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Seroprevalence of *Trypanosoma equiperdum* (Dourine) in and Around Asella, Oromia, Ethiopia.

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Abstract

A cross sectional study design based on serological test (CATT/T. *evansi*) was conducted from December, 2016 to April, 2017 in order to determine the seroprevalence of dourine in and around Asella. For this purpose , a total of 202 horse sera were collected randomly from these areas, and were subjected for testing with card agglutination test for trypanosomosis called CATT/*T. evansi*. The test was checked by using positive and negative controls before the whole field samples were tested. The positive results for the test were determined at cut-off point dilution of 1:4. From the 202 serum samples examined; 26 animals (15 males and 11 female) horses showed a positive result on CATT/*T. evansi*; so that 12.9 % of tested animals were seropositive for trypanosomal antibodies. Regardless of difficulties in differentiating between the infections caused by *T. equiperdum* and *T. evansi*, the findings of the present serological survey disclosed that there was a valuable *T. equiperdum* infection (dourine) in and around horses of Asella area. According to this study the seroprevalence of *T. equiperdum* (dourine) with respect to explanatory variables or risk factors; sex, age, body conditions, castration status, previous abortion and animal origin were found to have no significant difference ($p > 0.05$) on the sero prevalence of the disease using CATT/*T. evansi* test. This suggests that all animals had equal chance of exposure to the parasite. Even though there was no direct parasitological detection of *T. equiperdum*, the result of serological test conducted by using CATT /*T. evansi* provides strong evidence that Dourine is highly prevalent, most important problem in and round Asella of Arsi zone. Furthermore, in view of large number of horses in Ethiopia and the unrestricted movement of animals throughout the country it is likely that dourine may be more widespread in Ethiopia particularly in highlands of Arsi-Bale, than is currently realized. Moreover, further diagnostic tests should be performed to clearly visualize the prevalence and extent of T. equiperdum (Dourine).

Keywords: Asella, CATT/T. evansi, Dourine, Ethiopia, Horses, Seroprevalence, *T. equiperdum*

Introduction

The world equine population is estimated at 44 million donkeys, 11 million mules and 59 million horses. More than 97% of the world's donkey and mule populations, and over 72 % of the world's horse population is found in developing countries specially kept for draft purpose [1]. Ethiopia has more than 6 million donkeys, the second largest donkey population in the world next to china, 1.9 million horses and over 350,000 mules specifically kept for work [2].

In Ethiopia, equines have their greatest contribution in agriculture and transport sector of the national economy. They are used for various works such as carting goods and people, carrying packs and bricks,

and other materials, riding, tillage, weeding, and water carrying. Despite their tremendous contribution less attention has been paid to equines in terms of health care and husbandry managements. Of the major causes of economic losses and low productivity of livestock, the prevalence of large number of diseases in the country is considered to be the major one [3].

Among the multiple health and welfare problems affecting working equids, parasitic diseases are one of the major constraints to their productivity and work performance ; which often leads to high morbidity and mortality [4, 5]. Dourine (*Trypanosoma equiperdum*) infection is mostly a chronic or acute contagious disease of horses and other members of the family equidae. It is among the major parasitic diseases affecting horses in Ethiopia, which is caused by protozoan parasite Trypanosoma *equiperdum*. Of the non-tsetse transmitted African trypanosomosis, dourine is the only trypanosomosis that is not transmitted by invertebrate vector, but is transmitted exclusively during coitus [6]. Dourine can affect horses, mules and donkeys. The later are generally more resistant and often remain asymptomatic carriers [7].

The causative agent of dourine, *Trypanosoma equiperdum*, differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood and it is strictly limited to the equines (horses, donkeys and mules) under natural condition [8]. This parasite efficiently evades the host animals' immune system through the use of variable surface glycoproteins or VSGs [9].

