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Research paper

Development and evaluation of recombinant antigen and monoclonal antibody based competition ELISA for the sero- surveillance of surra in animals

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ABSTRACT

Trypanosoma evansi, a haemoflagellated protozoan parasite, is responsible for chronic as well as the acute debilitating disease called surra in a wide range of herbivores and carnivores including domestic and wild animals. Since the parasite is having wide host range, there is a need for diagnostic test which can detect the *T. evansi* specific antibody in different species of animals for generating sero-surveillance data. In the present study we developed and evaluated competitive enzyme immunoassay using monoclonal antibodies (MAbs) raised against recombinant variable surface glycoprotein (rVSG) of *T. evansi*. The immunoreactivity of the developed MAbs (IgG3-subtype) was evaluated by immunoblot as well as ELISA and subsequently used in the development and standardization of competitive ELISA (C-ELISA). Further, the serological data generated from the C-ELISA using reference samples constituting true positive or surely infected (35), true negative (45), sero-positive (225) and sero-negative (215) samples and was analyzed statistically. The true positivity/negativity was determined by thin blood smear examination and diagnostic PCR assay, While, seropositivity/seronegativity of the reference samples was determined through standard reference tests. The data showed the diagnostic sensitivity of 92.6% and specificity of 96.4% with Cohen's kappa value of 0.88. In order to determine the utility of C-ELISA in detecting *T. evansi* antibodies in different species of animals, the assay was further evaluated with 1361 field sera sample comprising bovine, horse, donkey and camel. Since the C-ELISA described herein has showed high sensitivity and specificity, this single test can be explored in the sero-surveillance of *T. evansi* in a wide range of animals.

1. Introduction

Trypanosoma evansi, a haemoflagellated protozoan parasite, is responsible for a fatal wasting disease known as surra in animals. Surra is known to cause havoc economic loss to the livestock industry (Field and Carrington 2009). *Trypanosoma evansi* has a worldwide distribution and widest host range among salivarian group which includes both domestic and wild animals. However, camel, horses, cattle, buffalo and carnivores such as dogs are considered as the most likely affected hosts to *T. evansi* infection (Holland et al. 2004). Although *T. evansi* is considered to be a livestock pathogen, there are reports documenting the incidence of human trypanosomosis (Truc et al. 1998, 2013; Joshi et al. 2005; Kaur et al. 2007; Van Vinh Chau et al. 2016). Transmission of the parasite in livestock is mainly by mechanical methods through biting flies such as *Tabanus* and *Stomoxys* (Sumba et al. 1998) and also by vampire bats in South and Central America (Hoare 1965). Surra is clinically characterized by recurrent fever, anemia, oedema, loss of appetite, muscular weakness and abortion. Recovered animals can maintain low levels of fluctuating parasites for years together and can serve as carrier of the parasite. These apparently healthy carrier animals can silently diffuse the disease to newer geographical areas and to other susceptible animals as the detection of carrier status is often impossible. Thus, *T. evansi* is considered as an inapparent spreading parasite. Hence, adopting effective control measures are the useful steps to stamp out the disease. In this circumstance, data regarding the sero-surveillance of surra/trypanosomosis in different species of animals is an essential part in implementing the effecting control measures.

Different serological tests based on antibody detection have been developed for the diagnosis of *T. evansi* infection, such as indirect immunofluorescent antibody test and the immune trypanolysis test (OIE, 2012), VSG RoTat 1.2 and native antigen based ELISA (Verloo et al. 2000, 2001; OIE, 2012), CATT/*T. evansi* (Songa and Hammers, 1988; Verloo et al. 2000), and ELISA/*T. evansi* (Verloo et al. 2000). Furthermore, several antigen detection tests have also been developed by

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several groups including ELISA for camels (Nantulya et al. 1989; Diall et al. 1992; Verloo et al. 2000) and buffaloes (Nantulya et al. 1989; Davison et al. 1999; Verloo et al. 2000). The ELISA using soluble (native) antigens qualifies as a universal test and more likely to identify the truly uninfected animals while; CATT/*T. evansi* is more likely to classify truly infected animals correctly (OIE, 2102). Hence, in the present study the newly developed assay has been statistically evaluated using the panel of reference samples which constitute true positive or surely infected, true negative, seropositive and seronegative samples. The true positive and negative samples were determined by parasitology and diagnostic PCR assay. While, seropositive and seronegative samples were determined through standard reference tests such as ELISA using soluble (native) antigen and CATT/*T. evansi*.

