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Trypanosoma evansi Infections: Towards Penside Diagnosis

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ABSTRACT

The mainstay for control of *Trypanosoma evansi* infections (surra) is chemotherapy and chemoprophylaxis. This strategy rests not only on availability of simple, efficacious drugs, but also on diagnosis. The parasitological techniques frequently employed in diagnosis, however, have very low sensitivity since most infections in the field are not associated with patent parasitaemia. An indirect latex agglutination test, Suratex®, for detecting circulating trypanosomal antigens in the blood of infected animals has the potential to circumvent this problem. The test is simple and rapid. It is carried out by mixing equal volumes of serum, plasma or whole blood with the Suratex® reagent on a test card, and the card rocked manually. In positive reactions, agglutination develops in 2 minutes. Suratex® has been shown to be specific, with a specificity value of 99%, based on studies carried out using sera from horses, camels and cattle from non-endemic areas. The sensitivity of Suratex® is high: 93-97% of blood samples from animals with parasitologically confirmed diagnosis give positive reactions, and most importantly, the test also diagnoses the latent infections which cannot be detected by the parasitological techniques.

INTRODUCTION

First reported by Evans (1880) to be associated with a disease in equines and camels locally known as "surra" in the Dera Ismail Khan in the Punjab, India, *Trypanosoma evansi* is today the most widely distributed pathogenic trypanosome species. It afflicts a wide range of domestic and wild animal species in many countries in South and Central America, Africa, the Middle East and Asia.

The mainstay for control of surra is chemotherapy and chemoprophylaxis. This in part rests on availability of suitable techniques for identifying the infected animals. However, surra is not easy to diagnose. Majority of infections (50-80%) are not associated with patent parasitaemia and so cannot be diagnosed by the parasitological techniques frequently employed (Benett 1933; Killick-Kendrick 1968; Nantulya 1990). This not only affects proper management of individual infected animals, it also makes it difficult to generate reliable epidemiological data on the actual prevalence and incidence of surra and its socio-economic impact in the endemic areas.

This paper summarizes the results of studies carried out by various investigators in evaluating a simple field assay, Suratex® (Brentec Diagnostics, Kenya), developed for the detection of *T. evansi* circulating antigens in serum, plasma or whole blood as means for diagnosis of current active infections (Nantulya 1994).

PRINCIPLE OF THE TEST

The Suratex® reagent is a suspension of latex particles which have been sensitized with a specific monoclonal antibody against a *T. evansi* somatic antigen. The antigen molecules in

the serum, plasma or whole blood are captured by the immobilized antibodies on latex particles. Since the antigen molecules identified have multiple combining sites, several latex particles aggregate, leading to the agglutination reaction.

TEST PROTOCOL

A drop (25-50μl) of serum, plasma or whole blood, is placed in a test circle on a special test card. An equal volume of Suratex® reagent is placed next to a drop of the specimen. The two drops are mixed and spread out using a stirring rod. The text card is then rocked manually and the reactions observed. Most reactions show up within 2 minutes (Nantulya 1994; Olaho-Mukani et al. 1996).

RESULTS

Specificity of Suratex®

The specificity of this assay has been determined in various studies by screening horse, camel and bovine field sera from trypanosomiasis-free areas. The results obtained by various investigators (Table 1) show that the test has high specificity of 99%.

Table 1 The specificity of Suratex®

Animals PPS	No. Tested	No. Negative	% Negative	Ref.
Camels	30	30	100	a
Camels	61	61	100	b
Cattle	98	96	98	c
Horses	25	25	100	c
Total	214	212	99	

[a]Nantulya (1994); [b]Olaho-Mukani et al. (1996); [c]Nantulya (unpublished)

Sensitivity of Suratex® in Diagnosis of Patent Infections

The sensitivity of the assay in diagnosis of patent infections was investigated in two separate studies. In the initial study (Nantulya 1994), sera from 33 camels with parasitologically confirmed diagnosis were tested for circulating *T. evansi* antigens. Thirty-two (97%) of the sera gave positive reactions (Table 2). Similar levels of sensitivity (Table 2) were recorded in subsequent studies by Olaho-Mukani et al. (1996), where 53

Table 2 The sensitivity of Suratex® in diagnosis of patent *Trypanosoma evasi* infection in dromedary camels

Diagnosis confirmed by positive	No. Tested	No. (%) Suratex®	Ref.
Buffy coat technique	33	32 (98)	a
Microhaematocrit centrifugation	58	53 (65)	b
Mouse inoculation	133	124 (93)	b
Total	224	209 (93)	

[a]Nantulya (1994); [b]Olaho-Mukani et al. (1996)

(95%) of 58 and 124 (93%) of 133 infected camels tested positive for antigens; indicating that 93-97% of animals with patent infections have detectable levels of circulating antigens as determined by Suratex®.

Sensitivity of Suratex® in Diagnosis of Sub-Patent Infections

In *T. evansi* infections, a large proportion of infected animals do not manifest detectable levels of parasitaemia. The potential for Suratex® to diagnose the sub-patent infections has been investigated. In studies carried out in Mali, 3 camel herds from different regions of the country (Nara, Menaka and Kidal) were screened for parasitaemia using the buffy coat technique (Murray et al. 1977), for anti-trypanosome antibodies using Card Agglutination Trypanosomiasis Test (CATT) (Magnus et al. 1978) and for circulating antigens using Suratex®. Nara is a high transmission area while Menaka and Kidal are low transmission areas. The results (Table 3) show that whereas the buffy coat technique diagnosed infections in only 18 (21.7%) of the 83 animals in Nara, Suratex® diagnosed more than twice the number (44; 55.0%). The CATT gave positive reactions with 66 (79.5%). In Menaka, a low transmission area, the buffy coat technique detected the infection in one camel. Suratex® too detected only this one animal, underpinning the specificity of the test. CATT, on the other hand, diagnosed 15 (23.8%). In Kidal, another low transmission area, none of the 87 animals had detectable infection by the buffy coat technique. Suratex® diagnosed the infection in 2 (2.3%) animals in this herd, while 20 (23.0%) gave positive reaction in CATT.

