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EVALUATION OF FORMOL GEL SLIDE TEST WITH REFERENCE TO BLOOD MICROSCOPY AND ENZYME LINKED IMMUNOSORBANT ASSAY FOR DIAGNOSIS OF SURRA IN CATTLE

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Surra

Cattle

Formol Gel Slide test

ELISA

Wet Blood Examination

ABSTRACT

The objective of the present study was to evaluate the conventional Formol Gel Slide Test (FGST) vis-a-vis Enzyme Linked Immunosorbant Assay (ELISA) in diagnosis of *Trypanosoma evansi* infection in cattle. In all, 51 adult Holstein-Friesian cattle were categorised in three groups i.e *T. evansi* positive symptomatic, parasite negative symptomatic and apparently healthy from 12 animal sheds at Guwahati, Assam. The FGST was performed with some modifications and results obtained were compared with blood smear findings and ELISA. The serum samples obtained from symptomatic cattle with or without detectable parasite in blood were found positive in the FGST as evidenced by immediate gellification and opacity development akin to white of a boiled egg. When compared to ELISA, the sera from clinical cases with high antibody titre (OD value >0.736) were found positive in FGST, while those from asymptomatic cattle with lower antibody titre, (OD value < 0.736) were all negative in the test. Thus the detection performance of blood microscopy, FGST and ELISA were found to be 3.92%, 5.88% and 17.64 % respectively. The study also suggests that ELISA as usual is more sensitive and suitable for screening of cattle herds during epidemiological investigation. However, the study revealed FGST to be a suitable, simple to perform and low cost screening test for field diagnosis of surra in clinically suspected cattle.

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1 Introduction

Trypanosoma evansi is one of the most pathogenic trypanosome that infects different animal species in many parts of the world including India. The haematophagus dipteran flies especially the tabanid flies are the mechanical vectors of this trypanosome, which is transmitted from animal to animal in nature. The infection in cattle and buffaloes are usually cryptic in nature and leads to a carrier state that usually remains unnoticed (Jaiswal et al., 2015), yet capable of affecting their production potentialities (Muraleedharan, 2015). The carrier status may often become patent in the face of multiple stresses (Rani et al., 2015) resulting into per acute, acute or chronic clinical forms. Epizootics of the infection with profound morbidity and mortality have been reported in different species of animals including cattle and buffaloes (Singh et al., 2014).

Diagnosis of surra in cattle and buffaloes is difficult due to cryptic nature of infection, which interferes in gathering adequate information on the epidemiology of the disease. Infected cattle in clinical cases often exhibit nervine symptoms, corneal opacity and oedema of dependant parts in addition to lethargy, intermittent fever, progressive anaemia and emaciation. The routine laboratory methods available at present for diagnosis of infection are the microscopic examination of blood in wet film, stained smear and buffy coat preparations. However, finding of parasite in blood is very uncertain due to latent carrier status, low grade or intermittent parasitaemia in clinical cases. Several serological tests have been developed for detection of parasite antigen or antibodies in *T. evansi* infected animals (Jeyabal et al., 2003; Kumar et al., 2013; Yadav et al., 2014). These tests although specific with varying sensitivities are more suitable for herd screening in a geographical location. The polymerase chain reaction (PCR) based molecular techniques although claimed to be the most sensitive and specific, might generate false negative result owing to the fluctuating parasitaemia (Tehseen et al., 2017). Thus, these advanced methods despite their sensitivity and specificity cannot be employed in all field situations (Bal et al., 2014) due to lack of trained personnel, well equipped laboratory and rapid diagnostic facilities. Several chemical tests based on detection of rise in serum immunoglobulin level were employed in the past to diagnose surra in animals (Gill, 1991; Iqbal et al., 2012). One of those, the Formol Gel Test (FGT) also known as Napier's aldehyde test described by Napier (1922) for field diagnosis of suspected cases of visceral leishmaniasis (VL) in human patient was applied for the first time in diagnosis of surra in camels in Algeria and Sudan (Bhatia & Shah, 2001). This test although abandoned due to its non-specificity is still known to provide dependable result that facilitates tentative diagnosis of camel surra and VL of man in developing countries where improved and rapid diagnostic facilities are scarce (Chappuis et al., 2005; Nazmulahasan et al., 2008). The performance of FGT in comparison with other improved serological and molecular methods has been evaluated for diagnosis of surra in camels and horses (Aslam et al., 2010; Tehseen et al., 2015). The present study was undertaken to evaluate the FGST with reference to blood microscopy and enzyme linked immunosorbant assay (ELISA) towards detection of *T. evansi* infection in cattle

