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Short Communication

SEROLOGICAL DETECTION OF *TRYPANOSOMA EVANSI* IN SMALL RUMINANTS AT DODOLA AND ASSASSA DISTRICTS OF ARSI-BALE HIGH LANDS OF ETHIOPIA

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ABSTRACT
The study was conducted in two districts of Oromiya regional state in Arsi-Bale highlands. The data were collected on the occurrence of *T.evansi* infection in sheep and goats. The disease has been detected by two serological tests (CATT and LATEX) and antibodies were demonstrated against *T.evansi* at least in one of the examined animals in two districts: Dodolla (Danaba, Wabe, Tulualewanso and Ganata) and Assassa (Bucho and Beyema kerensa). Blood samples from 55 goats and 96 sheep were collected randomly and animals considered in this study were sexually mature adults of varying ages living under traditional management systems of free grazing. Among the small ruminants examined, 52 (34.4%) were positive by CATT/ *T.evansi* test and 7(4.6%) were positive by LATEX/ *T.evansi* test. Out of 55 goats 18(32.7%) were positive for *T.evansi* by CATT/*T. evansi* test and 2(3.6%) were positive by LATEX/ *T.evansi* test and out of 96 sheep 34(35.4%) were positive by CATT/*T.evansi* test and 5(5.2%) were positive by LATEX/*T.evansi* test. Statistical analysis of the rate of infection on the basis of species and districts showed significant difference ($p<0.05$) by CATT test but there was no significant difference ($p>0.05$) by LATEX test. The study revealed that small ruminant *T.evansi* infection follows sub clinical course and shoats are potential reservoirs endangering other animals.

KEY WORDS: Arsi-Bale highlands, CATT, Goats, LATEX, Sheep, Small ruminant T.evansi.

INTRODUCTION

Ethiopia possesses 43.4 million cattle, 24.6 million sheep, 24.3 million goats, 9 million equines, 2.3 million camels and 50 million poultry ranking first in Africa and 9th in the world in livestock population (CSA, 2004). However, poor genetic potential, animal diseases and nutritionally induced disorders are among the most important factors retarding agricultural development and hence reducing production of food for human consumption. The enormous size of wastage can be easily understood from the fact that millions of cattle, sheep and goats die each year from diseases (Junior, 2005). Among such diseases, Trypanosomosis is a wide spread and economically important disease of man and his animals. Over recent years, there has been an increasing interest shown in this infection because it restrains the development of livestock potential of Africa (Moluneux and Ashford, 1983). Trypanosomosis is a serious often fatal disease of mainly domestic animals and humans that occur in large areas of Africa, Latin America, the Middle East and Asia. It is caused by species of flagellate protozoa belonging to the genus Trypanosome of the family Trypanosomatidae that multiply and inhabit in the blood stream, lymphatic vessels, and tissues including the cardiac muscle and central nervous system and are transmitted by vectors. It affects most of livestock species mainly cattle (Fischer and Say, 1989). In Africa, the most important agents of trypanosomosis in terms of economic loss in domestic livestock are tsetse transmitted species, such as *T.congolense, T.vivax* and *T.brucei brucei, T.brucei rhodesiences* and *T.brucei gambience* cause human sleeping sickness (Urquart *et al.,* 1987; Maudlin *et al.,* 2004). On the other hand, *T.evansi* is widely distributed hemoflagellate of veterinary importance that infects a variety of large mammals including camel, equines, buffaloes, cattle and deer. Among the equines horses are most seriously affected followed by mules and donkeys. It causes epidemics of disease called "surra", which is a great economic importance in Africa. The distribution of *T. evansi* in Africa extends into the tsetse area where it is difficult to differentiate it from *T. brucei* and the tsetse fly (Glossina species) like other blood sucking flies, can act as mechanical vectors in the areas where both *T. evansi* and tsetse flies occur (Junior, 2005). An essential factor in the mechanical transmission is interrupted feeding on the part of flies, which go quickly from one host to the other in order to become replete. Trypanosomes do not survive for more than 10 – 15 minutes in the proboscis of a fly (Soulsby, 1968). Transmission is enhanced when animals congregate (at water hole or river areas) or is closely herded and when they have high number of parasites in their blood. It is very difficult to control this insect population, because they exist in very large number. Therefore control measures are aimed at the host and they include detection and treatment of infected animal, prophylactic treatment of susceptible animals and their protection from biting flies (Radiostis *et al.,* 1994). Due to various reasons, small ruminants do not show overt clinical signs of disease under field condition as cattle do. However, infection in small ruminants has been reported from field surveys and the economic impact has been

