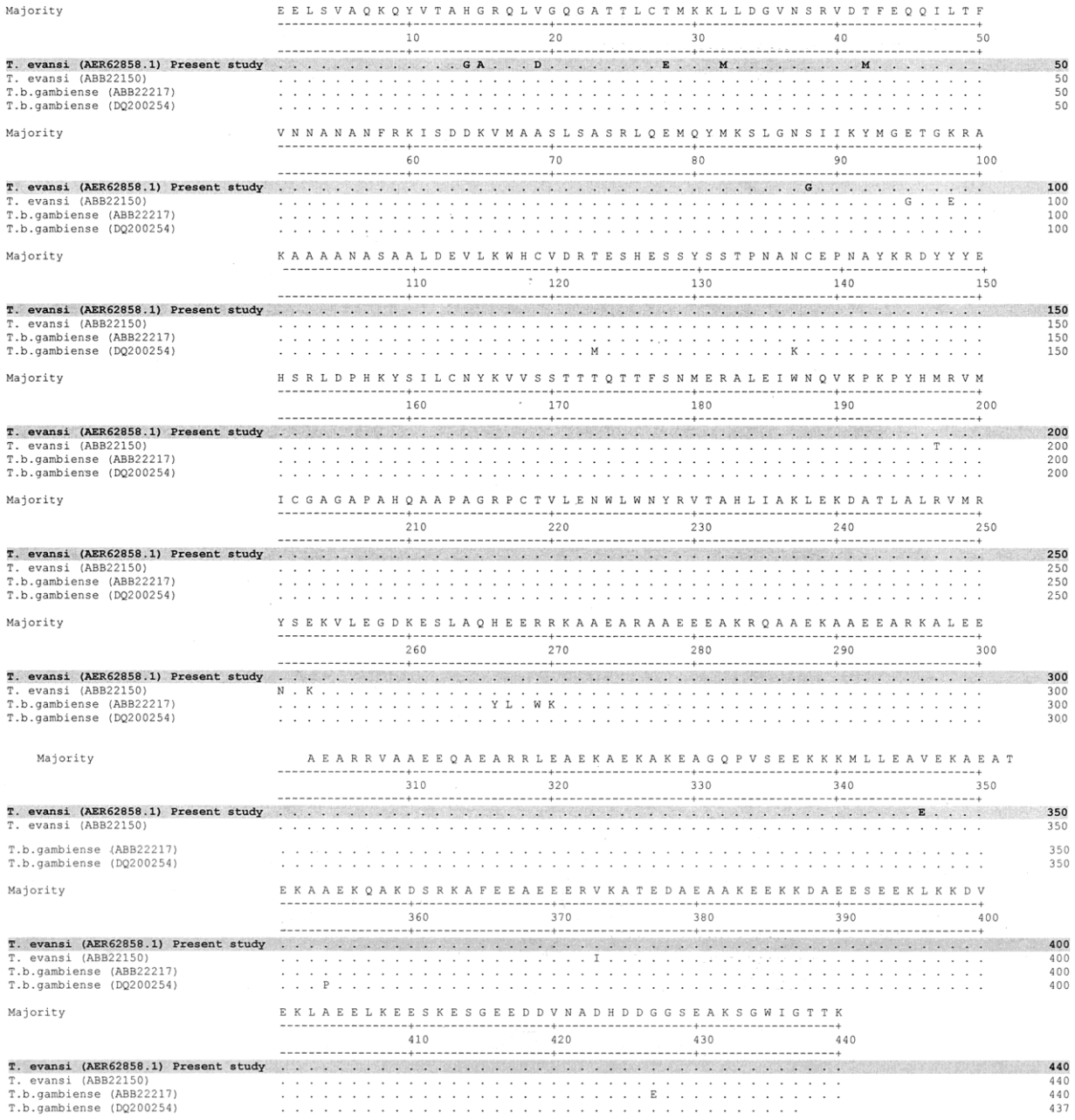


Fig. 5—Multiple sequence (deduced aa sequence) alignment of ISG-75ED. [Dot (.) represents the sequence identity, spaces or dash (–) denotes the gap generated during alignment, which are introduced for optimal comparison. The variation in aa sequence is represented by a single letter aa code and the present study is shaded]

*T. evansi*, the agglutination was observed up to 1:8 sera dilution with rabbit hyper immune sera raised against recombinant protein, while the hyper immune rabbit sera raised against lion and leopard isolates showed the minimum reactivity. The immunoreactivity in CATT/*T. evansi* was found to be more with buffalo immune serum over cattle hyper immune serum; this might be due to the abundant IgM produced during active infection which is mainly responsible for agglutination reaction[24]. The comparative reactivity of field sera sample with different diagnostic tests/antigens is shown in Table 1. Moreover, the serum samples from cattle infected with *Babesia bigemina* and *Theileria annulata* did not react with rISG-75ED, indicating that ISG-75 is specific to trypanosomes.

