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Serological Investigation of *Trypanosoma equiperdum* Infection (Dourine) in Horses of Selale, Northern Shewa, Oromia, Ethiopia

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Abstract: A cross-sectional serological study was conducted from December, 2015 to May, 2016 in order to investigate dourine in the selected horse breeding districts of Selale. A total of 176 horse sera were collected randomly from these districts and were subjected for testing by a card agglutination test for trypanosomosis called CATT/ *Trypanosoma 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 176 serum samples examined, 21 animals (14 male and 7 female horses) showed a positive result on CATT/ *T. evansi*; so that 11.9% of tested animals were seropositive for trypanosomal antibodies. Regardless of the difficulties in differentiating between the infections caused by *Trypanosoma equiperdum* and *Trypanosoma evansi*, the findings of the present serological survey in association with the indicative clinical signs, suggested that there was a considerable *T. equiperdum* infection (dourine) in the Selale horses. The present serological occurrence of the disease was due to possibly through unrestricted movement of the infected animals. Although it was initially designed to detect surra infections in camel, the serological test employed in the present study (CATT/ *T. evansi*) can also be a valuable method for detection of dourine. Thus, further detailed studies using advanced diagnostic methods should be carried out in order to clarify the confusions among the infections due to *T. equiperdum* (dourine) and *T. evansi* (surra); and the conditions for the movement and importation of animals from infected to non-infected areas should be defined.

Key words: Catt/ *Trypanosoma evansi* · Dourine · Ethiopia · Horses · Infection · Selale · *Trypanosoma* equiperdum

INTRODUCTION

Ethiopia is the richest country in livestock population in Africa, possessing 2.75 million horses, 5.02 million donkeys and 0.63 million mules and 1 million camels [1]. These vast numbers of working equids play crucial roles in both urban and rural areas of the country. Horses have a prominent position in the Ethiopian agricultural and transport systems as draft, pack and riding animals and in many cases, as the sole means of generating income for their resource-limited owners [2]. Nearly 90% of agricultural operations depend on manual labor 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[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]. Dourine (*Trypanosoma equiperdum* infection) is a chronic (mostly) or acute contagious disease of horses and other members of the family equidae. It is among the major parasitic diseases affecting the horses in Ethiopia, which is caused by a protozoal parasite called *Trypanosoma-equiperdum*. Of the non-tsetse transmitted African trypanosomes, dourine is the only trypanosomos is that is not transmitted by an invertebrate vector, but is transmitted almost exclusively during coitus [5].

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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 is strictly limited to the equine (horses, donkeys and mules) under natural condition [6]. This parasite efficiently evades the host animal's immune system through the use of variable surface glycoproteins or VSGs [7].

Clinical signs of dourine are highly variable in manifestation and severity. The disease is characterized mainly by swelling of the genitalia, cutaneous plaques and neurological signs but develop over weeks or months [8]. The clinical course and severity varies with the pathogenecity of the trypanosome, the resistance of the breed and the physical condition (nutritional status) of the horse and stress factors.

The incubation period ranges from two weeks till three months; clinical signs might be suppressed during cold months. Generally the disease is divided in to three phases: primary stage (Genital Oedema), secondary stage (Plaques and skin eruptions) and tertiary stage (Neurological signs). Anemia, cachexia and genital oedema are often seen at post mortem [9].

The first official report of the disease was made in 1980 when the Arsi Rural Development Unit requested the tsetse and trypanosomosis survey and control department to investigate a persistent disease problem in horses in the administrative regions of Arsi and Bale [10]. Since then, dourine has been found to be prevalent throughout the highlands of Ethiopia [11].

Diagnosis of *T. equiperdum* by standard parasitological techniques is difficult, due 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 [12]. 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 in the implementation of monitoring, treatment and control programmes [11, 13].

Despite their huge numbers and significant contribution to the communities and the national economy, the attention given to study the health aspects of Horses in Ethiopia is quite minimal [14]. Although investigation of dourine for its serological status in the horses of Selale Zone was conducted so far [15] no positive result was found regarding the suspected *T. equiperdum* infection in this study area. This research is therefore, needed to further assess and check whether the horses of Selale play a role in the distribution and prevalence of dourine in the endemic areas (Especially Arsi-Bale highlands).