shown to be substantial (Luckins, 1996) but more work should be done to determine the status and situation of small ruminants regarding the disease.

MATERIALS AND METHODS

The present study was conducted in the selected areas of Oromiya Regional State in Arsi-Bale highlands of Ethiopia. Arsi and Bale zones are located 175 Kms and 430 Kms away from Addis Ababa. Topographically, the altitude ranges from 500 to 4130 MSL, where a central plateau (2000-2500 MSL) predominates with a narrow lowland area. The area experiences a bimodal rainfall occurring from July to October and April to May. An average annual temperature of 20- 25^{o}C and rainfall of 200 mm in the lowlands whereas 10- 15^{o}C with a rainfall of 400 mm in the highlands are recorded. The study was conducted on 151 small ruminants (55 goats and 96 sheep). Animals considered in this study were sexually mature adults of varying ages of sheep and goats living under a traditional management system of free grazing. Approximately, 7-10 ml of blood samples were collected from the jugular vein of each animal using plain vacutainer tubes and needles after the site is wiped with cotton wool soaked in alcohol. The vacutainer tubes were labelled and set tilted on a table overnight at room temperature to allow clotting. Then the serum samples were filled into serum storage (Polypropylene sterile cryogenic) vials and stored at –20^{0} C until the serological tests were performed.

The test procedures followed and details of the diagnostic steps applied were those described in the bench protocol manual of the Prince Leopold Institute of Tropical Veterinary Medicine (ITM), Antwerp, Belgium. The laboratory procedures followed in the present serological survey of *T. evansi* in small ruminants.

Card Agglutination for Trypanosomosis Test (CATT/ *T. evansi*)

The CATT/ *T. evansi* is a direct rapid card agglutination test which uses formaldehyde fixed, freeze- dried trypanosomes expressing a predominant variable antigen type of *T. evansi* (RoTat 1.2) stained with Coomassie blue.

Reconstitution of the CATT antigen

§ Using the syringe, add 2.5 ml of CATT buffer to a vial of freeze dried CATT antigen
§ Immediately shake the vial for few seconds so as to obtain a homogenous suspension
§ Put a dropper on the vial. The antigen suspension is ready for use

Reconstitution of the controls

§ Using the syringe, add 0.5 ml of CATT buffer to a vial of the positive and negative control
§ After reconstitution of each vial of CATT antigen, test one drop of the positive control and one drop of the negative control to check the quality of the antigen.

Preparation of test samples

§ Prepare serial twofold dilutions 1:4, 1:8, 1:16, 1:32 and 1:64 of the test sample in CATT buffer
§ Using a micropipette put 25 µl of the serial twofold dilutions on a test area of the card
§ Add one drop (about 45 µl) of the well homogenized CATT antigen in each test area
§ Using a stirring rod, mix and spread out the reaction mixture to about 1 mm from the edge of test area. Wipe off the stirring rod after each use
§ Rotate the test card on a flat bed orbital for 5 minutes at 70 rpm

Evaluate the agglutination reaction as indicated below in Table 1 as follows:

Table 1: Result interpretation of CATT/ *T. evansi* test.