In course and clinical signs vary considerably depending on the virulence of the strain concerned. The course of the disease in horses is chronic, varying from a few months to 1-2 years. The clinical signs are market by periodic exacerbation and relapse, ending in death or, possibly recovery. Fever, local edema of the genitalia and mammary glands, cutaneous eruptions, in coordination, facial paralysis, ocular lesions, anemia and emaciation may all be observed. Edematous plaques, 5-8 cm in diameter and 1 cm thick, are pathognomonic [10, 11].

The first official report of the disease in Ethiopia was made in 1980 when the Arsi Rural Development Unit asked the Tsetse and Trypanosomosis Survey and Control Department to investigate a persistent disease problem in horses in the administrative regions of Arsi and Bale [12]. Since then, dourine has been found to be prevalent throughout the highlands of Ethiopia, particularly in the Arsi and Bale zones [13]. 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 [14].

Horses are very susceptible to *T. equiperdum* and usually die at the end of a chronic disease that may last for 1–2 years. Occasionally, acute forms that lead to death in 2–3 months are seen in thoroughbred horses. Donkeys and mules, despite being susceptible to infection, develop a mild syndrome or remain asymptomatic. The incubation period in horses ranges from 1 week up to 6 months [15].

Diagnosis of *T. equiperdum*, the causative agent of dourine in horses by standard parasitological techniques is extremely difficult owning to the low numbers of parasites present in the blood or tissues fluids and the frequent absence of clinical signs of disease. Demonstration of trypanosomal antibodies in the serum has become the most important parameter in determining the disease status of individual animals [16].

Diagnosis of equine trypanosomosis caused by the subgenus *Trypanozoo*n commence with the observation of clinical signs and symptoms. However, further specific diagnosis requires serological demonstration of the parasites' antibody and antigen reaction. Equines are considered to be the only natural host of *T. equiperdum* [10].

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 [14].

However, some of the above studies had isolated the trypanosomes in the blood of dourine suspected animals; there is need for further detailed studies in the diagnosis of *T. equiperdum.*

Therefore, the objectives of this research were:

☞ To determine the Seroprevalence of equine trypanosomosis (Dourine) in and around Asella , using CATT/*T. evansi*

☞ To asses host related risk factors associated with trypanosome infection in equine

Materials and Methods

Description of Study Areas

The present study was conducted from November, 2016 to April, 2017 in Asella town and its surrounding. The Town is a capital of Arsi Zone, Oromia regional state. It is located about 175 km south east of Addis Ababa at 6° 59' to 8° 49' N latitudes and 38° 41' to 40° 44' E longitudes. The altitude of the area ranges from 2500 to 3000 m.a.s.l. Asella and its surrounding is characterized by mid subtropical weather, with minimum and maximum temperature ranging from 8.4 to 22.6°C, and the relative humidity ranging from 43 to 60%. The average rainfall is 2000 mm. The area has a bimodal rainfall occurring from March to April (short rainy season) and July to October (long rainy season). According to Arsi Planning and Development Office [17], the area is densely populated, with livestock population of 85,893 cattle, 57,118 sheep, 10,725 goats, 7,841 horses, 15,642 donkeys, 517 mules and 35,489 poultry. The farmers in the area practice mixed crop-livestock farming system.

Study Animals and Sample size

The study animals which were selected in this study were horses found in and around the towns of Asella. The animals for sampling were accessed from the farmers' share of communal grazing land and marketing areas as well as veterinary clinics in association with the animal health personnel of the respective districts. The sample size was calculated according to the Thrusfield [18], by considering 19.66 % expected prevalence taken from previous study Hagos *et al*. [19] and 5% desired absolute precision at 95% confidence interval using the following formula:

$$N=\frac{1.96^2 Pexp\ (1-Pexp)}{d^2}$$

Where, N= required sample size, P_{exp} = expected prevalence, D = desired absolute precision, 1.96^2 = z-value for 95% confidence interval.

So that, the sample size to find the seroprevalence of T. *equiperdum* (Dourine) is 202.