2 Materials and Methods

The study was conducted in 12 cattle sheds owned by marginal farmers at Guwahati, Assam (India). In all, 51 adult cross bred (Holstein-Friesian) cows were made available for the investigation. The animals were reported to be apparently healthy, except for three animals from a shed, which had history of intermittent fever, dullness, anorexia, gradual drop in milk yield, oedema in hind legs and non responsive to symptomatic treatment. Blood of two of the clinical cases was also microscopically positive for *T. evansi*.

2.1 Sample collection

Individual blood samples were obtained from the jugular vein and placed partly in a heparinized tube and serum separator tube with clot activator. Heparinized blood samples were microscopically examined in wet film and Giemsa stained thin film preparations. Clotted blood tubes were centrifuged at 2000 rpm and sera separated out for storage at -20°C until use.

2.2 Blood microscopy

Wet blood film and giemsa stained smears were examined under high power (40X) and oil immersion (100X) for detection of *T. evansi*.

2.3 Formol Gel Slide Test (FGST)

This test was performed as per the method described by Chappuis et al. (2005) and Tehseen et al. (2015) with modifications. Briefly, 100 µl test serum was placed in a cavity microslide and 10µl of concentrated formalin (37% formaldehyde) was added and thereafter mixed by gentle tilting of the slide. Observation was made for 20 min to visualize gellification of the test serum and simultaneous development of opacity, if any. A positive test was indicated by the formation of gel that adhered to the slide and development of opacity like the white of a boiled egg. Formation of precipitation at the bottom of the slide with a clear surface fluid running off the slide, when tilted as seen in the test using foetal bovine serum (negative control) was considered negative (Figure 1).

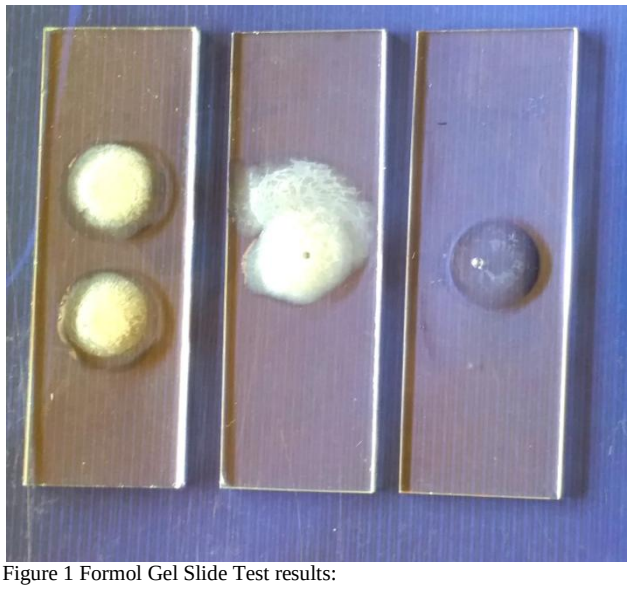

Figure 1 Formol Gel Slide Test results:
a(left)- Negative test serum showing precipitation with clear surface; b(middle)- Positive test serum showing gelification and opacity like white of a boiled egg;
c(right)- Negative control using foetal bovine serum.

2.4 Enzyme Linked Immunosorbant Assay (ELISA)

Briefly, a series of checkerboard titrations were conducted to determine the optimum concentration of *T. evansi* whole cell lysate antigen (WCL cattle origin) and conjugates for use in ELISA assay. ELISA plates (Nunc) were coated with 50 µl of 1.0 mg/ml of antigen in 0.1 M carbonate/bicarbonate buffer (pH 9.6) per well. Blocking was done with 100 µl of 5 % skimmed milk in PBST (SM-PBST) for 1 h at 37 °C. Subsequently, after washing 50 µl of test serum (1:100 diluted in 5 % SM-PBST) were added to each well and incubated for 1 h at 37 °C. Thereafter, 50 µl of 1:4000 diluted anti bovine IgG–peroxidase conjugate (Sigma) was added to each well after washing and the plates incubated for 1 h at 37 °C. Thereafter, substrate Tetra methyl benzidine (Genei, India) 50 µl was added and reaction was stopped by adding 50 µl 0.5 N H_2SO_4 to each well. The absorbance was read at 450 nm on ELISA reader (Thermo scientific, MULTISKAN GO, Finland) and results were expressed as mean OD of duplicate samples. The cut off values were determined using mean OD ± 3SD of uninfected serum samples from the herd.

