ASSESSMENT OF DIAGNOSTIC EFFICACY OF VARIOUS METHODS IN DETECTION OF *TRYPANOSOMA EVANSI* INFECTION IN BUFFALOES

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ABSTRACT

The present study was conducted to Assess the efficacy of various diagnostic techniques for that all the suspected cases of buffaloes coming to TVCC, U.P. Pandit Deen Dayal Upadhyaya pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura were examined by parasitological and serological methods. (viz. giemsa stained thin blood smear, buffy coat method and TELAT) for the diagnosis of trypanosomiasis in naturally infected buffaloes. The order of decreasing diagnostic efficacy during present investigation was found as: TELAT (52.02%) > Buffy Coat method (45.62%) > Giemsa stained thin blood smear (38.20%).

Keywords: Trypanosomiasis, TELAT, buffaloes, efficacy

INTRODUCTION

Trypanosomiasis is one of the most important hemoprotozoan diseases of bovines in India. This is caused by unicellular, flagellated protozoa of the genus *Trypanosoma*. It is an important vector born disease occurring in tropical and subtropical countries including India (Da Silva *et al*., 2009). Diagnosis of trypanosomiasis is based on clinical signs and demonstration of the parasites in the blood supplemented by hematological, biochemical and serological test. The clinical manifestations of Surra, although indicative, are not pathognomonic enough to confirm the disease without laboratory diagnosis (Dia *et al*., 1997). When there is high parasitaemia, the examination of wet blood films, stained blood smears and lymph node materials reveals the trypanosomes but in chronic cases such as the carrier status, examination of thick blood smears as well as methods of parasite concentration are required. The standard diagnostic test for *T. evansi* infection is the giemsa-stained thin blood-smear, which has a sensitivity of ~10^5 trypanosomes ml^{-1} of blood (Paris *et al*., 1982). The diagnostic capability can be significantly improved by adopting simple, low-cost alternatives, such as HCT (hematocrit centrifugation technique), which has a sensitivity of ~85 Trypanosomes ml^{-1} blood (Reid *et al*., 2001). The sensitivity of parasite detection can be enhanced by approximately tenfold when using buffy coat (Reid *et al*., 2001). In India, a monoclonal antibody based latex agglutination test (MAb-TELAT) with immense field applicability was developed to detect *T. evansi* antigens (Rayulu *et al*., 2007).

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MATERIALS AND METHODS

The study was performed at Teaching Veterinary Clinical Complex, Uttar Pradesh Pandit. Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura (TVCC, DUVASU, Mathura) from June 2013 to May 2014. Animals came to TVCC with history of suffering with fever, anorexia, loss of production and loss of body condition were selected for study. Blood samples were drawn from animal by usual technique of collection from ear tip and Jugular vein aseptically with a sterilized disposable syringe and needle. The sample from jugular vein was collected in two clean, dry blood collection vials containing EDTA as anticoagulant. In present study all the suspected cases of buffaloes were examined by parasitological and serological methods.

Diagnosis on the basis parasitological examinations in the suspected cases was done by thin blood smear examination and buffy coat examination technique. For thin blood smear examinations, a drop of blood was placed 20 mm from one end of a clean microscopic slide and a thin film was drawn. The film was air-dried briefly, fixed in absolute methanol for 2 minutes and allowed to dry. The smears were then stained by giemsa (one drop giemsa +1 ml PBS, pH 7.2) for 25 minutes. This preparation was poured off; the slide was washed in tap water and dried. Slides were visualized under microscope at 100x using immersion oil.

In buffy coat technique, blood was collected into micro-hematocrit centrifuge tubes containing anticoagulant and sealed with clay and centrifuged in microhaematocrite centrifuge at 12,000 rpm for 5 minutes. A smear was prepared by scratching and breaking the capillary tube 1mm below the surface of the buffy-coat and one drop of the buffy coat was expelled onto microscope slide, smeared and covered with a cover slip and examined under microscope at 40x.

A monoclonal antibody-based latex agglutination test for the diagnosis of Surra in domestic, zoo and wild animals (Invented at LLRUVAS, Hisar). It involves the detection of *T. evansi* circulating antigens in sera samples from infected animals. Standard protocol was followed as proposed by inventor. Reagent was shaked well before use and aliquot of required volume (20 µl per test sample) was transferred into the empty vial supplied in the kit. The stock reagent was transferred back in refrigerator at 4ºC. The reagent (20 µl) was put on a clean glass cavity slide and mixed with equal amount of serum sample obtained from the suspected animal. Similarly positive and negative serum control were used, ensuring that reagent should not auto agglutinate. Observed for up to five minutes for agglutination to occur in strong positive serum samples. The reagent was mixed intermittently (for 15 seconds preferably 3 to 4 minutes after start of the test) by swirling motion of the slide for agglutination to occur. Agglutination was indicated by appearance of granules or curdle-like aggregates in the solution mixed with the serum sample that contained *T. evansi* circulating antigen. The blue reagent turned watery with blue granules settling out from the solution. The samples showing agglutination within 5, 10 and 15 minutes were marked as positive. All other samples which did not show the agglutination within 15 minutes were declared as negative.

