Parasitological and serological survey on trypanosomosis (surra) in camels in dry and wet areas of Bale Zone, Oromyia Region, Ethiopia

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SUMMARY

A cross sectional study on camel trypanosomosis was conducted in Dello-Mena and Sawena districts of Bale Zone Oromyia Region, Ethiopia from September 2007 to June 2008. The study was carried out to determine the serological and parasitological prevalence of surra and to compare them in two areas of different ecological characteristics (wet and dry). Blood samples were collected from 619 camels and examined for the presence of agglutinating antibodies against T. evansi using card agglutination test for trypanosomiasis (CATT/T. evansi), and a parasite detectionuffy-coat method (BCM). The overall parasitological and serological prevalence of camel trypanosomosis was found to be 12.12% and 24.88%, respectively. Camels in Dello-Mena had a significantly higher serological as well as parasitological prevalence of surra than in Sawena district. Thus it was found that camels in riverine areas of Dello-Mena were more at risk of infection than herds in non-riverine areas of Sawena. There was a statistically significant difference (P<0.001) in the mean PCV of parasitologically positive and negative camels. However, the mean PCV of seropositive but parasitologically negative camels did not differ from the mean PCV of sero negative and parasitologically negative camels. More than 1/3 of the positive camels in BCM were negative in CATT/T. evansi indicating the existence of non RoTat 1.2 T. evansi isolates from camels in Ethiopia. From the results of the present study it can be inferred that camel trypanosomosis is endemic in Dello-Mena and Sawena districts of Oromyia Region, Ethiopia where it demands further study on identification of the principal vector species responsible for transmission and investigating the potential for vector control.

Keywords: Camel, Trypanosomosis, BCM, CATT/T. evansi, Bale Zone, Ethiopia.

Introduction

In Ethiopia, as in most dry lands of Africa and Asia, camels are the principal source of income and food for millions of pastoralists. In addition, camels play a central role in providing draught power and determining the wealth and social status of pastoralists. Ethiopia’s camel population is estimated to be one million head. In Ethiopia camels inhabit almost all peripheral drier lowlands that generally fall below 1,500 metres above sea level. These areas include the major parts of the Somali and Afar National Regional States and some parts of the Oromyia National Regional State [25].

In Ethiopia, camels are kept in the arid low lands, which cover 50% of the country and the home range of over 2 million pastoralists. Due to the fact that the production is usually a migratory system in remote areas with harsh living conditions and poor infrastructure, the animals are presumed to be inaccessible for veterinary service delivery. In Ethiopia until recently there has been very little systematic research to assess the impact of diseases on camel productivity [22].

Trypanosomosis (Surra) is one of the major diseases in camels. This parasitic infection is caused by Trypanosoma evansi, which is transmitted mechanically by haematophagous biting flies, especially tabanids. The disease causes a great impairment in productivity in camel herds and even mortality in the acute form. Surra is widespread in different parts of the world and poses a major constraint to camel productivity [6]. Available information on the prevalence of surra caused by Trypanosoma evansi in many countries of the world as reported, are: Kenya (28%) [17]; Ethiopia (21%) [28]; India (22%) [19] and Sudan (33%) [6].

RÉSUMÉ

Prévalence sérologique et parasitaire de la trypanosomose chez le chameau en zone humide et sèche dans la région d’Oromyia, Ethiopie
Une étude croisée sur la trypanosomose des chameaux a été réalisée dans les district de Dello-Mena et Sawena qui se trouvent dans la région de Oromyia en Ethiopie, pendant la période allant de septembre 2007 à juin 2008. La prévalence sérologique et parasitologique a été déterminée et comparée à des données écologiques des régions étudiées. Des échantillons sanguins ont été collectés sur 619 animaux et analysés pour la présence d’anticorps agglutinants envers T. evansi (Test d’agglutination sur carte). Une détection parasitaire a aussi été réalisée. La prévalence du parasite était de 12.12 % alors que la prévalence sérologique était de 24.88 %. Les chameaux de la région de Dello-Mena ont une prévalence parasitaire significativement plus importante que ceux de la région de Sawena. Dans cette dernière zone, le taux d’infestation est plus important dans les zones humides.Il ressort de la présente étude que la trypanosomose sévit à l’état endémique chez les camélidés dans les district de Dello-Mena et Sawena en Ethiopie.