This is because, most of the Arsi-Bale horses suffering from this disease are originated from Selale via marketing [11] and hence Selale horses were suspected if they are seropositive for dourine at their origin site. This study could further help us to set up an important step towards conducting a detailed research and diagnostic methods on the status and identification of the specific causative agent of the infection in the area.

In light of the above background information and justifications, the objective of the present study was initiated to investigate the occurrence of *T. equiperdum* infection (Dourine) in selected horse breeding districts of Selale Zone.

MATERIALS AND METHODS

Description of the Study Area: The study was carried out in three sleeted districts (Sululta, Wuchale and Jida) involving five different localities (Chancho, Mukaturi,Gumbichu, Gebrielgute and Sirti) of Selale. Fiche is a capital town of North Shea zone where all the study areas were conducted. Fiche has a latitude and longitude of 9°48'N and 38°44'E, respectively; and an elevation between 2,738 and 2,782 metres above sea level, respectively [16].

According to Fiche Station meteorological data, the average rainfall amount of the district is about 1200mm and maximum and minimum rainfall is about 1651mm and 1115mm, respectively. Temperature of the district ranges from a minimum of 11.5 °C to a maximum of 35 °C. The total livestock population is estimated as 2290 horses, 14650 donkey, 380 mules, 119650 cattle, 33250 sheep,17295 goat, 72270 chicken and 1915 bee hives [17].

Study Animals: The study animals which were involved in this study were horses prevailing in and around the towns of the three selected districts of Selale. The animals for sampling were accessed from the farmers' share of

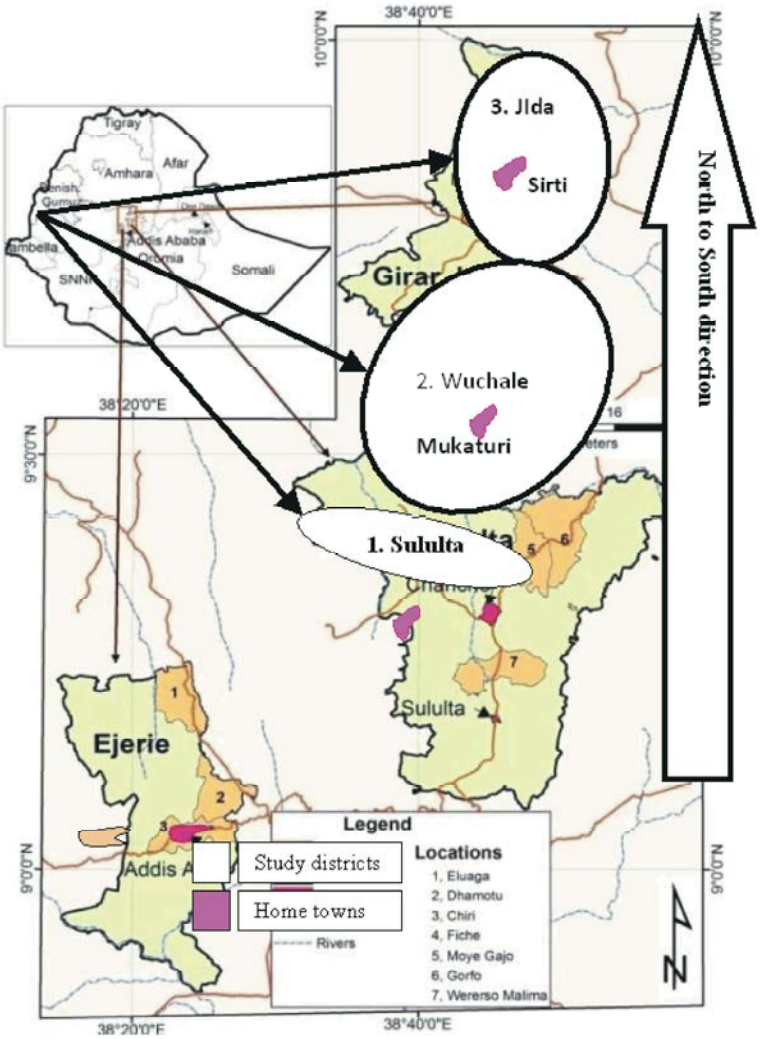

Fig. 1: The three selected districts of the study area (Selale) having five sampling points: Chancho from Sululta (1); Mukaturi, Gumbichu and Gebriel-Gutefrom Wuchale(2) and Sirti from Jida(3) Source: Feyissa *et al.* [16]

communal grazing and marketing areas, Veterinary clinics, Peasant Associations (PAs) in collaboration with the animal health personnel of the respective district.