Assessment of diagnostic efficacy

Diagnostic efficacies of giemsa stained thin blood smear; buffy coat method and TE-LAT were evaluated on the basis of % positivity shown by individual diagnostic test.

RESULTS AND DISCUSSION

In present study giemsa stained thin blood smear examinations in suspected cases of trypanosomiasis in buffaloes revealed the presence of *T. evansi* with efficacy of 28.20%. The present findings are more or less similar with the findings of Mandal *et al*. (1977) who reported 36.9% occurrence of surra in buffaloes by blood smear examination after giemsa staining. Carlos *et al*. (1990) reported 45.6% efficacy with giemsa stained blood smear which is higher than the present findings. Laha *et al*. (2009) detected 5.3%, 9.4% and 40.6% infections in cattle, buffalo and horses by examination of giemsa-stained blood smears. The present findings are much higher than the findings by the work done by Das *et al*. (1998) who reported 2.63%, Dhami *et al*. (1999) reported 1.34%, Agarwal *et al*. (2003) reported 7.49% prevalence, Awandkar *et al*. (2004) reported 1.73%, Muraleedharan *et al*. (2005) reported 0.04% and by Shahzad *et al.* (2010) finding was 3.5% by the giemsa stained thin blood smear. It may be due to the reason that most of the cases that came to TVCC, DUVASU, Mathura, were in acute stage which were showing high degree of parasitaemia with prominent clinical signs. It may also be due to the fact that owners usually came to the TVCC only when their animals started showing prominent clinical signs like high fever, respiratory distress with loss of production etc. Mathura and its surrounding areas are more prone for exposure to biting flies (Tabanid flies); because of the fact that in this area there is higher vector density due to its agro climatic condition and Yamuna belt, therefore, the animals might be getting acute infection during grazing time.

$$\text{\% Positivity} = \frac{\text{No of positive cases given by a particular diagnostic test (n)}}{\text{total no suspected cases (N)}} X100$$

Buffy coat examination in suspected cases of trypanosomiasis in buffaloes revealed the presence of *T. evansi* with efficacy 45.62%. similar findings were earlier reported by Hollanda (2001) found the case sensitivity of the buffy-coat technique (BCT) to be 38.6%, Carlos *et al*. (1990) found an efficacy of 63.4%. The buffy coat technique detected more number of cases of *T. evansi* infection compared with giemsa stained blood smears examination. It could attribute to the reason that in most of the hosts, *T. evansi* can induce mild clinical or subclinical carrier infections with low parasitaemia and in such conditions concentrations methods like buffy coat technique become necessary. The application of parasite concentration methods like buffy coat techniques is recommended (OIE, 2000) to diagnose the *T. evansi* infection as an alternative method including the serological techniques. Dwivedi (2004) stressed the importance of buffy coat technique for the identification of subclinical or carrier state of *T. evansi* infection in bovines.

In present study TE-LAT examination in suspected cases of trypanosomiasis in buffaloes revealed the presence of circulating *T. evansi* parasite antigen in 463 cases showing an efficacy of 52.02%. All the blood smear and buffy coat positive cases were also found positive with TE-LAT however, healthy control showed negative reaction indicating the higher sensitivity of TE-LAT than other techniques applied. The findings of present investigations are in accordance with the findings earlier reported by Shyma *et al*. (2012); Rayulu *et al.* (2007). Reema *et al*. (2012) inferred that TE-LAT as being simple to perform, rapid, convenient and cost-effective could be quite suitable for diagnosis of trypanosomiasis at field level.

In order of decreasing diagnostic efficacy during present investigation the results obtained were as follows: TE-LAT > Buffy Coat method

Table. 1. Percent positivity (efficacy) of various diagnostic tests in the diagnosis of trypanosomiasis in buffaloes.

Diagnostic tests	Buffaloes (Total- 890)	
	No. positive	% positive
BLOOD SMEAR	340	38.20
BUFFY COAT	406	45.62
TELAT	463	52.02