Aging of horses was made based on the description provided by. Therefore, animals between 4-6 years of age were considered as young adults, while animals more than 6 years old as adults. Body condition scoring of the horses also was made based on the description provided by [20].

Study design and Sampling Strategies

A cross sectional study design was based on serological survey was conducted from *December,* 2016 to May, 2017 on the horses prevailing in the study area. The selected sites were: Beritti Bilalo and Asella town (Tiyo district). The sampling points of the study area were selected a randomly in collaboration with the animal health personell of the respective areas on the basis of participant cooperation, logistics, the farmers share of communal grazing land and accessibility. All the horses sampled for this study were those lived under a traditional management system of free grazing.

Sample collection and storage methods

Blood samples were collected from the jugular vein of horses using plain vacutainer tubes and needle, after the site had been wiped with cotton wool soaked in alcohol for serological test. The plain vacutainer tubes were labeled and the blood was allowed to clot overnight at room temperature before the serum was separated and collected into its container, test tubes. The serum samples were then stored in sterile polypropylene cryogenic vials at -20^{o}c, and transported under a cold chain using ice-box (cool box) according to OIE [21] to the diagnostic laboratory until they are tested using a card agglutination test for trypanosomosis called CATT/T.*evansi.*

Serological Survey

Card agglutination Test for Trypanosomosis (CATT/T. evansi)

The sample testing on investigation of (Dourine) *T. equiperdum* infection was conducted at the Bishoftu CVMA Ethio-Belgium VLRI-funded PhD project laboratory, which was established by Ethiopian and Belgium collaborative project funded by the Flemish

Inter-University Council-University Cooperation (VLRI-UOS) by using a card agglutination test called CATT/*T. evansi*, which was initially designed to detect T. *evansi* infection (surra), but can also be a valuable method for detection of *T. equiperdum* (dourine) [10].

CATT/*T. evansi* is a rapid, direct card agglutination test which uses formaldehyde fixed Commassie stained, freeze-dried antigen of *T.evansi* VAT RoTat 1.2 [22]. In the CATT/*T. evansi*, 50 micro litter of serum was diluted with PBS (Phosphate buffered saline solution) and was mixed with 50 micro litter of the reagent (CATT antigen) on a test card; spread over approximately 1.5cm and shaken with electrical rotator arranged at 70 rotation per minute for 5 minutes. The test was checked with positive and negative controls before the whole samples were tested. In the test, positive results were determined at cut-off point dilutions 1:4. The presence of trypanosomal antibodies was revealed by macroscopic agglutination [23, 24].

CATT/T.*evansi* is used as a screening test for trypanosomosis infection with *T. equiperdum* results in production of circulating antibodies against several surface antigens of the parasite. Such antigens can be demonstrated in the serum of the infected host by production of a visible agglutination reaction (within about 4 minutes) on the circular area of the test card when the antigen reacts with serum antibody produced against trypanosomal infections [22].

