



Sero prevalence of surra in camels using flagellar antigen in some areas of Rajasthan: A pilot survey

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Abstract

Background: Surra is caused by *Trypanosoma evansi* and is considered as one of the important disease in camels in India. Very often camel acts as the carrier of the infection with very low level of parasitaemia which cannot be detected by conventional blood smear examination. **Methodology:** In the present study, a pilot sero-epidemiological study was conducted among camels for trypanosomosis in Rajasthan using purified flagellar (FLA) antigen based indirect enzyme linked immunosorbent assay (I-ELISA) and compared with standard CATT/*T. evansi* test. The diagnostic potentiality of purified FLA antigen based I-ELISA (FLA-I-ELISA) were evaluated using 230 camel sera samples from different districts of Rajasthan including Jaipur, Udaipur, Bikaner, Jodhpur, and Ajmer. **Results:** The test showed an overall 23.04% sero positivity (S. P). In Jaipur, S. P was found to be 23.07%, in Udaipur 25.5%, in Bikaner 18.18%, in Jodhpur 25.3% and in Ajmer, S. P was found to be 23.33%. **Conclusion:** Thus, epidemiological survey were done in camels with purified FLA-I-ELISA test in different districts of Rajasthan and revealed that, Udaipur showed the maximum sero positivity for trypanosomosis.

Keywords: *Trypanosoma evansi*; surra; purified flagellar (FLA) antigen; I-ELISA; sero-epidemiology.

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1. Introduction

Surra, caused by *Trypanosoma evansi* is transmitted by the bites of tabanids flies and is considered as one of the important disease in camels in south-east Asia¹. The clinical symptoms of surra includes anemia, recurrent fever, oedema, weight loss, loss of appetite, poor draughtability, infertility, abortion and even death of animals. On different host, the pathogenic effects of surra vary accordingly to the virulence of the strain of parasite, local epidemiological condition and the susceptibility of the host species^{2,3}. When parasitaemia is high, the clinical form of the disease can easily be detected by the conventional Giemsa stained blood smear examination, which is widely practiced in the field. The untreated recovered animals can harbor low parasitaemia with latent infection which is apparently symptomless, but if there is any stress to the immunity of the animal the

infection may flare up to the clinical infection with high parasitaemia. Moreover such carrier animals may act as a source of infection to other healthy animals through fly bites. Also it has been reported that there is a significant reduction in animal production in carrier animals⁴. Therefore, for successful stamping out of the disease and for its subsequent treatment, there is an urgent need for the development of improved serological test for the detection of carrier status of animals which may be very much useful to screen out a large number of animal populations. Many diagnostic tools have been developed in past for the detection of carrier status of infection for trypanosomosis such as several serological test like indirect fluorescent antibody test (IFAT) or enzyme linked immunosorbent assay (ELISA), card agglutination test (CATT/*T. evansi*) etc, nucleic acid detection by PCR^{5,6} and parasitological diagnostic tests like thick or wet blood smear examination tests. As by parasitological tests, only clinical stages of infection can be diagnosed satisfactorily and not the latent or chronic infection⁷, serological test such as ELISA qualifies as a universal test for the detection of trypanosomosis as it is not strain specific². Flagellar portion protrudes from the basal body

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of *T. evansi* and is involved with the attachment to the host surfaces and elicit host antibody response⁸. Several flagellar pocket associated proteins have been identified and found to contribute to trafficking and virulence⁹. Moreover, it has been reported that, paraflagellar rod protein may be the critical organelle mediating attachment to vector cell surface¹⁰. Several groups have cloned and expressed paraflagellar rod protein gene 1 (PFR1) and paraflagellar rod protein gene 2 (PFR 2) in prokaryotic system^{11,12}. In present study, purified flagellar based indirect ELISA (FLA-I-ELISA) was developed and detailed epidemiological study was carried out in camels from five different districts of Rajasthan including Jaipur, Udaipur, Bikaner, Jodhpur, and Ajmer for the diagnosis of antibody of surra.

2. Materials and methods

2. 1. Preparation of *Trypanosoma evansi* stabilate and experimental animals

For *T. evansi* stabilate preparation, buffalo isolates from Karnataka were used which were maintained in Parasitology Laboratory, NIVEDI, Bangalore, India. . The parasites were propagated and purified^{5,6,13-15} to isolate the FLA antigen.

2. 2. Preparation of purified flagellar (FLA) antigen of *T. evansi* from whole trypanosomes

Whole trypanosomes/parasite was isolated from blood through standard protocol using diethyl amino ethyl (DEAE) cellulose column chromatography¹³. Purified flagellar (FLA) antigen was prepared from whole parasite¹⁶⁻¹⁸ and was confirmed by Giemsa stained blood smear examination. The protein concentration of FLA antigen was estimated¹⁹ and were preserved for further use.

