

Seroepidemiological survey of trypanozoon infection in horses in the suspected dourine-infected Bale highlands of the Oromia region, Ethiopia

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Summary

This paper presents the results of a seroepidemiological survey of trypanozoon infection in horses carried out between September 2007 and June 2008. The survey was conducted to determine the seroprevalence of anti-trypanozoon antibodies in 880 serum samples collected randomly from selected horse-breeding districts of the Bale highlands of Ethiopia. The seroprevalence of trypanozoon infection was found to be 173 (19.66%) and 140 (15.91%) for the CATT/*T. evansi* and LATEX/*T. evansi* tests, respectively. The high seroprevalence of trypanozoon infection strongly indicates that the infection is endemic. Neither test can differentiate between anti-trypanozoon antibodies caused by infection with *T. equiperdum* (the causative agent of dourine) and those of *T. evansi* (the causative agent of surra). The findings of the present study suggest that field-applicable screening serological tests such as the CATT/*T. evansi* and LATEX/*T. evansi* could be useful for epidemiological studies and the control of trypanozoon infection.

Keywords

CATT/*T. evansi* – Ethiopia – Horse – LATEX/*T. evansi* – Seroepidemiology – Trypanozoon infection.

Introduction

Ethiopia has a very large equine population of approximately 5.2 million donkeys, 2.8 million horses and 0.65 million mules. Equine animals are extremely important in Ethiopian agriculture and for the national economy. Nearly 90% of agricultural operations depend on manual labour, and because of the rugged mountainous terrain of the country these animals are still the main

method used to transport both people and agricultural products (9, 11).

In developing countries such as Ethiopia the contribution of equine animals is extremely diverse. They can carry heavy loads, draw carts, serve as a means of personal transport and provide a taxi service; consequently, they contribute significantly to the national economy (10). The Arsi and Bale people, like many others in the remote highland areas

of Ethiopia, where communications are poor, use horses as pack animals and for work on farms (9).

Of the non-tsetse-transmitted African trypanosomes, dourine is the only trypanosomosis that is not transmitted by an invertebrate vector. The causative agent of dourine, *Trypanosoma equiperdum*, differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood (14). The first official report of the disease in Ethiopia was made in 1980 when the Arsi Rural Development Unit asked the Tsetse and Trypanosomiasis Survey and Control Department to investigate a persistent disease problem in horses in the administrative regions of Arsi and Bale (15). Since then, dourine has been found to be prevalent throughout the highlands of Ethiopia, particularly in the Arsi and Bale zones (1). Multiple cases have been found to be positive on the serological complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA), or to be positive on a trypanozoon polymerase chain reaction, yet aparasitaemic horses have also been reported in the Arsi and Bale zones (7). So far there has been no study conducted in the highlands of Bale to investigate the occurrence of *T. evansi* infection in horses or in other domestic animals. However, adjacent districts in the Bale lowlands, such as Barebre, Dello-Mena and Harena-Buluk, are known to be endemic for surra (trypanosomosis caused by *T. evansi*) (3). Diagnosis of *T. equiperdum* by standard parasitological techniques is difficult, owing to the low numbers of parasites present in the blood or tissue fluids and the frequent absence of clinical signs of disease. Therefore, the demonstration of trypanosomal antibodies in the serum has become the most important parameter in determining the disease status of individual animals (4). The main reason for the use of serological tests for the diagnosis of trypanosomosis is to overcome the low sensitivity of parasitological tests in detecting chronic infection.

The difficulty in the diagnosis of *T. equiperdum* has led to difficulties in obtaining reliable data on the prevalence and distribution of the disease, and for the implementation of monitoring, treatment and control programmes. Moreover, shortages of trypanocidal drugs and the absence of vaccines against trypanosomosis have hampered the control and prevention of the disease in endemic areas (7). Hence, the objective of the present study was to determine the seroprevalence of anti-trypanozoon antibodies in selected horse-breeding districts of the Bale highlands of Ethiopia.

Materials and methods

Study area

The present survey was conducted in Oromia Regional State within the Bale highlands of Ethiopia, mainly in four

selected horse-breeding districts, namely, Agarfa, Dinsho, Goba and Sinana. The Bale highlands are located about 430 km south-east of Addis Ababa. Topographically, the altitude ranges from 2,800 to 4,377 metres above sea level. The area experiences a bimodal pattern of rainfall, which occurs from March to May and July to October. Agriculture is the mainstay of the livelihood of the people and the leading economic activity of the area, with a mixed farming system. The highest proportion of the total agricultural activity involves crop–livestock production (3).

Study design and sampling strategies

A cross-sectional study design was employed for serological examination. Sampling points within the four study sites were selected in collaboration with the animal health personnel of the respective district. Thus, a purposive sampling strategy was used on the basis of participant cooperation, logistics, the farmer's share of communal grazing land, and accessibility. A total of 880 horses were sampled; 25% (220 animals) were sampled in each selected district. All horses (528 males and 352 females) used in this study were sexually mature and lived under a traditional management system of free grazing.