Mots clés : Chameau, trypanosomose, BCM, CATT/T. evansi, Zone de Bale, Ethiopie.
Hence, considering the growing reliance on camels and the importance of trypanosomosis in the pastoral production systems, the present study was undertaken to determine the prevalence and ecological variation of camel trypanosomosis in Dello-Mena and Sawena districts of Bale Zone, Oromia Region, Ethiopia.

Materials and Methods

STUDY AREA

The present study was conducted in two selected districts of Bale Zone namely Dello-Mena and Sawena of the Oromia Regional State South east of Ethiopia about 430 kms away from Addis Ababa. The two study districts were selected purposively to represent the camel rearing districts of the zone based on their camel population and ecological conditions. The altitude of the study area ranges from 850 to 2800 m a.s.l., where the lowland area predominates with a narrow strip of high land area in the Northern part of Dello-Mena district. The area experiences a bimodal rainfall occurring from September to November and March to June. An average annual temperature of 20-25°C and rainfall of 200 mm are recorded in Dello-Mena. Vegetation of the area changes with altitude ranging from scattered trees and bushes in the low land to dense woody forest area in the high land. Dello-Mena district is endowed with several rivers, nine perennial rivers flow across the district namely: Welmel, Yadot, Erba-1, Erba-2, Deyu, Denda, Doya, Gomgoma and Shawae. The rivers and other deep wells, ephemeral ponds, seasonal streams are sources of water for livestock and people. Unlike Dello-Mena, surface water is a serious problem in Sawena district, where only seasonal streams, ephemeral ponds and shallow temporary wells are sources of water in the rainy season and these usually dry out after a few days during the dry season. Dello-Mena district has an agricultural vocation and a mixed farming system with crop-livestock production, where as Sawena has a pastoral vocation with livestock rearing being the dominant economic activity of the district [2].

STUDY DESIGN AND SAMPLING STRATEGIES

A cross-sectional study design based on parasitological and serological examination was conducted in a total of 619 randomly selected camels from two camel-rearing districts of Bale Zone from September 2007 to June 2008. The camels were sampled proportionally from Dello-Mena district, 319 (51.5%) and from Sawena district 300 (48.5%) based on camel population size. The estimated camel population in Dello-Mena and Sawena districts was 51,500 and 48,500 heads, respectively [2]. Accordingly, 19 peasant associations, 10 from Dello-Mena and 9 from Sawena districts were randomly selected and considered as study population. The average population of camels in each peasant association was 1700 and 1550 from Dello-Mena and Sawena districts, respectively [2]. Selection of individual camels for the study purpose was executed on basis of systematic random sampling methods where every tenth animal was considered. However, a number of young animals and dominant males could not be caught. Study animals were physically restrained and 10 ml of blood was collected from the jugular vein using heparinized and plain vacutainer tubes.

EXAMINATION PROCEDURES

PCV and parasitology

Blood samples were drawn into paired heparinized micro-haematocrit capillary tubes up to ¾ of their length. One end of the tubes was then sealed with cristaseal (Hawksly, England). The tubes were symmetrically loaded in the haematocrit centrifuge, with the sealed end outwards, centrifuged at 12,000 rpm for 5 minutes. PCV levels of individual samples were determined on haematocrit reader (Hawksly, England) and the values were expressed in percentages. Following the determination of PCV both capillary tubes were examined for the presence of motile trypanosomes using the buffy-coat method (BCM) [12].