Study Design and Sample Size: A cross-sectional study design based on serological survey was conducted from December, 2015 to May, 2016 on the study population prevailing in the study area. Sampling points within the three districts of the study site were selected a purposivey in collaboration with the animal health personnel of the respective district on the basis of participant cooperation, logistics, the farmer's share of communal grazing land and accessibility. All the horses sampled for this study were those kept under a traditional management system of free grazing. The total number of horses required for this study is calculated based on the formula given by Thrusfield [20]. The minimum sample size, which enables me to detect at least one diseased animal in the population, was calculated as follows [20].

$$n = (1-(1-\alpha)^{1/d}) \times [(N-d/2) +1]$$

where:

n = Required sample size

α = Is the desired confidence level (That is the probability of finding at least one seropositive in the sample)

N = Population size of equines in a given district

d = Prevalence (Expected) of the disease when it exists in a population x N (Population size).

Fig. 2: Mixing of the diluted serum with the buffered antigen drop on the test card.
Source: Own camera photo captured during serological examination at CVMAVLIR Ethio- Belgium-funded PhD project laboratory.

Considering α (Confidence level) to be 99%, an average total population of 10,000 horses in a district and 5% prevalence of the disease when it exists in a population, the minimum sample size which enables us to detect at least one diseased animal in the population was calculated to be 58 and hence a total of 174 animals were required for sampling, but 176 horses (109 male and 67 female)were sampled randomly and examined from the three selected districts (Involving five different sampling points) of the study area.

Clinical Observation and Interview: During sampling, the animals were observed and checked for any clinically suggestive or pathognomonic signs of dourine such as cutaneous plaques (Abnormal patches grown inside the skin), swelling of the genitalia, marked emaciation and neurological signs. The animals were also recorded for their body condition score (BCS), estimated age, sex and origin (District). The age groups were categorized as young, adult and old, which referred to animals of under 5 years old; 5-9 years old and older than 10 years respectively [21]. Poor referred to very thin and thin body condition scales (BCS of 1-3). Moderate referred to less thin, moderate and moderately fleshy body condition scales (BCS of 4-6); whereas good body condition referred to, fleshy, fat and extremely fat body condition scales (BCS of 7-9) [22]. The animal owners, clinicians and veterinarians in the respective districts were interviewed for information regarding any case reported or recorded with such suggestive clinical signs of the disease.

Sample Collection and Storage Methods: 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 labeled and the blood was allowed to clot overnight at room temperature before the serum was separated and collected into its container. The serum samples were then stored in sterile polypropylene cryogenic vials at -20°C and transported under cold-chain using cool-box (Ice box) according to OIE [23] to the diagnostic laboratory until they are tested using a card agglutination test for trypanosomosis called CATT/ *T. evansi*.

Serological Survey

CATT/ T. Evansi: The sample testing (Screening) on investigation of *T. equiperdum* infection was conducted at the Bishoftu CVMA Ethio-Belgium VLRI-funded PhD project laboratory, which was established by a Ethiopian and Belgian 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* infection (Dourine).

CATT/ *T. evansi* is a rapid, direct card agglutination test which uses formaldehyde fixed, Coomassie stained, freeze-dried antigen of *T. evansi* VAT RoTat 1.2 [23]. In the CATT/ *T. evansi*, 50 µl of serum was diluted with PBS (Phosphate buffered saline solution) and was mixed with 50 µl of the reagent (CATT antigen) on a test card; spread over approximately 1.5 cm and shaken with an electrical rotator (Brought from Belgium, ITM, Antwerp) at 70 rotations per minute for 5 minutes [24]. 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 [25].

