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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.