Sero

Study on the seroprevalence of camel trypanosomosis was conducted using the 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. The positive samples were determined at cut-off point dilutions 1:4 and above [3]. Accordingly, 25 μl of camel serum, diluted 1:4 in CATT-buffer, was pipetted onto a reaction zone of a plastic coated test card. After adding one drop (about 45 μl) of CATT reagent, the reaction mixture was spread out by means of a stirring rod and allowed to react on a Card Test Rotator for 5 min at 70 rpm.

Statistical analysis

Statistical analysis was performed to determine the prevalence (percentage), the level of agreement between diagnostic tests (Kappa statistics, κ) and the influence of ecological characteristics on prevalence (chi-square test). P-value less than 0.05 was considered as a statistically significant difference between considered variables [23].

Results

The overall parasitological prevalence of surra in the Dello-Mena and Sawena districts of Bale Zone was 12.1% (75/619) (Table I). Parasitemia varied on the microscope field between a few parasites per slide and over 10 trypanosomes per field. The respective parasitological prevalence was 16.3% (52/319) and 7.7% (23/300) from Dello-Mena and Sawena districts, respectively. Out of a total of 619 camels tested by card agglutination test for the detection of agglutinating antibodies against T. evansi the overall sero prevalence was found to be 24.9% (Table I).
PCV AND CAMEL TRYPANOSOMOSIS

A statistically significant difference ($P<0.05$) in mean PCV was observed between parasitologically positive (22.6%) and negative (25%) camels (Table II).

Based on the trypanosomosis status using serological and parasitological tests mean PCV of the camels was compared. Accordingly, mean PCV of 21.6% and 25.1% was recorded for serologically and parasitologically positive, and serologically and parasitologically negative camels, respectively (Table III). This was found to show a statistically significant difference ($P<0.05$) between the groups.

DEGREE OF CONCORDANCE BETWEEN PARASITOLOGICAL AND SEROLOGICAL TESTS

Out of 75 animals with positive results in the parasitological test, 54 and 21 were positive and negative using CATT/$T. evansi$, respectively (Table 4). Statistical test using kappa indicated a moderate agreement between the two tests ($k=4$). The relative sensitivity of the CATT/$T.evansi$ test employed in the present study was found to be 54/75 (72%) (Table IV).
Discussion

The present study involving parasitological and serological examinations provided a strong evidence that camel trypanosomosis caused by *T. evansi* is widespread and a major threat to the well-being and productivity of the camel population in Dello-Mena and Sawena districts of Bale Zone Oromiya Region, Ethiopia.

The overall parasitological prevalence of 12.1% obtained in this study is in agreement with previous findings in different parts of Ethiopia (12.5%) [21], 10.2% [8] and 10.9% [24]. However, the present result is higher than reports made by other investigators 6.54% [1] and 5% [5]. The highest parasitological prevalence observed in this study may be linked to ecological conditions of the study area especially in Dello-Mena district where there are numerous animal watering points and the existence of wooded area near permanent and temporary waterways might have created favorable environment for the insect vectors of *T. evansi* [2]. This suggests a correlation between the prevalence of infection and the existence of wooded areas near permanent or temporary waterways where the animals can go to drink. The occurrence of surra is influenced by climatic and ecological conditions, based on the distribution and abundance of the insect vectors. It has a marked seasonal incidence related to wet and humid condition and increased activity of biting flies during the wet season. Local epidemics of surra occur where conditions exist for the spread of infection with *T. evansi*, such as when many animals are stabled together or close herded and particularly when the biting fly population is abundant during the wet season [9]. Surveys of Tabanus in the various tropical areas have shown a definite correlation between the seasonal outbreaks of *T. evansi* infections and the increase in number of Tabanus during the rains. Thus, rainfall, suitable moisture-retaining clay soil and surface water pools also support the development of suitable camel browsing conditions, where Acacia senegal shrubs grow in abundance [17].