Moreover, surface glycoprotein genes such as variable surface glycoprotein (Sengupta et al. 2010, 2012, 2014, 2016), and invariant surface glycoprotein (Rudramurthy et al. 2013, 2015, 2017a) have also been targeted for PCR based and serological based diagnosis of trypanosomosis. Several serodiagnostic tests based on recombinant antigens have also been developed recently using surface glycoproteins of trypanosomes (Tran et al. 2009), VSG has already been expressed in different host cells including insect cell line (Urakawa et al. 2001), yeast (Roge et al., 2013; Sengupta et al. 2016) and *E. coli* (Sengupta et al. 2012, 2014) and has been used for sero-surveillance in indirect ELISA. In the present study competitive ELISA (C-ELISA) has been developed utilizing MAbs raised against recombinant VSG expressed in *Pichia pastoris* and was evaluated with a panel of sera sample collected from different species of animals.

2. Materials and methods

2.1. T. evansi stabilates and recombinant VSG

T. evansi isolated from buffalo and maintained in the Parasitology Laboratory of ICAR-NIVEDI, Bengaluru, India, was used in the present study. *T. evansi* isolate was propagated as per the methodology described by Sengupta et al. (2010). Recombinant VSG was heterologously expressed in *P. pastoris*- X-33 (Sengupta et al. 2016) and used for the development of MAbs. Animal experiments were conducted with the prior permission from the Institutional Animal Ethics Committee (IAEC). The experimental animals were maintained and handled strictly by adhering to the standards of animal ethics committee.

2.2. Whole cell lysate (WCL) and VSG RoTat 1.2 antigens

The WCL of *T. evansi* (buffalo isolate) was prepared as per the protocol mentioned by Rudramurthy et al., (2015). In brief, the purified parasite pellets ($\sim 8 \times 10^5$ trypanosomes mL^{-1}) were suspended in phosphate buffered saline (PBS, pH. 7.2) and ultrasonicated (Soniprep.150, MSE Sanyo, UK). The sonicated material was centrifuged at 9500 x g at 4 °C for ½ h and the supernatant was collected and used as WCL (native) antigen. The protein concentration in the supernatant was estimated (Lowry et al. 1951) and preserved (in aliquots) at −80 °C till further use. The WCL antigen was used as standard reference antigen (OIE, 2012) in ELISA (at 500 ng/well) to evaluate the newly developed assay and also in the comparative evaluation of the assay using field sera sample.

Furthermore, the VSG RoTat 1.2 antigen was procured from the Koning Leopold Institute of Tropical Medicine, Antwerp, Belgium (OIE reference laboratory of surra) and was explored in ELISA as per the manufacturer's instruction. The VSG RoTat 1.2 antigen was used in ELISA at 600 ng/well for comparative evaluation the new assay using field sera sample.

2.3. Sera sample

Immune serum raised in buffalo against *T. evansi* (buffalo isolate) by Sengupta et al., (2012) and preserved in the laboratory is used in the characterization of C-ELISA. The reference field serum samples (260 each for positive and negative) constitute true positive or surely infected, true negative, sero-positive and sero-negative samples. The true positive samples constituting cattle (3), buffalo (4) and camel (2) and true negative samples constituting cattle (16), buffalo (15) and camel (14) were screened by parasitology through thin blood smear examination and diagnostic PCR assay by following the standard protocol (OIE, 2012; Sengupta et al. 2010). Further, 26 samples from cattle (8), buffalo (9) and camel (9) were positive only by diagnostic PCR and negative by thin blood smear examination were considered as surely infected and were also included in the reference set. For diagnostic PCR assay, the DNA was isolated from all the blood samples and subjected to diagnostic PCR using VSG specific diagnostic primers by following the protocol described by Sengupta et al. (2010) and the amplified product was subjected to agarose gel electrophoresis to determine the 400 bp DNA. The remaining reference samples constitute 225 sero-positive and 215 sero-negative samples. The seropositivity/seronegativity of the serum samples for surra was determined through indirect ELISA using standard reference antigen such as native (WCL) antigen and also by CATT/*T. evansi*. Indirect ELISA and CATT/*T. evansi* has been carried out as per the standard protocol mentioned earlier (Rudramurthy et al. 2015). The new assay (C-ELISA) was evaluated using the above mentioned reference samples.