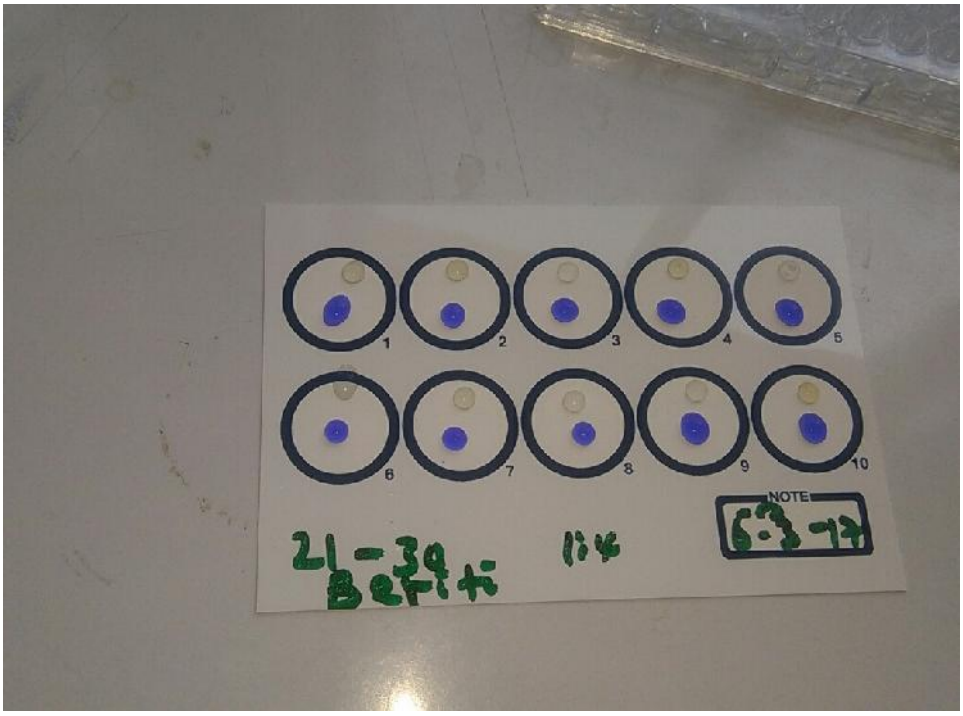

Figure (1) Preparation of the serum sample and the buffered antigen on the test card for mixing.
Source: Own camera photo captured during serological examination at CVMA, VLIR Ethio-Belgium-funded PhD Project laboratory

Data analysis

All collected raw data recorded in this study were entered to a Microsoft Excel database system and analysed descriptively using SPSS Version 20 used for analysis. Many attribute data that imported to database system, includes age, sex, castration status, previous abortion, Animal origin and laboratory result. The seroprevalence of the disease was calculated by dividing the number of positive animals to the total number of animals tested. Variation in the seroprevalence of dourine between, age, sex, Body condition, castration status and animal origin were determined by using a chi-square (x^2) statistics. A p-value of less than 0.05 at 95% confidence interval is considered as statistically significant [25].

Results

Results of CATT/T. evansi test

Since, the CATT/*T. evansi* principally detects IgM (agglutinating pentavalent Immunoglobulin, the half life of which is short) (OIE, Terrestrial Manual, 2012), the findings of the present survey disclosed (pointed out) that there were either early infections, or late infections with recent circulation of parasites antibodies against *T. equiperdum* infection in the horses' blood.

The results of the employed serological test (CATT/*T.evansi*) for *T. equiperdum* regardless of the difficulties in differentiating the infections caused by *T. equiperdum* and *T. evansi* revealed that 12.9% of the tested animals were seropositive for *T. equiperdum*. This seroposistivity of antibodies against *T. equiperdum* (Dourine) provides strong circumstantial evidence that dourine which is known to occur previously in the horses of Arsi Bale highlands of Ethiopia (Hagos *et al*., 2005) is spreading to adjacent areas of the country.

According to sex of the horses, the seroprevalence on the CATT/*T.evansi* was found to be (15 %) and (9.8%) for male and female horses, respectively. The seroprevalence among the three study areas was found (14.3%), (12.9%) and (11.4%) for the horses of Beritti, Tiyo and Bilalo respectively. Among age groups, the seroprevalence of *T. equiperdum* (Dourine) varied between 8.82% (young) to 13.7% (adult). Based on BCs, the CATT/*T. evansi* test, positive result was ranged between 13.3% to 8.89% representing the animals of poor and good BCs, respectively. In general, there was no statistically significant association of seroprevalence on the suspected risk factors.

The presence of serum antibodies against *T. equiperdum* (the parasite antigen) was revealed by macroscopic agglutination reaction formed on the circular zone of the test card. (**Figure 1** and Annex 3, picture….).