2. 3. Card agglutination test for Trypanosomosis (CATT/*T. evansi*)

The CATT/ *T. evansi* kit obtained from the Koning Leopold Institute of Tropical Medicine, Antwerp, Belgium (OIE reference Laboratory of Surra) uses freeze dried trypanosomes of *T. evansi* VSG RoTat 1. 2^{20,21}. This kit was used as per manufacturer's instruction.

2. 4. Preparation of hyperimmune sera (HIS) in animal

In the present study, HIS against purified FLA antigen of *T. evansi* was raised in rat by using standard protocol¹³. All the experimental animals were dealt as per the standards of animal ethics; feed and drinking water were given *ad libitum*.

2. 5. Collection of field camel sera samples

Field camel sera samples (230) were collected randomly from five different districts of Rajasthan including Jaipur (52), Udaipur (40), Bikaner (33), Jodhpur (75), and

Ajmer (30) were subjected to FLA based indirect ELISA (FLA-I-ELISA) and CATT/*T. evansi* in duplicates for the screening of antibodies of *T. evansi* for trypanosomosis. After sampling, the collected camel sera samples were preserved at -80°C for further use.

2. 6. Characterization of purified FLA antigen of *T. evansi*

2. 6. 1. SDS PAGE analysis of purified FLA antigen of *T. evansi*

Purified FLA antigen (50µl) was loaded into 10% polyacrylamide gel and were subjected to SDS PAGE electrophoresis to detect purified FLA antigen as described by following the standard protocol²². The electrophoresed gel was stained with PAGE blue staining solution (Invitrogen, USA), followed by destaining.

2. 6. 2. Indirect- Enzyme linked immunosorbent assay (I-ELISA)

Using I-ELISA, the immunoreactivity of the purified FLA antigen was determined. The antigen concentration, sera dilution and conjugate dilution for I-ELISA were optimized by checker board titration. The concentration of purified FLA antigen was chosen at 200ng/well, dilution of HIS/field sera samples at 1:100 and conjugate dilution was chosen at 1:1500 respectively. In brief, the microtitre plates (maxisorp[®], nunc) were coated overnight at 4°C with purified FLA antigen of *T. evansi* (200ng/well) in PBS (pH=7. 2). After overnight incubation, the microtitre plates were washed three times with washing buffer [0. 25% (v/v) Tween- 20 in PBS, pH 7. 2] and blocked with 3% blocking buffer (skimmed milk powder in PBS were used) for one hour at 37°C. After incubation, the plates were washed and HIS raised in rat diluted at 1:100 dilution with half strength of blocking buffer was added and incubated at 37°C for one hour as described above. Later, the respective secondary antibody such as anti-rat IgG (for rat sera) and Protein G (for camel sera samples) conjugate with horse peroxidase (diluted as per manufacturer's instruction (sigma)) was added (100µl/well) and incubated for one hour at 37°C. The plates were washed and 100µl/well enzyme substrate (chromogenic) solution (5 mg of O- phenyl diamine dihydrochloride (sigma) and 0. 03% (v/v) H₂O₂) was added to develop the colour. The reaction in the plates were stopped after few minutes by adding 1M H₂SO₄ (100µl/well) and O. D was recorded at 492 nm in an ELISA reader (bench mark microplate reader, Biorad). Thus 230 field camel sera samples of different places in Rajasthan were subjected to FLA-I-ELISA in duplicate and were compared against CATT/*T. evansi* for epidemiological study for the detection of antibody against *T. evansi*.

2. 7. Statistical Analysis

A group of field camel sera samples collected from Jaipur, Udaipur, Bikaner, Jodhpur and Ajmer were subjected to FLA-I-ELISA and CATT/*T. evansi* test for the various statistical analysis of the test.