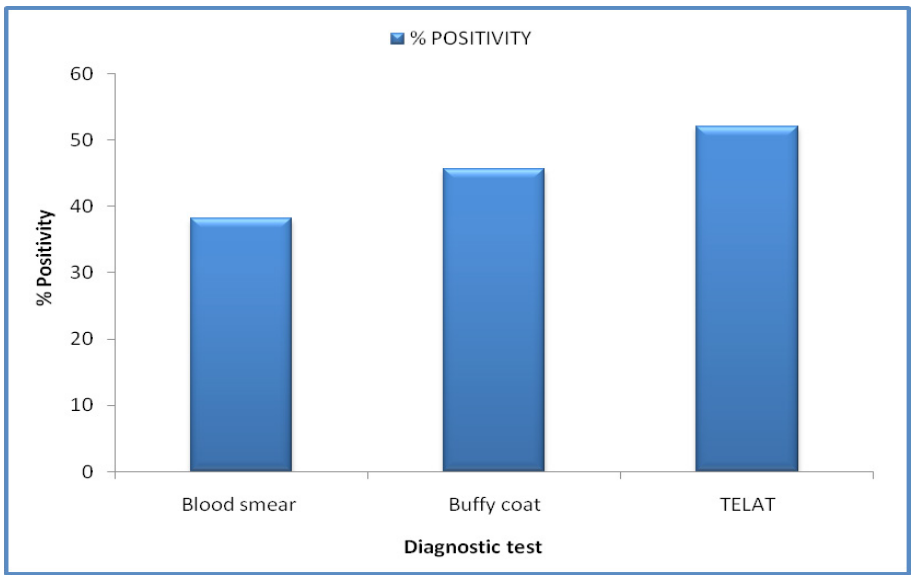

Figure 1. Showing comparative efficacy of three diagnostic tests.

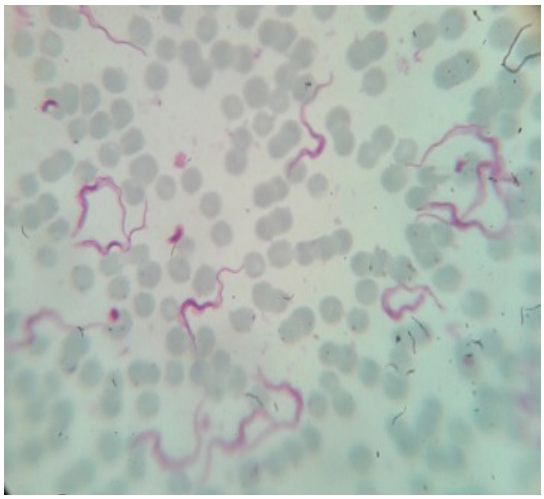

Figure 2. Blood smear showing *T. evansi.*

Figure 3. Capillary tube showing buffy coat.

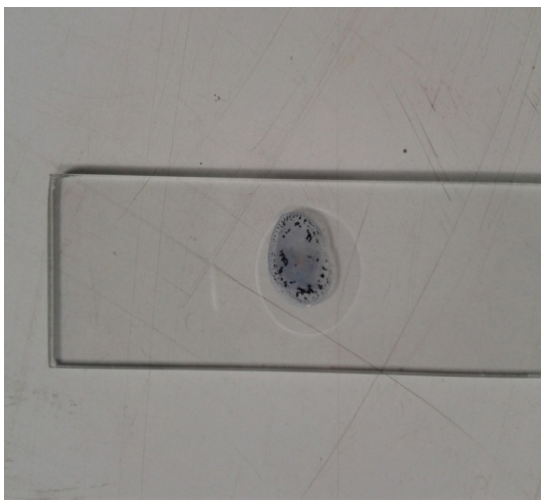

Figure 4. TE-LAT (Positive).

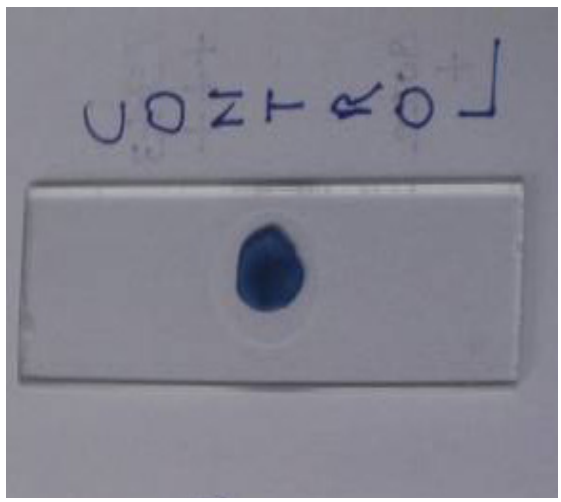

Figure 5. TE-LAT (Control).

> Giemsa stained thin blood smear. Similar kind of findings were earlier reported by Shyma *et al*. (2012) who found that diagnostic sensitivity of TE-LAT was more than WBF and PCR. Carlos *et al*. (1990) performed a comparative study between different parasitological methods for diagnosis of *T. evansi* and found that case sensitivity in order of mouse inoculation test > haematocrit centrifuge technique > buffy coat method > wet blood films > giemsa-stained smears. However, Paris *et al.* (1982) evaluated the order of diagnostic sensitivity as follows: dark ground buffy coat technique > haematocrit centrifuge technique > thick film > thin film > wet film.

In present study the diagnostic efficacy of giemsa stained thick blood smear was found less in comparison with the buffy coat and TE-LAT. It is may be due to the fact that microscopic detection of parasites in the blood are not always effective since trypanosomes are frequently absent from peripheral blood (Kendrick, 1968). In the present study, the buffy coat technique detected more number of cases of *T. evansi* infection compared with the giemsa stained thin blood smears examination. It may be due to the reason that, *T. evansi* can induce mild clinical or subclinical carrier infections with low parasitaemia and in this conditions concentrations methods like buffy coat technique become necessary. Therefore, it can be said that serological test like TE-LAT is of immense value in the detection of *T. evansi* infections than the parasitological examination methods.

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