Table 3 Comparative analysis of the prevalence of surra in 3 regions of Mali using the microhaematocrit centrifugation technique (HTC), CATT and Suratex®

Region	No. Tested	No. Parasite positive by HCT	*No. (%) Positive by CATT	*No.(%) Positive by Suratex®
Nara	83	18 (21.7)	66 (79.5)	44 (55.0)
Mena	63	1 (1.6)	15 (23.8)	1 (1.6)**
Kial	87	0 (0)	20 (23.0)	2 (2.3)

*All grades of positivity counted, including weak one; ** Same animal positive for parasites

Seeking to understand the basis for positive Suratex® reactions in non-parasitaemic animals, all the positive reactions were cross-tabulated against CATT results: 30 (96.8%) of 31 Suratex®-positive non-parasitaemic animals also tested positive for anti-trypanosome antibodies as revealed by CATT, indicating that these were true infections even though they were not associated with detectable parasitaemia.

In another study carried out in Kenya, Olaho-Mukani et al. (1996) screened 8 camel herds from endemic areas for parasitaemia using the microhaematrocit centrifugation technique (MHCT) and mouse inoculation (MI), and for circulating antigens using antigen ELISA and Suratex®. Of the 450 animals examined, 58 (13.0%), 133 (30.0%), 247 (54.9%) and 232 (51.5%) were positive by MHCT, MI, Suratex® and antigen ELISA, respectively. These results once again showed that Suratex® had higher sensitivity than parasitological diagnosis: it diagnosed twice the number of animals detected by mouse inoculation and 4 times the number diagnosed by MHCT. Thus the test was able to diagnose also the sub-patent infections and this was corroborated by the antigen ELISA results

(Olaho-Mukani et al. 1996).

Relationship Between the Degree of Parasitaemia and Suratex® Reactivity

In the study carried out in Mali, the degree of parasitaemia in all confirmed cases was cross-tabulated against Suratex® and CATT reactivity. The results given in Table 4 show that animals that test negative by Suratex® tend to have levels of parasitaemia hat can be readily detected by simple parasitological techniques like microhaematocrit centrifugation. The Suratex®-negative, parasitaemic animals also had low titers of anti-trypanosome antibodies.

Table 4 Correlation between degree of Parasitaemia and Suratex® reactivity

Animal No.	Buffy Coat	Suratex
43	++++	+
52	++++	-
38	++++	-
23	+++	-
45	++	++
86	++	-
27	+++	+++
82	++	+++
78	++	+++
85	++	+++
79	++	+++
34	++	+++
80	++	+++
74	++	+++
55	++	+
59	++	-
72	++	++
17	++	++
16	+	+

+/+++ = degree of reactivity

DISCUSSION

Being tissue dwellers, *T. evansi* organisms preferentially sequester in organs like the bone marrow, lymph nodes, spleen, liver, central nervous system, kidneys etc. As the result, *T. evansi* infections, particularly the chronic infections, are often not associated with patent parasitaemia. It has been estimated that upto 50-80% of the infections cannot be diagnosed by parasitological techniques. This makes it difficult not only to identify the individual animals to be treated, but it also compromises capacity to define the epidemiology and socio-economic impact of surra in endemic countries.

The studies reviewed show that Suratex®, a simple, indirect latex agglutination test for detection of *T. evansi* circulating, somatic antigen can be used for diagnosis of *T. evansi* infections. Its specificity is high (99%) and it diagnoses the infection in 93-97% of animals with parasitologically confirmed infections (Nantulya 1994; Olaho-Mukani et al. 1996). In

all the studies described, however, Suratex® consistently gave higher prevalence rates in endemic areas compared to parasitological diagnosis. The nagging question is whether the Suratex® reactions observed in non-parasitaemic animals were false positive or not. The answer is provided by two studies. In the studies carried out in Mali, 30 (96.8%) of the 31 Suratex® positive non-parasitaemic animals also tested positive for anti-trypanosome antibodies by CATT. Moreover, Olaho-Mukani et al. (1996) showed that Suratex® positive, non-parasitaemic animals were also antigen-ELISA positive. We conclude that these reactions represent true sub-patent infections. Hence, this test has a high enough level of sensitivity to diagnose not only the patent but also the sub-patent infections. This assay goes a long way towards solving current diagnostic inadequacies arising from inherent inability of parasitological techniques to detect sub-patent infections.

In the study carried out in Mali, Suratex® and CATT were compared. The CATT consistently gave higher prevalence rates compared to Suratex®. The explanation for this difference may lie in the fact that the two assays measure different things: the CATT is a measure of exposure while Suratex® diagnoses current/active infections. Since anti-trypanosome antibodies remain in circulation for a long time after chemotherapeutic cure, the extra CATT positive sera could have come from treated and cured animals.

It was noted in all the studies described that a few animals with patent infections tested negative for circulating antigens. These tended to be animals with high levels of parasitaemia. Such animals also had low antibody reactivity in CATT. Thus it is possible that these may represent peracute infections. Since these infections are readily detected by simple parasitological methods, combination of Suratex® with simple parasite detection pen-side techniques such as the wet blood film examination or microhaematocrit centrifugation could diagnose all the active/current infections.

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