Agglutination	Test result
+++	Strongly positive (very strong agglutination)
++	Positive (strong agglutination)
+	Positive (moderate agglutination)
±	Weakly Positive (weak agglutination)
-	Negative (absence of agglutination)

LATEX (LATEX/ *T. evansi*) test

LATEX agglutination/*T. evansi* is a rapid antibody detecting indirect agglutination test, in which the antigen consists of purified variable surface glycoproteins (VSG) of *T. evansi* Vat RoTat 1.2 covalently coupled to latex particles (0.9 micron in diameter). This method is more specific in testing for *T. evansi* than the CATT method (Verloo *et al*., 2001).

Reagents

Latex: Lyophilized latex suspension coated with semi-purified variable surface antigens from *T. evansi* VSG Ro Tat 1.2 trypanosomes.

Buffer: Phosphate buffered saline with 0.02 % sodium azide (PBS) for negative control, reconstitution of the positive control and dilution of the test sera.

Positive control: Freeze-dried goat immnunization serum.

Reconstitution of Lyophilized latex reagent

§ Resuspend the latex reagent with 1 ml of buffer (PBS). Mix gently for 30 seconds. Use the same day.

Reconstitution of the positive control

§ Dissolve the content of the positive control vial in 0.5 ml of PBS. No further dilution is needed. If not used the same day, store at – 20^{0} C.

Dilution of the test samples

§ Prepare serum dilutions 1:2, 1:4, 1: 8, 1:16, 1: 32 and 1: 64 in buffer (PBS) in a microplate as follows.
§ Dispense 40 µl of PBS buffer in each well of columns 1 to 4.
§ In well A1, dispense 30 µl of serum to dilute, mix properly and transfer 30 µl to well A2, mix and transfer 30 µl in well A3, mix and transfer 30 µl in well A4. Dilute 7 other blood samples in the column B to H in the same way. Use the other half of the microplate, from column 6 to, to dilute 8 other blood samples.

Execution of the test

§ The test on serum is performed with 20 µl of dilutions 1:2, 1:4, 1:8 and1:16.
§ Adjust the speed of the rotator at 70 rpm.
§ Dispense 20 µl well mixed latex suspension onto a spot of a test card. Add 20 µl of test sample, positive control or negative control (PBS). With a plastic stick,

mix well and spread over ± 1cm diameter. Wipe the stick with paper between each sample.

§ Rock the card on a rotator for 5 minutes. To prevent evaporation put the cover over the card.

Reading the test result

Evaluate the agglutination reaction as indicated below in Table 2 as follows:

Table 2: Result interpretation of LATEX/ *T. evansi* test.

Code	Agglutination	Result
0	Absent	Negative
1	Hardly visible	Negative
2	Manifest	Weakly positive
3	Intense	Positive
4	Almost complete	Strongly Positive

RESULTS

The overall occurrence of *T. evansi* infection in both species using CATT/ *T. evansi* test was 34.4%. Using this test 35.4% sheep and 32.7% goats were found to be positive but the rate of infection between two species showed no statically significant difference (P>0.05)(table 1). Using LATEX / *T.evansi* test the over all occurrence of *T .evansi* infection in both species was 4.6% The rate of infection was found to be 5.2% in sheep and 3.6% in goats but there was no stastical significance difference (P>0.05)(table 1).

Table3:-Relative frequency of small ruminant *T.evansi* infection using CATT test & LATEX /*T. evansi* test.

Species	CATT/*T. evansi* test		Total	% of positive cases
	positive	negative		
Sheep	34	62	96	35.4%
Goat	18	37	55	32.7%
LATEX /*T. evansi* test				
Sheep	5	91	96	5.2%
Goat	2	53	55	3.6%

p>0.05

Out of the 151 small ruminants in the study areas described, the rate of *T evansi* infection using CATT / *T evansi* test was 25% (Dodola) and 61.5% (Assassa) and the rate of *T.evansi* infection using LATEX /*T.evansi* test was 3.6% (Dodola) and 7.7% (Assassa). The occurrence of *T.evansi* infection in small ruminants in Dodola and Assassa districts was statically different (P<0.05) when CATT test is used but there was no stastical difference when LATEX test is used (P>0.05)(tables 3 and 4).