Field sera sample from bovine (599), horse (51), camel (624) and donkey (87) were collected randomly from apparently healthy animals from different states of India during 2011–2014 as shown in Table 1. The sera included in the present study were screened for surra by serological tests such as indirect ELISA using WCL and VSG RoTat 1.2 antigens, CATT/*T. evansi* and competitive ELISA in duplicate for comparative evaluation.

Table 1
Immunoreactivity of different sera sample in indirect ELISA (WCL and VSG RoTat 1.2 antigens), competitive ELISA and CATT/*T. evansi*.

Tests→		Indirect ELISA using				Competitive ELISA		CATT/ *T. evansi*		χ2 value	*P* value
		WCL Antigen		VSG RoTat 1.2 antigen							
↓States	Species	P	N	P	N	P	N	P	N		
Karnataka	Bovine	92	227	92	227	94	225	93	226	0.042	0.997
	Horse	3	48	2	49	3	48	3	48	0.288	0.962
	Donkey	5	82	6	81	6	81	6	81	0.14	0.986
Odisha	Bovine	10	44	11	43	10	44	9	45	0.245	0.969
West Bengal	Bovine	83	143	84	142	85	141	87	139	0.165	0.983
Rajasthan	Camel	71	553	72	552	76	548	74	550	0.228	0.972

P: Positive, N: Negative, χ2: Chi square, P value: Significance value.

3. Production of MAbs

3.1. Immunization of BALB/c mice and preparation of splenocytes

Four BALB/c mice (8–12 weeks old) were used for the immunization and another two were maintained as healthy control. The immunization was carried out as per the protocol mentioned earlier (Ligi et al. 2016). Briefly, primary immunization was given subcutaneously with emulsified recombinant VSG (200 μg/mice) followed by two booster doses (200 μg/mice) having 21 days apart. The third booster injection was given intraperitoneally with 1000 μg/mice of recombinant antigen 3–4 days prior to fusion. Further, the splenocytes were prepared from the immunized mice by following the protocol mentioned earlier (Ligi et al. 2016). The splenocytes were counted in haemocytometer by diluting the aliquot of suspension in RBC lysis buffer. Meanwhile, the sera was collected from the mice and used as a positive control.

3.2. Preparation of B-cell myeloma and feeder layer

In the present study, SP2/O cell line which are hypoxanthine, aminopterine and thymidine (HAT) sensitive ($HGPRT^-/TK^-$ and non-Ig secretor) were used for the production of hybridoma clone as per the standard protocol (Ligi et al. 2016). Briefly, SP2/O cells which were grown up to log phase in IMDM were harvested and washed twice with the media resuspended in IMDM without any supplements and finally counted using haemocytometer. The macrophages which are used as feeder layer were prepared from the peritoneal exudate of the non-immunized BALB/c mice and the serum collected from the mice was used as negative control.

3.3. Production of hybridoma clones

The hybridoma clones were produced by the fusion of SP2/O cells with splenocytes by following the protocol mentioned earlier (Ligi et al. 2016). In brief, splenocytes (20 million) and SP2/O cells (2 million) were mixed in a screw capped tube containing 10 mL of IMDM and then the cells were pelleted by spinning at 214 g for 10 min at room temperature. After removing the residual medium from the cell pellet, 500 μL of polyethylene glycol (PEG) and dimethyl sulphoxide (DMSO) mixture (Sigma Aldrich) was added (within 1 min) drop wise and exposed for an additional one min. Five milliliter of IMDM (without any supplementation) was added to the tube slowly after one minute incubation. The cells were then transferred to humidified CO_2 (5%) incubator and incubated for 90 min. The cells were pelleted out (at 214 g for 10 min at room temperature) and suspended in IMDM, containing sodium hypoxanthine (0.1 mM), aminopterine (0.4 μM), thymidine (0.016 mM) (Gibco®Life technologies), 20% fetal calf serum (FCS) and L-glutamate (20 mM). The cells were then plated out in 96 well culture plate (0.2 mL per well), containing feeder layer/macrophages (4×10^6 cells/96 well plate). Meanwhile, two wells containing only SP2/O cells and macrophages were maintained as controls. The plates were then incubated in a humidified CO_2 (5%) incubator at 37 °C for 7 days, after 7th day the medium was replaced with IMDM, containing all the supplements as mentioned above except aminopterine and then transferred to humidified CO_2 (5%) incubator.