The significant difference observed in PCV between infected and non infected camels suggest that anaemia is one of the pathologies of surra. Anaemia appears to be a major component of the pathology of surra [7]. Its development and persistence in the course of the disease induce anoxic conditions which manifest signs of dysfunction in various organs as a result of a fall in tissue pH and vascular damage. This is followed by the release of large quantities of cytoplasmic and mitochondrial enzymes, especially aspartate alanine transferase (AST) and alanine transferase (ALT), among others, into serum, causing further cellular and tissue damage. The net effect associated with the above changes is immunosuppression which later develops and predisposes the animals to other infections and death if untreated.

Anaemia is a major component of the pathology of surra and of African trypanosomoses generally. In the early phases of *Trypanosoma evansi* infections of camels the anaemia is haemolytic and haemophagocytic. Its development and persistence in the course of the disease induce anoxic conditions which manifest signs of dysfunction in various organs as a result of a fall in tissue pH and vascular damage. In the late stages, anaemia continues to be a major factor, with probably additional causes [7].

Contrary to the parasitological findings a large proportion of camels were found to be seropositive. The poor sensitivity of parasitological methods, often less than 50% [10, 11, 13], to detect low level parasitemia in the chronic stage of the disease, the possible extra vascular location of trypanosomes and CATT/*T. evansi* can not distinguish current from cured infection [9] as detectable level of antibodies can still be found in self cured animals or after treatment with trypanocidal drugs might explain this enormous difference. The overall higher seroprevalence (24.9%) compared to the parasitological result (12.1%) recorded in the present study could also be associated to the inability of the serological test to differentiate antibodies against *T. evansi* during past or present infection. Taking in to account the high specificity of CATT/*T. evansi* at serum dilution 1:4 [27], the observed 24.9% seroprevalence indicates a serious prevalence of *T. evansi* among camel population Ethiopia, which is not necessarily associated with severe outbreaks. Likewise the VSG of trypanosome can elicit high antibody titer which could clear 99% of the parasite that is followed by antigenic shift [4]. Thus during the high antibody assault the level of parasitemia could be low not to be detected parasitologically rendering low sensitivity of the test [20].

The results of the present study clearly indicated that more than 1/3 of the parasitologically (BCM) positive camels were negative in serology (CATT/*T. evansi* RoTat 1.2). This strongly suggests the existence of non RoTat1.2 *T. evansi* isolates from camels in Ethiopia. The variant surface glycoprotein (VSG) of *T. evansi* Rode Trypanozoon antigen type (RoTat) 1.2 is a diagnostic antigen for *T. evansi* from diverse geographical regions. Serological and PCR tests based on the VSG of RoTat 1.2 have shown high specificity and sensitivity in studies in different geographical regions of the world [3, 26, 27]. Exceptions are a number of *T. evansi* isolated in Kenya. It has been shown however, that a number of *T. evansi* in Kenya were not detected by tests based on RoTat1.2 VSG and four of the isolates lacked the RoTat1.2 VSG gene [15, 16]. CATT/*T. evansi* RoTat1.2 is generally considered as a highly sensitive test most often more than 90% sensitivity in parasitologically positive animals. However, sensitivity of CATT/*T. evansi* RoTat1.2 in the present study was found to be 72%. This lower sensitivity observation is in agreement with previous studies in Kenya who reported 65.5% and 68.6% sensitivity, respectively emphasizing the need to address the problem of diagnosis of *T. evansi* in the region [14, 18].

Hence, the present study indicated that camel trypanosomosis caused by *T. evansi* is endemic in Dello-Mena and Sawena districts with a significantly higher prevalence in Dello-Mena than Sawena district. The degree of disease prevalence is probably linked to the presence of permanent and temporary waterways and the plant canopy where the animals can trek. Thus it was found that camels in riverine areas are more at risk of infection than herds in non-riverine areas. Low PCV value was strongly associated with positive trypanosomosis status of the camel. Considering the widespread existence of surra and its impact on camel productivity further study on identification of the principal vector species responsible for transmission and investigating the potential for vector control is recommended.
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