Serological survey

Blood samples were collected from the jugular vein of the horses using plain vacutainer tubes and needles, after the site had been wiped with cotton wool soaked in alcohol. The plain vacutainer tubes were labelled and the blood was allowed to clot overnight at room temperature before the serum was separated by centrifugation. The serum samples were stored in sterile polypropylene cryogenic vials at -20°C until they were tested using card agglutination tests for trypanosomosis (CATT/*T. evansi* and LATEX/*T. evansi*). The testing was conducted at the Debre Zeit Faculty of Veterinary Medicine molecular parasitology laboratory, which was established by a Belgian–Ethiopian collaborative project funded by the Flemish Inter-University Council – University Development Cooperation (VLIR–UOS). For both tests (CATT/*T. evansi* and LATEX/*T. evansi*), positive results were determined at cut-off point dilutions of 1:8. The tests were checked using positive and negative controls before the field samples were tested (2).

Statistical analysis

Differences in the estimated prevalence between animals of different sexes and among the study districts were analysed statistically using the chi-square test for independence (13). A p-value of <0.05 was taken to be statistically significant.

Results

The results of the survey disclosed an overall seroprevalence of 173/880 (19.66%) and 140/880 (15.91%) for the CATT/*T. evansi* and LATEX/*T. evansi* tests, respectively (Table I). With regard to the sex of the horses, the seroprevalence of anti-trypanozoon antibodies obtained using the CATT/*T. evansi* test was found to be 98/528 (18.56%) and 75/352 (21.31%) for male and female horses, respectively. Similarly, the seroprevalence of anti-trypanozoon antibodies based on the LATEX/*T. evansi* test was 88/528 (16.67%) and 52/352 (14.77%) for male and female horses, respectively (Table I). The seroprevalence of anti-trypanozoon antibodies among the four study districts was found to be in the range of 18.64% to 20.91% and 15% to 16.82% for the CATT/*T. evansi* and LATEX/*T. evansi* tests, respectively (Table II).

Table I
Details of the seroprevalence of anti-trypanozoon antibodies in male and female horses in selected districts of the Bale highlands of Ethiopia on the basis of CATT/*T. evansi* and LATEX/*T. evansi* tests

Type of horse	Number of horses tested	Number of positive tests (seroprevalence)	
		CATT/ <i>T. evansi</i> test	LATEX/ <i>T. evansi</i> test
Male	528	98 (18.56%) ^(a)	88 (16.67%) ^(a)
Female	352	75 (21.31%) ^(a)	52 (14.77%) ^(a)
Total	880	173 (19.66%)	140 (15.91%)

χ^2 between sexes using CATT/*T. evansi* test = 2.65 (d. f. 1) $p > 0.05$

χ^2 between sexes using LATEX/*T. evansi* test = 1.08 (d. f. 1) $p > 0.05$

(a) no significant difference in seroprevalence ($p > 0.05$)

Table II
Seroprevalence, by district, of anti-trypanozoon antibodies in horses in selected districts of the Bale highlands of Ethiopia on the basis of the CATT/*T. evansi* and LATEX/*T. evansi* tests

Study districts	Number of horses tested	Number of positive tests (seroprevalence)	
		CATT/ <i>T. evansi</i> test	LATEX/ <i>T. evansi</i> test
Agarfa	220	42 (19.09%) ^(a)	34 (15.45%) ^(a)
Dinsho	220	41 (18.64%) ^(a)	36 (16.36%) ^(a)
Goba	220	44 (20.00%) ^(a)	33 (15.00%) ^(a)
Sinana	220	46 (20.91%) ^(a)	37 (16.82%) ^(a)
Total	880	173 (19.66%)	140 (15.91%)

χ^2 CATT/*T. evansi* seroprevalence between study districts = 2.13 (d. f. 3) $p > 0.05$

χ^2 LATEX/*T. evansi* seroprevalence between study districts = 1.54 (d. f. 3) $p > 0.05$

(a) no significant difference in seroprevalence ($p > 0.05$)

Discussion

The present survey disclosed a relatively high seroprevalence of trypanozoon infection in the Bale highlands. The seroprevalence of anti-trypanozoon antibodies was found to be 19.66% and 15.91% by using the CATT/*T. evansi* and LATEX/*T. evansi* tests, respectively. This seroprevalence is in agreement with the previous reports based on indirect methods (antibody and antigen detection) in the Arsi–Bale highlands of Ethiopia (1, 16).

Statistically significant variation was not observed when the seroprevalence of anti-trypanozoon antibodies in both the CATT/*T. evansi* and LATEX/*T. evansi* tests was compared among the examined animals with respect to sex and study district. This might be attributed to the fact that the animals of both sexes and all study districts were equally exposed to the parasites, and this indicates uniform spread of the disease.

Although there was no statistically significant difference in seropositivity between the CATT/*T. evansi* and LATEX/*T. evansi* tests, a relatively low prevalence was observed in the latter test. This might be due to the fact that the LATEX/*T. evansi* test is more specific than the CATT/*T. evansi* test (6).