3.4. Screening of hybridoma clones and selection of monoclones

The clones appeared in the plate were screened by indirect ELISA on the 10th day for rVSG specific antibody production. The positive clones obtained were expanded and preserved in IMDM containing FCS (40%) and DMSO (10%) till further use. By dilution cloning technique the monoclones were selected from the positive hybridoma. For dilution cloning, the hybridoma cells were counted and 100 cells [suspended in 20 mL IMDM (with all supplements)] were seeded in 96 wells (200 μL/well) culture plate (~one cell/well). The plate was transferred to humidified CO_2 (5%) incubator and incubated till the appearance of clones. The wells with single clone (monoclone) were screened for rVSG specific antibodies by indirect ELISA, expanded further and preserved in IMDM containing FCS (40%) and DMSO (10%) till further use. For large scale production of MAbs the cells were grown (250 mL culture flasks) till the IMDM color changes to yellow and further tested by indirect ELISA. The MAbs from hybridoma culture was collected by centrifugation at 3000 rpm for 3 min and preserved at -80 °C till further use.

Indirect ELISA was carried out as per the standard protocol. In brief, the microtiter plate/s (Maxisorp®, Nunc) were coated (100 μL/well) overnight with purified rVSG (3 μg/well) antigen in PBS (pH 7.2) at 4 °C. Culture supernatant from each well (from 96 well plates), including control wells (SP2/O, spleen and macrophage) was added (100 μL/well) and incubated for 1 h at 37 °C, followed by washing. After washing, appropriately diluted anti-mouse antibody (100 μL/well) conjugated with horseradish peroxidase (Sigma) was added and incubated for 1 h at 37 °C followed by addition of substrate solution. The reaction was stopped by the addition of 1 M H_2SO_4 and the plates were read at 492 nm in an ELISA reader. The MAbs obtained from two clones named as MVSG121and MVSG122 (high reactivity) were preserved at -80 °C till further use.

4. Characterization of MAbs by immunoassay

MAbs (MVSG121and MVSG122) were characterized by immunoassay as per the standard protocol (Sambrook and Russell, 2001). The electrophoresed proteins (recombinant VSG and control antigens) were transferred onto nitrocellulose membrane. The membrane was then treated with undiluted culture supernatant from MVSG121, MVSG122 and SP2/O clones. The substrate solution containing diamino benzidine tetrahydrochloride was added finally to develop the immunoblot.

4.1. Isotyping of MAbs

Monoclonal antibodies (MVSG121and MVSG122) were isotyped using mouse isotyping antibodies (IgG1, IgG2a, IgG2b, IgG3, IgA and IgGM) (Sigma Aldrich) by antigen mediated ELISA as per manufacturer's instructions. Briefly microtiter plates (Maxisorp®, Nunc) were coated with 100 μL/well of rVSG (3 μg/well) for overnight at 4 °C. The undiluted MAb (culture supernatant from monoclone) was added into each well (100 μL/well) and incubated for 2 h at 37 °C followed by the addition of isotyping antibodies (100 μL/well). After washing anti goat IgG was added each well (100 μL/well) and incubated for 30 min at 37 °C, followed by the addition of substrate solution (100 μL/well). The plate was then read in ELISA reader at 492 nm.

4.2. Development of C-ELISA

Competitive ELISA was developed exploring rVSG and MAb (MVSG121). The MVSG121 showed high reactivity with rVSG by indirect ELISA compared to MVSG122, hence MVSG121 was explored in the development of new diagnostic assay. The optimum concentration of antigen (rVSG), serum dilution and MVSG121 dilution were determined by checker board titration using immune serum (buffalo and cattle) raised against *T. evansi* (buffalo isolate). Polystyrene microtiter plates (Maxisorp®, Nunc) were coated with 6 μg of purified rVSG antigen in PBS (pH 7.2) at 4 °C overnight. After overnight incubation the microtiter plates were washed three times with PBS containing 0.5% Tween 20 (PBST) to remove any unbound antigen. Subsequently the wells were treated with 3% bovine serum albumin in PBS (150 μL/well) and incubated at 37 °C for 1 h to block the nonspecific reactions. After washing the plates, test sera sample (20 μL of test serum, 20 μL of blocking buffer and 60 μL of MVSG121) were added in duplicate and incubated at 37 °C for 1 h followed by washing. Control wells such as,