3. Results and discussion

Purified FLA fractions of *T. evansi* showed nine bands in SDS- PAGE analysis ranging from 18 -130KDa at 18, 20, 30, 50, 55, 60, 75, 90 and 130KDa (Figure I). In the earlier studies in some geographical regions, 4 to 12 polypeptide bands in the range of 17. 6 to 80. 2 kDa were found in different isolate for flagellar antigen¹⁷. By I-ELISA, the purified FLA antigen with hyperimmune sera showed high reactivity of O. D value of >1. 2 and control healthy rat sera remained non-reactive with O. D value of <0. 2 respectively. In SDS PAGE and ELISA, the purified FLA antigen of *T. evansi* has showed immunoreactivity with the panel of sera samples consisting of experimentally produced field samples including camels. Using winepicope software, the degree of agreement was determined by chi square test. Out of 230 field camel sera samples, 53 samples were found to be positive by FLA-I-ELISA test while approximately, 56 sera samples were found to be positive by CATT/*T. evansi* test. The overall seroprevalence (S. P) of trypanosomosis in camels in Rajasthan was found to be 23. 04% and 24. 34% respectively by I-ELISA and CATT/*T. evansi* tests. Moreover, district wise sero-prevalence survey by FLA-I-ELISA in Rajasthan state of India showed 23. 07% S. P in Jaipur, followed by 25. 5% S. P in Udaipur, 18. 18% in Bikaner, 25. 3% S. P in Jodhpur and 23. 33% S. P in Ajmer. Overall, district wise sero-prevalence survey in Rajasthan showed the dominant statistics with Udaipur of 25. 5% S. P followed by Jodhpur with 25. 3%, Ajmer with 23. 33%, Jaipur with 23. 07% and Bikaner with 18. 18% (Table I). In Rajasthan, no significant variations were observed between sero-positivity and negatively among two tests ($\chi^2=0. 108$, $df=1$, $p>0. 05$). When district wise analysis was carried out in Rajasthan, it was found that among camels, no significant difference observed in both the tests ($p>0. 05$). The developed ELISA showed high

agreement in screening of samples from animals when compared with CATT/*T. evansi* test. However a large panel of sera samples is required to determine the exact sensitivity and specificity of the test.

Conclusion

Thus, according to the statistical analysis it was revealed that FLA antigen based I-ELISA test can be explored for the detection of antibody of *T. evansi* for surra for the epidemiological study in camels in Rajasthan in India. However a large panel of samples is required to be tested for detail study.

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Conflict of interest

The author's declares none.

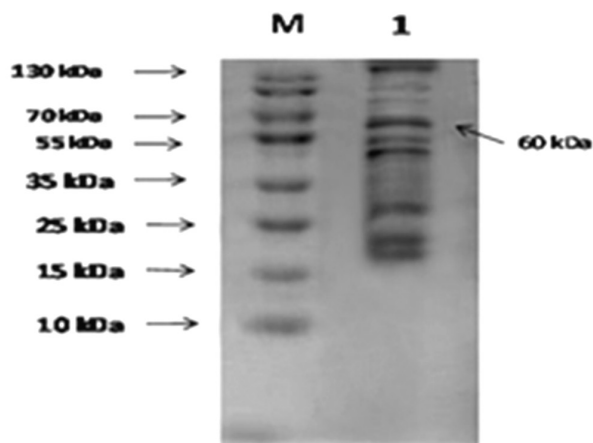


Figure I: SDS-PAGE analysis of purified flagellar antigen (FLA) Lane 1 = Purified FLA antigen, Lane M= Pre-stained protein ladder (Jose et al., 2015)²³

Table I: Comparison of FLA-I-ELISA and CATT/*T. evansi* test of the camel samples collected from various districts of Rajasthan

Test →	FLA-I- ELISA			CATT/ <i>T.evansi</i>		
	P	N	% Positive	P	N	% Positive
District wise (Rajasthan)						
Jaipur	12	40	23.07	13	39	25
Udaipur	9	31	25.5	9	31	22.5
Bikaner	6	27	18.18	7	26	21.21
Jodhpur	19	56	25.3	21	54	28
Ajmer	7	23	23.33	6	24	20
Total	53	177	23.04	56	174	24.34