Though, ISG-75 from *T. brucei gambiense* has been expressed earlier[10] in *P. pastoris* (GS115 M5), it has been reported that the expressed protein was cleaved into two (40-45 kDa and 60-65 kDa) fragments and lacked first 147 aa at the N-terminal region of 40-45 kDa fragment. Further, it is reported that the expressed protein remained non-reactive with positive camel serum samples[10]. However, in the present study, the expressed protein (rISG-75ED) remained intact (nondegraded). This may be attributed to different expression host system. Moreover, recent study by Sengupta *et al*.[25] revealed the expression of intact recombinant VSG in *P. pastoris* and its potential application in the diagnosis of surra. Besides, rISG-75ED reacted with cattle, buffalo, camel, goat and horse sera, indicating the presence of ISG-75 specific epitopes for bovines, camels and horses at the amino-terminal region of the intact protein. Hence, the expression of intact amino-terminal region of ISG-75 ED is significant for serodiagnosis trypanosomosis.

The seroepidemiology and chi square ($\chi^2$) analysis showed that, the trypanosomosis is more prevalent in West Bengal (eastern India) compared to other regions of India (Fig. 6). However, the seropositivity (SP) of trypanosomosis in buffalo was found to be more in Karnataka (SP=31.9%, $\chi^2$=0.236, df=2, *P* >0.05) and Maharashtra (SP=20.8%, $\chi^2$=0.274, df=2, *P* >0.05), compared to other regions of India. The eastern and southern parts of India revealed high seropositivity of trypanosomosis in animals, when compared to other regions of India.

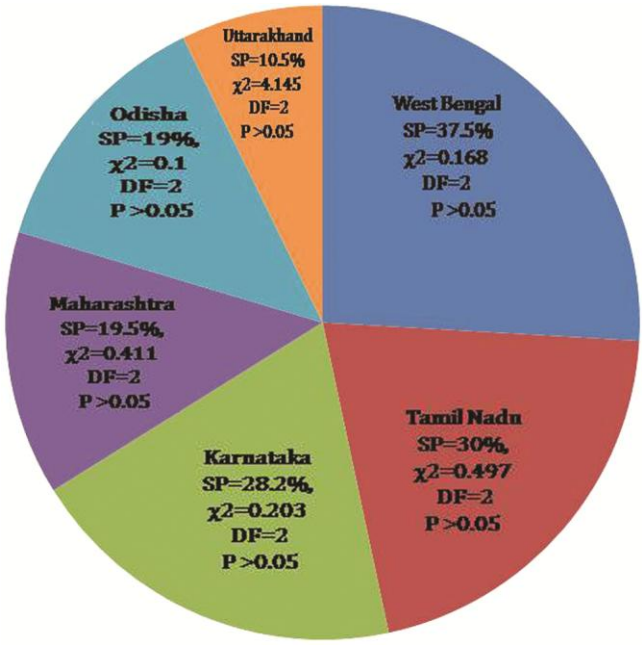


Fig. 6— Trypanosomosis; seroepidemiology and chi-square analysis. [SP, seropositivity; χ2, chi square; DF, degree of freedom; and P, significance value]

In the present study, rISG-75ED was characterized using naturally infected bovine, camel and horse serum samples, which is highly significant in demonstrating the immunoreactivity of the expressed protein. To the best of authors' knowledge and literature search, this is the first attempt of expressing an extracellular domain of ISG-75 gene from *T. evansi* in *P. pastoris*. The present finding can be used as a rapid, reliable, potential and promising perspective tool for future application in the serodiagnosis of trypanosomosis in animals caused by *T. evansi.* It is well established that, ISG-75 is highly conserved among all the *Trypanozoon* species and subspecies. The serological tests developed using ISG-75 can be used in the detection of trypanosomosis caused by other species of trypanosomes[4]. Hence, the developed assay also carries its potential in the detection of trypanosomosis caused by other species of trypanosomes.

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