negative, strongly positive, weakly positive, MAb control (Cm) and conjugate control were also run simultaneously. Anti-mouse antibody conjugated with horseradish peroxidase (diluted as per manufacturer's instruction) was added to each well (100 µL/well) and incubated at 37 °C for 1 h followed by the addition of substrate solution (100 µL/well). The plates were incubated till the visible color develops in the Cm wells and then the reaction was stopped by the addition of 1 M H_2SO_4 solution (100 µL/well). The plates were then read at 492 nm in an ELISA reader. The OD values obtained were converted to percentage inhibition (PI) values by using the formula: PI = 100 – {(OD in test well ÷ OD in Cm well) × 100} (Singh et al. 2004).

4.3. Determination of diagnostic sensitivity and specificity of C-ELISA

The frequency distribution graph (Wright et al. 1993) was prepared using reference serum samples to determine the positive–negative cut-off PI, diagnostic sensitivity and specifity of C-ELISA. The reference serum samples were subjected to C-ELISA and the serological data (PI values) obtained was statistically analyzed through frequency distribution graph to evaluate the new assay (C-ELISA).

The specificity of the developed test was determined using cattle serum samples clinically infected with *Theileria annulata* and *Babesia bigemina*. Furthermore, Chi square ($\chi 2$) analysis (Snedecor and Cochran 1968) was also carried out using the data (species wise) obtained from indirect ELISA using WCL and VSG Ro Tat 1.2 antigens, C-ELISA and CATT/*T. evansi* to determine the significance of the new assay.

5. Results

5.1. Production and characterization of MAbs.

Two monoclonal hybridoma clones were generated against rVSG such as, MVSG121 and MVSG122. Both MVSG121 and MVSG122 belong to isotype of IgG, subtype IgG3. MVSG121 showed high reactivity with rVSG by indirect ELISA compared to MVSG122. While, the control clones (SP2/O, spleen and macrophages) did not react with MAbs from both clones. Immunoblot analysis revealed that the both MVSG121 (Fig. 1) and MVSG122 (Fig not shown) are highly specific and reacted with rVSG with absence of immunoreactivity of with control proteins (Fig. 1).

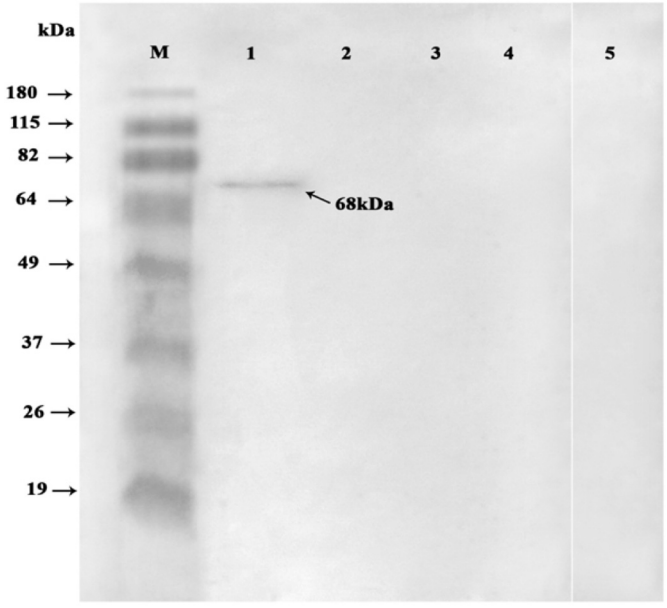

Fig. 1. Immunoblot analysis: Lane M = prestained protein ladder, lanes 1, 2, 3 and 4 = MAb (MVSG121) versus rVSG and control proteins, lane 5 = SP2/O versus rVSG.