Data Analysis: All the data were analyzed by using the computer statistical program called Statistical Package for Social Science (SPSS) version 20 [26]. The differences in the serological survey results between animal groups of different sex, age, body condition score and among the study districts were statistically analyzed using the chi-square (x^2) test for significance [27]. A P-value of < 0.05 was taken to be statistically significant with 95% confidence interval.

RESULTS

Results of Clinical Observation and Interview: The results of the interview held with animal owners and veterinary professionals in the clinic of the respective districts and clinical observation of horses revealed the presence of some suggestive signs of dourine such as marked emaciation, weakness and incoordination or asymmetrical posture (Annex 4; Picture 8.4.1). The occurrence of dourine in Jida district was suspected and announced by the veterinary professionals; who found it to be a newly observed disease in a horse brought to the veterinary clinic in Sirti, home town of the district. The veterinary professionals informed that the infection was a chronic case with subabdomial oedema which retained resistant for any of the available therapeuticals in the clinic; but got a relief up on treatment with a trypanocidal agent, Veriben (*Diminazin aceturate*).

The cutaneous form of the disease, which is mainly characterized by raised, edematous skin plaques sometimes called 'silver dollar plaques' marked on the skin, was also observed on the skin of the lower parts of the abdomen (Figure 3).

Results Of Serological Survey: As the CATT/T.evansi principally detects IgM (Agglutinating pentavalentimmunoglobulins, the half-life of which is short) [23]. The findings of the present survey suggested that there were either early infections, or late infections with recent circulation of parasites (Antibodies against *T. equiperdum*) in the horses' blood.

The results of the employed serological survey (CATT/*T.evansi*) for *T. equiperdum* regardless of the difficulties in differentiating between the infections caused by *T. equiperdum* and *T.evansi*, revealed that 11.9% of the tested animals were seropositive for *T.equiperdum* infection. This seropositivity of antibodies against *T. equiperdum* infection is the first to disclose the introduction and establishment of *T. equiperdum* infection (Dourine) in the horses of Selale.

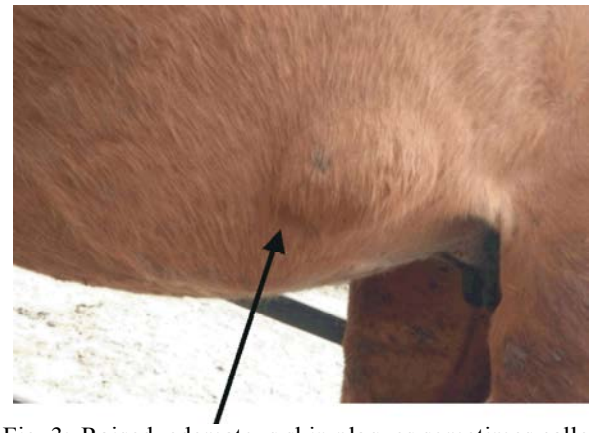

Fig. 3: Raised, edematous skin plaques sometimes called 'silver dollar plaques', on the skin of the lower parts of the abdomen which are often regarded as pathognomonic symptoms for dourine (Camera photo captured during sampling from Jida (Sirti) Veterinary clinic).

With regard to the sex ofthe horses, the seropositivity on the CATT/*T. evansi* was found to be (13.5%) and (9.2%) for male and female horses, respectively. The seropositivity among the three study districts was found to be 18.3%, 12.1% and 5.2% for Sululta (Chancho),Wuchale (Gumbichu, Mukaturi and Gebrielgute) and Jida (Sirti) districts with their sampling points, respectively. Among the age groups, theseropositive results varied between 10.0% (young) to 12.4% (Old). On the basis of BCS, the CATT/ *T.evansi*-positive result was in the range from 9.4% to14.3% representing animals of poor and good BCS, respectively. Generally, there was no statistically significant dependence of seropositivity on the suspected risk factors.

The presence of serum antibodies produced against *T.equiperdum* infection (The parasite antigens) was revealed by macroscopic agglutination reaction formed on the circular zone of the test card (Figure 4 and Annex 3; Picture 8. 3.1).