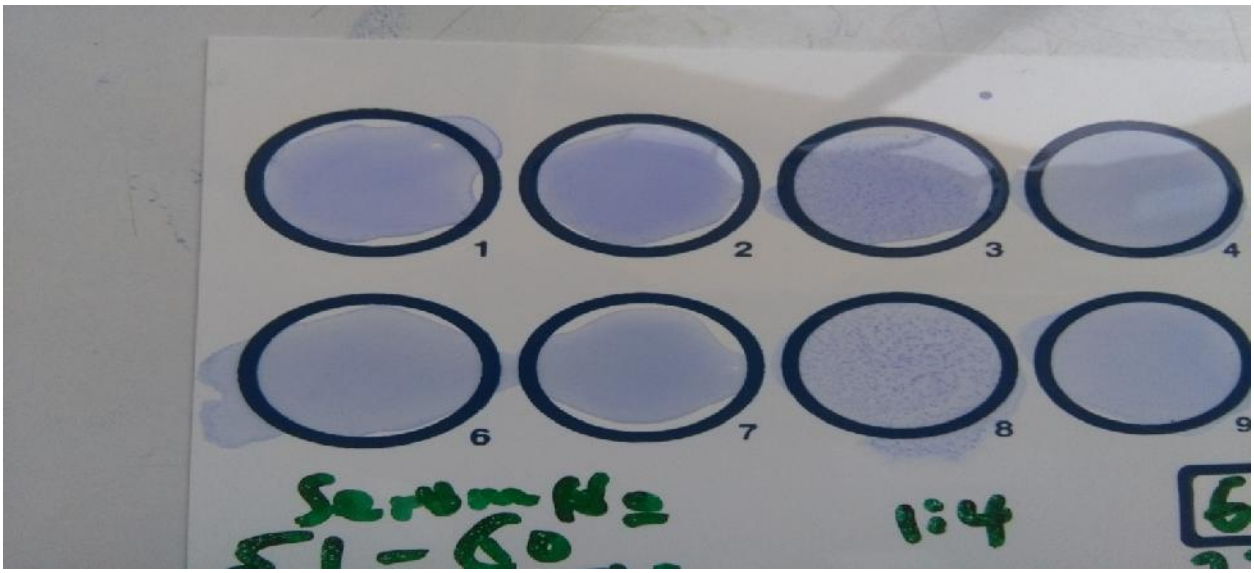

Figure 2: A visible agglutination reaction formed on circle of the test card (camera photo captured as of March 29, 2017 during serological survey by CATT/T. *evansi*).

Table 1: Seroprevalence of Dourine (*T. equiperdum*) of horses in and around Asella on the basis of CATT/ T. *evansi* test with associated risk factors.

Factors	Animals tested tested	Prevalence	P- value	Chi square
Districts				
Beritti	70	10(14.3%)		
Tiyo	62	8(12.9%)	0.88	0.25
Bilalo	70	8 (11.43%)		
sex				
M	98	15(15.3%)	0.32	1.10
F	104	11(10.6%)		
Age				
Young	34	3 (8.82%)	0.44	0.59
Adult	168	23 (13.7%)		
Body conditions				
Good	89	8(8.98 %)		
Moderate	98	16(16.32%)	0.32	2.24
poor	15	2(13.3%)		
Castration status				
Yes	68	10(14.7%)	0.61	0.26
No	32	6(18.75%)		
Previous Abortion Yes				
No	15	1(6.76%)	0.16	0.68
	90	9(10%)		
Animal origin				
Born at home	83	(10.84%)	0.47	0.512
Bought from local market	119	17(14.28%)		
Overall prevalence	202	26/202 (12.9%)		

Pearson's chi square (x^2) test for seroprevalence of T. *equiperdum* with the associated risk factors (explanatory variables; sex, age, body condition score, castration status, animal origin,) revealed that there is the variation in seroposistivity was statistically insignificant (p> 0.05) on the basis of CATT/T.*evansi* test.