Table 4:- Rate of small ruminant *T. evansi* infection in two districts (CATT/*T. evansi* test)

Districts	CATT /*T. evansi* test		Total
	Negative	Positive	
Dodola	84	28 (25%)	112
Assassa	15	24 (61.5%)	39
Total	99	52 (34.4%)	151

P< 0.05

Table 5: Rate of *T.evansi* infection of small ruminants in two districts of Arsi-Bale high lands (LATEX / *T. evansi* test)

Districts	**LATEX /T. evansi test**		**Total**
	Negative	Positive	
Dodola	108	4(3.6**%**)	112
Assassa	36	3(7.7**%**)	39
Total	144	7(4.6**%**)	151

P> 0.05

DISCUSSION

Over 90% of sheep and goats in Sub-Saharan Africa are found in East and West Africa where they supply 30% of the total requirements of meat and 15% of milk supply in the region In Ethiopia they provide 46% of the national meat production and 58 % of the value of hide and skin production (which is the second most important source of agricultural export income after coffee). Small ruminants provide 12.5% of the value of livestock products consumed on the farms and 48% of cash income generated by live stock production though they represented only 6..6% of the capital invested in farm livestock (Dinka, 2003).

Among the various constraints that hamper full utilization of the potential of small ruminants is disease. But little data is available on the prevalence of diseases of sheep and goats which limit their production in Ethiopia and neighboring countries (Dinka, 2003).

The impact of trypanosomes in the production of small ruminants is less investigated. This study thus demonstrates the possible role of small ruminants in the epidemiology of Trypanosomosis as well as its potential role in the reduction of out puts from small ruminants.

There is previous evidence that small ruminants naturally acquire trypanosomal infections and this shows that trypanosomosis constitutes one of the major wide spread constraints on their production (Bealby *et al.*, 1996 ; Masiga, 1996).

In this study, the overall rate of *T.evansi* infection in small ruminants was 34.4% using CATT/*T.evansi* test and 4.6% using LATEX test. This variation depends on the relative sensitivity and specificity of the the tests (Moudlin *et al.*, 2004).

Infected sheep and goat had good body conditions and there was no clinical sign observed during this work and this is in agreement with what has been reported by Nawathie *et al* (1995) from West Africa under field conditions which means, infection due to *T.evansi* remains to be sub clinical.

In many areas of the world, small ruminants are reared near or in close contact to highly susceptible species (particularly, cattle, dromedaries and horses) hence, their role as possible carriers of trypanosomosis is largely discussed (Gutierrez *et al.*, 2006).

Even if these is no significant difference, the occurrence of *T.evansi* infection in sheep(5.2%) is slightly higher than goats(3.5%).This agrees with the findings of Osaer *et al* (1994) who concluded that “in the epidemiological studies, the natural prevalence of trypanosomosis is usually lower in goats than sheep”. Snow *et al* (1996) explained the low incidence of infection in goats exposed to natural challenge as it is due to the fact that goats are more protected from tsetse and other biting files because of their anti- feeding behavior such as leg kicks and stamping, tail and ear flicks, head movement and particularly skin rippling than sheep. On the other hand, the susceptibility

of sheep may be attributed to their docile nature that favors the feeding of flies.

There was statistically significant difference between sites (peasant associations and districts) and CATT /*T.evansi* test (table 3) but there was no significant difference when detected by LATEX / *T. evansi* test (table 4). This is due to the relative difference of sensitivity and specificity of the tests (Maudlin *et al.,* 2004).Even though, there was no statistically significant difference (P>0.05)between the two districts using LATEX test, the rate of infection is slightly higher in Assassa (7.7%) than in Dodola (3.6%).This may be due to the relative difference in altitude of the districts i.e. Assassa is lower than Dodola and may favor the multiplication of biting flies.

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