5.2. Diagnostic sensitivity and specificity of C-ELISA.

The optimum concentration of antigen (rVSG), immune serum (buffalo and bovine) and MVSG121 were found to be 6 µg/well, 20 µL and 60 µL respectively. The positive –negative cut-off PI value was found to be > 40 (Fig. 2). The diagnostic sensitivity and specificity as revealed by the frequency distribution graph was found to be respectively 92.6% (95% confidence interval (CI), 89.5%, 95.7%) and 96.4% (95% CI, 94.1%, 98.7%), at > 40 PI. The Cohen's kappa co-efficient of agreement was found to be 0.88. The positive and negative predictive values were found to be respectively 96.5% and 92.3%. Furthermore, the Chi square ($\chi 2$) analysis also revealed no significant difference between indirect ELISA (with WCL and VSG Ro Tat 1.2 antigens), C-ELISA and CATT/*T. evansi* (Table 1) and the developed assay is in agreement with the standard reference test for surra.

6. Discussion

The disease incidence and the severity vary with the strain of the parasite as well as the species of host affected. The cell surface of the *T. evansi* is densely packed with a thick (12-15 nm) uniform coat of VSG which exhibits extensive antigenic variation. VSG is regarded as the interface between the host immune system and the parasite which help the later to remain undetected by the host immune system. In susceptible animals the VSG is expressed in all stages of infection irrespective of early, mid or later stages (Verloo et al. 2001). Moreover, the antigenicity of VSG is responsible for eliciting the host immune system for sufficient level of antibody production (Gadelha et al. 2011). However, VSG genes undergoes continuous shift in the antigenic expression site during clonal multiplication of the parasite within the host. In view of this, the present study is aimed at the expression of complete ORF of VSG gene. This is advantageous for the development of serological tests, since the recombinant VSG can bind efficiently with any anti-VSG antibodies. Moreover, as expression of VSG is exhibited during early, mid and later stage of the infection, the developed test will be helpful to detect the disease in all stages.

Earlier studies reveal that VSGs can act as predominant antigens in the development of antibody detection tests for sleeping sickness and surra (Magnus et al. 1978; Verloo et al. 2001). Previously, studies have been carried out for the heterologous expression of VSG in *E. coli* (Sengupta et al. 2014) as well as in *Pichia pastoris* (Sengupta et al. 2016) and explored as candidate antigen in the diagnosis of *T. evansi* infection (Songa and Hammers, 1988; Lejon et al. 2005). As *T. evansi* has wide host range it is essentially required to develop a single unique test that can be used for screening the sera from different species of animals. Keeping this as background, in the present study, development of competitive ELISA has been attempted exploring recombinant VSG expressed in *P. pastoris* and MAbs (MVSG121) raised against recombinant protein (rVSG). Since VSG is considered as one of the most immunodominant antigens, diagnostic assays based on monoclonal antibody will be having more sensitivity and specificity compared to tests based on polyclonal antibodies.

The competitive enzyme immunoassays based on recombinant antigens and MAbs have tremendous potentiality in the diagnosis of parasitic diseases. The C-ELISA in combination with recombinant antigens play significant role especially in the parasitic diseases, wherein the diagnostic antigen production cannot be done in cell culture. Furthermore, heterologous expression of recombinant antigen precludes the use of experimental animals in antigen preparation (Knowles and Gorham 1993). Moreover, the specificity achieved by MAbs based competitive ELISA can be very high, since antibodies are raised against a single epitope, which is known to be an organism specific. Several groups have successfully produced MAbs against different antigens of trypanosomes, such as against invariant antigens of vector forms of