In the above table , poor referred to very thin and thin body condition score (BCs 1-3), Moderate referred to less thin , moderate and moderately fleshy body condition scales(BCs of 4-6), where as good body condition referred to fleshy, fat and extremely fat body condition scales (BCs of 7-9) (Henneke *et al*., 1983). Pearson's chi-square (x^2) test revealed that variation in seroprevalence among animal groups of different BCs was statistically insignificant (P>0.05; x^2=2.242, df =2).

Similarly, aging of horses was made based on the description provided by Coombs[20]. Therefore, animals between 4-6 years of age were considered as young adults, while animals more than 6 years old as adults. Body condition scoring of the horses also was made based on the description provided by Coombs [20].

Discussion

In spite of the difficulties in differentiating between *T. equiperdum* and *T. evansi*,[12], the findings of the present serolological by CATT/T. *evansi* disclosed that there was a valuable *T. equiperdum* infection (Dourine) in horse of Asella. As CATT/T. *evansi* principally detects IgM (agglutinating pentavalent immunoglobulins the half life of which is short [22], the results of the present study also pointed that there were either early infections, or late infections with the recent circulation of parasite (*T. equiperdum*) in the horses' blood.

The overall seroprevalence of *T. equiperdum* was found to be 12.9% by using the CATT/*T. evansi* test. This seroprevalence was in agreement with the previous reports based on indirect methods (antibody and antigen detection) in the Arsi-Bale highlands of Ethiopia [7]. This relatively high seroprevalence of Dourine founded through CATT/*T. evansi* result provided the strong evidence that *T. equiperdum* which was highly prevalent in horses of Arsi- Bale highlands of Ethiopia [26, 27] Hagos *et al*. [26]; Degefa [27] spreading to adjacent areas of the country.

This seroposistivity was in agreement with the previous researches based on CATT/T.evansi, LATEX/T. evansi and ELISA/ *T. evansi* serological tests in the same area, which is so far reported that *T. equiperdum* was present in the Arsi-Bale highlands [19] (Hagos *et al.* [19], which reported On overall seroposistivity (on CATT/*T. evansi*) of 19.6 % in dourine infected horses of Bale highlands.

Statistically significant variation was not observed when the seroprevalence of anti-trypanozoon antibodies in CATT/*T. evansi* test was compared among the examined animals with respect to explanatory or risk factors such as sex, age, study districts, castration status, previous abortion and animal origin. This might be attributed the fact that the animals of both sex, age, body conditions, castration status, previous abortion and all study areas were equally exposed to the parasites, and this also indicates uniform spread of the disease.

The seroprevalence result is almost consistent with the previous reports based on indirect methods (antibody and antigen detection) in the Arsi-Bale highlands of Ethiopia [13, 26, 28, 29].

Until now, the only officially approved test for dourine remains the complement fixation test (CFT), although it is generally accepted that this test cannot discriminate between T. *evansi* and T. *equiperdum*. Currently, neither serological parasitological nor DNA-based tests allow a subspecies identification within the subgenus *Trypanozoon*; therefore, no definitive diagnosis can be given for T. *equiperdum* [14, 29]. The limitations and difficulties of the serological test to differentiate Trypanosoma in equine infections due to *equiperdum, T. evansi* and *T. b. brucei* have been described by Zablotskij *et al*. [12] and whether the examined animals in this study are infected with *T. equiperdum* (the causative agent of dourine) or with *T. evansi* (the causative agent of surra) is under question.

Considering study areas, the horses of Beritti and Bilalo showed highest (14.3%) and lowest (11.3%) positive results, respectively in the CATT/*T. evansi* test. This could be attributed to the variation among the chance of exposure (sexual contact) to carrier introduced animals. The body condition with moderate showed that a relatively higher (16.32 %) seroprevalence results than good body condition (8.89%). Thus, it is possible to suggest that the animals under moderate conditions had relatively higher number of previous mating and genital contacts, which possibly increases chance of acquiring infection from an infected or carrier host [19]. The seroprevalence in male (15.3%) and female (10%) respectively, could be associated with the chance of mating of low number of stallions with many mares horses number of sampled animals.