Difficulties in differentiation of *T. equiperdum* within the subgenus *Trypanozoon* using CFT have been described by several experts, although this is the only official diagnostic test used in the international trade of equine animals (15). Currently, neither serological, parasitological nor DNA-based tests allow subspecies identification within the subgenus *Trypanozoon*, therefore no definitive diagnosis can be obtained for *T. equiperdum* (5, 8). Recently, it has been proven that most so-called *T. equiperdum* strains also express isovariant antigenic types (isoVATs) of *T. evansi* Rhode *Trypanosoma* antigen type (RoTat) 1.2 (6). In a recent study conducted in Ethiopia, a very high concordance was observed between the serological results of both the CATT/*T. evansi* and the ELISA/RoTat 1.2 and the clinical (dourine) status of the animals examined (12). Therefore, the CATT/*T. evansi* and LATEX/*T. evansi* tests appear to be valuable for the detection of anti-trypanozoon antibodies, regardless of whether the causative agent is *T. evansi* (surra) or *T. equiperdum* (dourine) (5, 6).

The high seroprevalence of anti-trypanozoon antibodies observed in all the selected horse-breeding districts of the Bale highlands provides strong circumstantial evidence that trypanozoon infection is a highly prevalent endemic disease and a potential threat to the equine population. This is the first report of such seroprevalence in these districts. The uniform and widespread distribution of the disease in this area could be attributed to the unrestricted animal movement from neighbouring districts for trade

and transport purposes, uncontrolled animal breeding, and the absence of effective trypanocidal drugs.

Given the current difficulties in diagnosis, particularly for parasite isolation and differentiation of *T. evansi*, further detailed studies should be conducted to isolate new parasite strains and to explore the possibility of molecular diagnosis of *T. equiperdum*.

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Étude séro-épidémiologique de l'infection due au sous-genre *Trypanozoon* chez le cheval dans une zone présumée infectée de dourine dans les hauts plateaux de Balé, région d'Oromia, Éthiopie

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Résumé

Une étude séro-épidémiologique de l'infection due au sous-genre *Trypanozoon* chez le cheval a été réalisée dans la région d'Oromia en Éthiopie entre les mois de septembre 2007 et juin 2008. Elle avait pour objet de déterminer la prévalence sérologique des anticorps dirigés contre *Trypanozoon* dans des échantillons sanguins prélevés de manière aléatoire sur 880 chevaux provenant de plusieurs districts des hauts plateaux du Balé dédiés à l'élevage des chevaux. Les taux de prévalence sérologique de l'infection due au sous-genre *Trypanozoon* obtenus au moyen du test d'agglutination sur carte (CATT)/*T. evansi* et de l'épreuve d'agglutination au latex/*T. evansi* étaient respectivement de 19,66 % (173 échantillons positifs) et de 15,91 % (140 échantillons positifs). Ces taux élevés indiquent clairement que l'infection due à *Trypanozoon* sévit à l'état endémique. Aucun des deux tests utilisés ne permet de différencier les anticorps dirigés contre *T. equiperdum* (agent causal de la dourine) de ceux dirigés contre *T. evansi* (agent causal du surra). Les résultats de cette enquête semblent confirmer l'intérêt du dépistage sérologique de terrain au moyen de tests comme le CATT/*T. evansi* et l'épreuve d'agglutination au latex/*T. evansi*, aussi bien dans le cadre d'études épidémiologiques que pour la lutte contre ces infections.

Mots-clés

Cheval – Épreuve d'agglutination au latex/*T. evansi* – Éthiopie – Infection à *Trypanozoon* – Séro-épidémiologie – Test d'agglutination sur carte/*T. evansi*.



Estudio seroepidemiológico de la tripanosomiasis en caballos de las tierras altas etiópicas de Bale (región de Oromia) presumiblemente afectadas por la durina

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Resumen

Los autores exponen los resultados de un estudio seroepidemiológico de las tripanosomiasis en caballos realizado entre septiembre de 2007 y junio de 2008. Para ello se determinó la seroprevalencia de anticuerpos contra tripanosomas en 880 muestras de suero extraídas aleatoriamente en ciertos distritos del altiplano de Bale (Etiopía) dedicados a la cría caballar. Al aplicar las pruebas de CATT/*T. evansi* y LATEX/*T. evansi*, los niveles observados de seroprevalencia de tripanosomiasis fueron de 173 (19,66%) y 140 (15,91%), respectivamente. Estos elevados guarismos avalan la tesis de la endemidad de la infección. Ninguna de las dos pruebas permite distinguir entre los anticuerpos generados por *T. equiperdum* (agente etiológico de la durina) y los causados por *T. evansi* (agente de la surra). Del estudio se desprende que pruebas de cribado serológico sobre el terreno como las susodichas CATT/*T. evansi* y LATEX/*T. evansi* podrían ser útiles tanto para estudios epidemiológicos como en la lucha contra las tripanosomiasis.

Palabras clave

Caballo – CATT/*T. evansi* – Etiopía – Infección por tripanosomas – LATEX/*T. evansi* – Seroepidemiología.



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