3 Results and Discussion

The results of blood microscopy, FGST and ELISA are presented in Table 1. Out of the 12 cattle sheds examined, animals from 6 (50 %) sheds were found positive for antibodies to *T. evansi* in ELISA. Animals of only one shed tested positive to *T. evansi* in giemsa stained blood smear examination and serum analysis by FGST (Table 1). Three animals from this affected shed were reported clinically ill and their serum samples were found positive in both the serological tests conducted. However, out of three, one animal showed presence of *T. evansi* parasites in giemsa stained smear examination. Further, test wise, the infection was recorded in 3.92%, 5.88% and 17.64 % of animals tested by microscopic examination, FGST and ELISA respectively (Figure 2). No study comparing the performance of FGST and ELISA has been reported in cattle sera. However, the results are in agreement with that of Aslam et al. (2010) who observed a good correlation among FGT, Ab-ELISA and PCR performed in horse suspected for *T. evansi* infection by FGT. Tehseen et al. (2015) also observed uniform result in FGST and ELISA conducted in camels.

The results of microscopic examination, ELISA and FGST were found to be uniform in two of the symptomatic cattle. However, despite FGST and ELISA positive result, the blood of the third symptomatic cattle was found parasite free, which might be due to inconsistent and fluctuating parasitaemia (Yadav et al., 2016). The ELISA data showed that 3/9 (33.3%) cattle sera with high antibody titre (O.D value >0.736) from symptomatic cattle were FGST positive (Table 2). Moreover, low antibody titre of sera from apparently healthy cattle as observed in the present study may be attributed to *T. evansi* carrier stage of infection.

Table 1 Performance of blood microscopy, FGST and ELISA conducted in cattle in detection of infection.

Area	No. of Animal sheds attended	No. of animal sampled	Percent animal positivity to *T. evansi*		
			Blood microscopy	FGST	ELISA
I Ganeshguri	7	26	-	-	11.53 (3)
II Jatiya	3	15	-	-	6.6 (1)
III Ambari	1	5	40.0 (2)	60.0 (3)	80.0 (4)
IV Cycle Factory	1	5	-	-	20.0 (1)
Total	12	51	3.92 (2)	5.88 (3)	17.64 (9)

Figures in parenthesis indicate number of positive cattle.