P: Positive, N: Negative

References

- Holland W G , Thanh N G , My L N , Do T T , Goddeeris B M & Vercruyse J, Prevalence of *Trypanosoma evansi* in water buffaloes in remote areas in northern Vietnam using PCR and serological methods. *Trop Anim Health Prod*, **36** (2004) 45-48.
- OIE, *Trypanosoma evansi* (surra). In: Manual of diagnostic tests and vaccines for terrestrial animals (2012). World organization for animal health, Paris, cited at: <http://www.oie.int>.
- Desquesnes M, Holzmuller P, Lai D H , Dargantes A , Lun Z R & Jittaplapong S , *Trypanosoma evansi* and Surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *BioMed Res Int*, Article ID **194176** (2013): 22.
- Pholpark S, Pholpark M, Polsar C, Paengpassa Y & Kashiwazaki Y, Influence of *Trypanosoma evansi* on milk yield of dairy cattle in northeast Thailand. *Prev Vet Med*, **42** (1999): 39-44.
- Sengupta P P, Balumahendiran M, Suryanarayana V V S, Raghavendra A G, Shome B R, Ganjendragad M R & Prabhudas K, PCR-based diagnosis of surra-targeting VSG gene: Experimental studies in small laboratory rodents and buffalo. *Vet Parasitol*, **171** (2010): 22-31.
- Rudramurthy G R, Sengupta P P, Balamurugan V, Prabhudas K & Rahman H, PCR based diagnosis of trypanosomiasis exploring invariant surface glycoprotein (ISG) 75 gene. *Vet Parasitol*, **193** (2013): 47-58.
- Fernandez D, Gonzalez-Baradat B, Eleizalde M, Gonzalez-Marcano E, Perrone T & Mendoza M, *Trypanosoma evansi*: a comparison of PCR and parasitological diagnostic tests in experimentally infected mice. *Exp Parasitol*, **121**(2009):1-7.
- Bastin P, Pullen T J, Moreira – Leita F F & Gull K, Inside and outside of the trypanosome flagellum: a multifunctional organelle. *Microb Infect*, **2**(2000), 1865- 1874.
- Field M C, Carrington M. The trypanosome flagellar pocket. *Nat Rev Microbiol*. **7**(2009): 775-786.
- Gadelha C, Wickstead B, de Souza W, Gull K & Cunha-e-Silva N, Cryptic paraflagellar rod in endosymbiont-containing kinetoplastid protozoa. *Eukaryot Cell*, **4**(2005), 516–525.
- Maharana B R, Rao J R, Tewari A K, Singh Harkirat, Isolation and characterization of paraflagellar rod protein gene (PFR 1) in *Trypanosoma evansi* and its conservation among their kinetoplastid parasites. *Indian J Anim Res*, **45**(4)(2011a): 283-288.
- Maharana B R, Rao J R, Tewari A K, Singh Harkirat, Cloning and expression of paraflagellar ROD protein 2 (PFR 2) gene of *Trypanosoma evansi*. *J Vet Parasitol*, **25**(2)(2011 b): 118-123.
- Sengupta P P, Balumahendiran M, Balamurugan V, Rudramurthy G R & Prabhudas K, Expressed truncated N-terminal variable surface glycoprotein (VSG) of *Trypanosoma evansi* in *E. coli* exhibits immuno-reactivity. *Vet Parasitol*, **187**(2012), 1– 8.
- Sengupta P P, Rudramurthy G R, Ligi M, Roy M, Balamurugan V, Krishnamoorthy P, Nagalingam M, Singh L & Rahman H, Sero-diagnosis of surra exploiting recombinant VSG antigen based ELISA for surveillance. *Vet Parasitol*, **205**(2014), 490-498.
- Rudramurthy G R, Sengupta P P, Metilda B, Balamurugan V, Prabhudas K & Rahman H, Development of an enzyme immunoassay using recombinant invariant surface glycoprotein (rISG) 75 for serodiagnosis of bovine trypanosomosis. *Indian J Exp Biol*, **53**(2015), 7-15.
- Purohit S K, Jatkar P R & Lodha K R, Isolation of flagella and cell membrane of *Trypanosoma evansi*. *Indian Vet J*, **64**(1987), 18-20.
- Singh V, Singh A & Chhabra M B, Polypeptide profiles and antigenic characterization of cell membrane and flagellar preparations of different stocks of *Trypanosoma evansi*. *Vet Parasitol*, **56** (1995): 269-279.
- Rayulu V C, Singh A & Chaudhri S S, Monoclonal antibody based immunoassays for the detection of circulating antigens of *Trypanosoma evansi* in buffaloes. *Ital J Anim Sci*, **6**(2007): 907-910.
- Lowry O H, Rosenbrough N J, Farr A L & Randall R J, Protein measurement with Folin Phenol reagent. *J Biol Chem*, **193**(1951): 265-275.
- Bajyana Songa E & Hamers R, A card agglutination test (CATT) for veterinary use based on an early VAT Ro Tat 1.2 of *Trypanosoma evansi*. *Ann Soc Belg Med Trop*, **68**(1988): 233-240.
- Verloo D, Magnus E & Buscher P, General expression of RoTat 1.2 variable antigen type in *Trypanosoma evansi* isolates from different origin. *Vet Parasitol*, **97**(2001): 183-189.
- Sambrook J & Russell D, Molecular cloning: a laboratory manual (2001). Cold Spring Harbor Laboratory Press, New York.
- Jose L, Sengupta PP, Rudramurthy GR & Rahman H, A pilot sero-survey for surra in livestock in Karnataka by ELISA using flagellar antigen of *Trypanosoma evansi*. *Int J Fund Appl Sci*, **4** (2015): 99-103.