With the regard to castration status, the seroprevalence of Dourine in the sampled horses was found; castrated (14.7%), and uncastrated (18.75%), respectively. This seroprevalence of *T. equiperdum* result could be due to those uncastrated horse might make sexual contact with a number of mares at a time when they are sexually active. The result of previous abortion showed the seroprevalence of *T. equiperdum* (aborted= 6.76%) and (not aborted 10 %); thus, the result could be due to the chance of sampling or due to concurrent disease conditions.

Whether the examined animals in this study are infected with *T. equiperdum* (the causative agent of dourine) or with *T. evansi* (the causative agent of surra) remains an open question. In conclusion, given the current difficulties in diagnosis, further detailed studies need to be conducted to isolate new parasite

strains using sensitive parasitological techniques, such as the mini-anion exchange chromatography test, and to explore the possibilities of molecular diagnosis of *T. equiperdum* or *T. evansi*.

Since the distribution of dourine is not restricted by environmental factors and it is possible through unrestricted movement of infected and carrier animals for infection to become established almost anywhere [11]. A large number of horses are moved and purchased from the Arsi-Bale highlands and transported into adjacent highlands of Ethiopia by local merchants for trade purpose. This could be due to the fact Arsi-Bale highlands are known for equine breeding [26].

Recently, it has been proven that most so-called *T. equiperdum* strains also express isoVATs of *T. evansi* RoTat 1.2. Therefore, the CATT/*T. evansi* may prove to be a good test for equine trypanosomosis, regardless whether the causative agent is *T. evansi* (surra) or *T. equiperdum* (dourine) (Claes *et al*., 2003). Thus, it appears that the serological test employed in the present study (CATT/T. *evansi*) can be valuable method for detection of Dourine, although it was initially designed to detect surra infections in camel [10].

Conclusion and Recommendations

Dourine is among the multiple health and welfare problems affecting working equids, parasitic diseases are one of the major constraints to their productivity and work performance, which often leads to high morbidity and mortality. Currently dourine is spreading and becoming a potential threat to the equines in the study site through unrestricted movement of animals outside of the endemic foci for trade and transportation purpose. Diagnosis of *T. equiperdum*, the causative agent of dourine in horses by standard parasitological techniques is difficult owning to the low numbers of parasites present in the blood or tissues fluids and the frequent absence of clinical signs of disease. Demonstration of trypanosomal antibodies in the serum has become the most important parameter in determining the disease status of individual animals. Regardless of the difficulties in differentiating between *T. equiperdum* and *T. evansi*, the findings of the present seroprevalence by CATT/*T. evansi* disclosed that there was a valuable *T. equiperdum* infection (Dourine) in horses of Asella. Since, CATT/*T. evansi* principally detects IgM (agglutinating pentavalent immunoglobulins the half life of which is short, although it was for first time designed and used to detect surra infection in camel; the results of the present study also pointed that there were either early infections, or late infections with the recent circulation of parasite (*T. equiperdum*) in the horses' blood. It also appears the serological test employed in the present study (CATT/T. evansi) can be valuable for detection of dourine, which helps to determine seroprevalence of this disease, which initially designed to detect surra infections in camel. As the distribution of the dourine not limited by environmental factors; it is possible through un restricted movement of infected or carrier animals, or through uncontrolled animal breeding.

Therefore, in light of the above conclusions the following recommendations were forwarded:

- ☞ Given in to account the current difficulties in diagnosis particularly parasite isolation and differentiation with *T. evansi*, further detailed studies need to be conducted to isolate new parasite strains, using sensitive parasitological and serological techniques such as the mini-Anion Exchange Chromatography Test (mAECT), and to explore the possibilities of molecular diagnosis of *T. equiperdum*

- ☞ There should be community awareness creation and extension service specifically focusing on to stop using clinical dourine cases for breeding purpose, apply strict animal movement control and castration of sick and recovered males.

- ☞ Further detailed study should be done to determine the socio-economic impact of the disease.

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