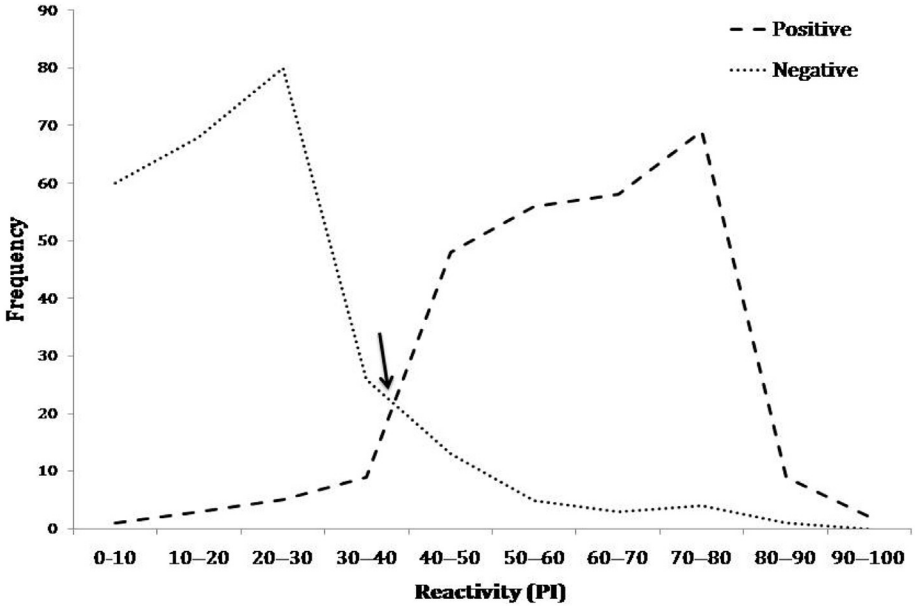

Fig. 2. Frequency distribution graph establishing diagnostic sensitivity and specificity of competitive ELISA.

Trypanosoma simiae (Bosompem et al. 1995), against metacyclic trypomastigotes stage of *Trypanosoma cruzi* (Manas et al. 1986), associated antigens against *Trypanosoma cruzi* (Takasu et al. 1989) and against cell membrane antigen of *T. evansi* (Rayulu et al., 2007). Earlier study also showed that inhibition ELISA (developed using MAbs and rISG) exhibited diagnostic sensitivity and specificity of 98.8% and 99.2% respectively (Rudramurthy et al. 2017b). Moreover, monoclonal antibodies play significant role in the discrimination of two different serological tests (discriminative serological test), thus could help in the development of more advanced and specific immunodiagnostic test. Earlier study by Lemesre et al. (1986), reported the development of a competitive antibody enzyme immunoassay for serodiagnosis of Chagas' disease using *T. cruzi* species-specific MAbs. The reported assay exhibited high sensitivity of 96.6% and also had significant potential to differentiae the Chagas' disease from any other cross-reacting parasitic diseases.

In the developed test, there is competition between polyclonal antibodies in the test sera and developed MAbs. It has been reported that, polyclonal antibodies exhibit high affinity towards its antigen than MAbs (Lipman et al. 2005). Hence, in positive cases, polyclonal antibodies will compete for antigen binding with MAbs and as a result MAbs will remain unbound. However, in negative cases, the absence of specific/polyclonal antibodies allows MAbs to bind with the antigen. The sensitivity and specificity of the new assay were found to be 92.6% and 96.4% respectively. Moreover, the developed assay is in agreement with the standard reference test for surra since, the Chi square ($\chi 2$) analysis revealed no significant difference between competitive ELISA, indirect ELISA (using WCL and VSG RoTat 1.2 antigens) and also with CATT/*T. evansi*. The indirect ELISA developed earlier using rVSG (ELISA/rVSG) exhibited diagnostic sensitivity and specificity of 95.4% and 93.8% respectively (Sengupta et al. 2016). Though the sensitivity remains low with C-ELISA compared to ELISA/rVSG, the specificity is found to be higher with C-ELISA. Moreover, by using single anti-mouse immunoglobulin conjugate, serum from any animal species can be tested with shorter turnaround time and lower expense. Hence, competitive ELISA has additional advantages over those of conventional serological assays and MAbs act as important reagents in diagnosis as well as treatment of diseases. Furthermore, the monoclonal antibodies developed in the present study could also be employed in the development of antigen capture ELISA to detect the current infection.

7. Conclusion

In conclusion, the trypanosomes which were once considered as non-infective/non pathogenic to humans are emerging as potential pathogenic human parasites. Hence, the developed assay may be explored in the detection of atypical human trypanosomosis caused by *T. evansi*. However, the test needs further evaluation before being used with human samples. As per the literature, this is the first ever endeavor of producing monoclonal antibody against variable surface glycoprotein of *T.evansi*. The developed MAbs (MVSG121) showed higher combination of diagnostic sensitivity and specificity. Therefore the new assay can be exploited as a reliable, potential and promising perspective tool for future application in the serodiagnosis of trypanosomosis for surveillance study in a wide range of animals.

Conflict of interest statement

The Authors declare no conflict of interest statement.

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