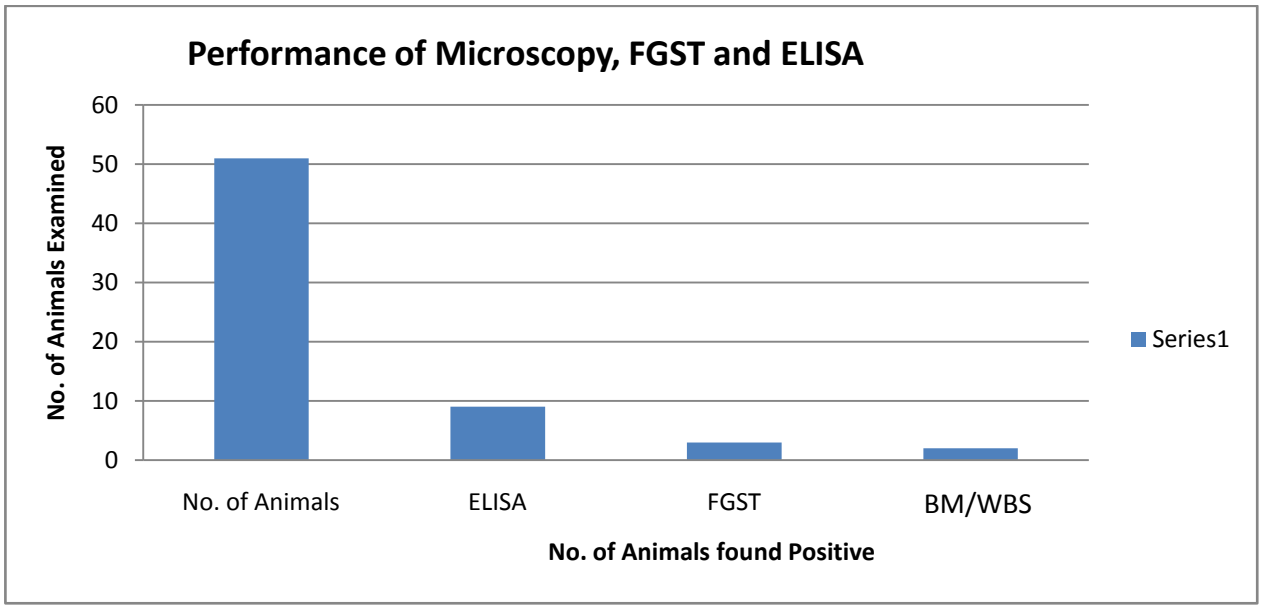

Figure 2 Bar diagram showing performance of Blood Microscopy, FGST and ELISA in diagnosing *T. evansi* infection in cattle.

Table 2 Performance of Formol Gel Slide test in relation to positive ELISA in cattle serum samples

Animal Sl. No.	Serum sample No.	OD value [1] in ELISA	Result of FGST
1	11	0.2563	-ve
2	21	0.2202	-ve
3	25	0.7356	-ve
4	27 (a)*	0.84425	+ve
	27(b)**	0.30635	-ve
5	28(a)*	1.0799	+ve
	28 (b)**	0.5312	-ve
6	29	1.07245	+ve
7	43	0.2277	-ve
8	45	0.2588	-ve
9	52	0.4933	-ve

Blood Sampling: * Pre treatment, Aug., 2014; ** Post treatment (Seven month) March, 2015, [1] Cut off value 0.22075 at 450 nm

Positive FGST result obtained in line with other tests performed in the present study indicates that the test is still useful and may be supporting evidence for diagnosis of clinical surra. The present findings are consistent with those observed in the similar tests conducted in parasitologically proven or suspected cases of VL in human (Chappuis et al., 2005; Parveen et al., 2007). There have been contradicting reports on validity of FGST for diagnosis of surra in cattle (Ray & Bhaskaran, 1953; Gill, 1991). However, satisfactory result obtained in clinical cases of the present study agrees to the earlier reports in camels and horses (OIE, 2010; Aslam et al., 2010; Tehseen et al., 2015) and also in cattle (Goel & Singh, 1971; Krishnappa et al., 2002) and dog (Poul, 1950; Farrar et al., 2013). Uniform result that can be obtained in hosts irrespective of their species re-affirms that the test is a useful one as an indicator of hyperglobulinaemia, an important biochemical alteration observed in trypanosomiasis and visceral leishmaniasis (Singla et al., 2000; Kaur & Juyal, 2003; Chappuis et al., 2005; Bal et al., 2014). Thus the present study suggests that FGST is a simple test and can be performed even under field condition at a very negligible cost for a quick decision in individual clinical cases of suspected surra in cattle also.

Compared to the north western and northern India (Singh & Chabbra, 2008), documentation of clinical surra from the north eastern region of India is very scanty. This might be due to very small equine population and absence of camel which are the primary hosts of *T. evansi*. However, this region has been experiencing regular inward transportation of high yielding cattle for milk production from the surra endemic north India. Increasing unregulated cattle transportation for private trade might be a prime factor (Fevre et al., 2006; Selby et al., 2013) responsible for spread of surra to this region. The recent report of clinical surra (Sarmah et al., 2015) and further evidence of positive results as observed in the clinical and apparently healthy

cattle in the present study may raise an alarm to the field veterinarians on the threat of dormant *T. evansi* infection in cattle and its possible excerbation under different stress related conditions.

FGST a rapid and simple low cost traditional tool may still find place in tentative diagnosis of clinical surra in cattle, similar to that of camels and horses of developing or under developed countries where more efficient rapid tests are yet to take off for field diagnosis.

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Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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