The following tables indicate the results of serological survey conducted on investigation of dourine (*T.equiperdum* infection) in association with their respective risk factors:

Pearson's chi-square (*x2*) test for serological occurrence of antibodies against *T.equiperdum* infection among the districts revealed that the variation in seropositivity was statistically insignificant (P >0.05).

In the above table, poor referred to very thin and thin body condition scales (BCS of 1-3). Moderate referred to less thin, moderate and moderately fleshy body condition

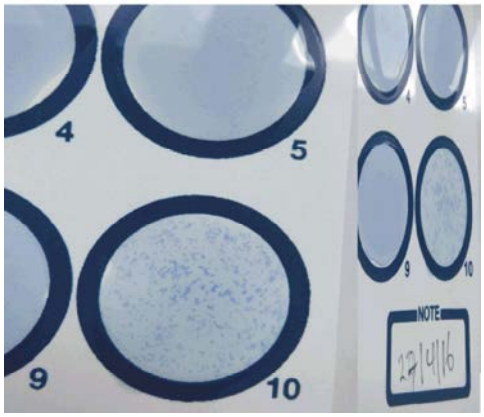

Fig. 4: A visible agglutination reaction formed on circle 10 of the test card (Camera photo captured as of April 27, 2016 during serological survey by CATT/*T. evansi*)

Table 1: Results of serological survey (CATT/*T. evansi*) on investigation of *T. equiperdum* infection by the study districts.

Study District	Nº. of samples tested	Nº. (%) of seropositive samples	P-value
Sululta/Chancho	60	11(18.3)	
Wuchale/Mukaturi	58	7(12.1)	0.088
Jida/Sirti	58	3(5.2)	
Total	176	21(11.9)	

Table 2: The results of serological survey (CATT/*T. evansi*) on investigation of *T. equiperdum* infection among the animal's body condition score (BCS)

BCS	Nº. of samples tested	Nº. (%) of seropositive samples	P-value
Good	35	5(14.3)	
Moderate	77	10(13.0)	0.717
Poor	64	6(9.4)	
Total	176	21(11.9)	

Table 3: The results of serological survey (CATT/*T.evansi*) on investigation of *T.equiperdum* infection by age groups

Age group	Nº. of samples tested	Nº. (%) of seropositive samples	P-value
Young	10	1(10.0)	
Adult	69	8(11.6)	0.970
Old	97	12(12.4)	
Total	176	21(11.9)	

Table 4: The results of serological survey (CATT/*T.evansi*) on investigation of *T.equiperdum* infection by gender

Sex	Nº. of samples tested	Nº (%) of seropositive samples	P-value
Male	111	15(13.5)	
Female	65	6(9.2)	0.398
Total	176	21(11.9)	

scales (BCS of 4-6), whereas good body condition referred to fleshy, fat and extremely fat body condition scales (BCS of 7-9) [22]. Pearson's chi-square (*x2*) test revealed that variation in seropositivity among animal groups of different BCS was statistically insignificant (P> 0.05).

Young, adult and old referred to animals of under 5years old; 5-9 years old; and ≥10 years old respectively [21]. Pearson's chi-square (*x2)* test revealed that variation in seropositivity among the animals of different age groups was statistically insignificant (P> 0.05).

Chi-square *(x^2)* testforseropositivity between the sex groups revealed that the variation on the serological survey results among the groups was statistically insignificant (P >0.05).

DISCUSSION

Regardless of the difficulties in differentiating between *T. equiperdum* and *T. evansi* [28] the findings of the present serological survey in association with the

observed indicative clinical findings, disclosed that there was a valuable *T. equiperdum* infection (Dourine) in the horses of Selale. As CATT/ *T. evansi* principally detects IgM (Agglutinating pentavalentimmunoglobulins the half-life of which is short [23] the results of the present survey also pointed out that there were either early infections, or late infections with recent circulation of the parasite (*T. equiperdum*) in the horses' blood.

The overall seropositivity of antibodies against *T. equiperdum* infection was found to be 11.9% by using CATT/ *T. evansi*. This serological occurrence is the first report to disclose the introduction of *T. equiperdum* infection (Dourine) in the horses of Selale. This a relatively high and an alarming serological occurrence found through clinical observation, interview-based and serological survey (By CATT/ *T. evansi*), provided a strong circumstantial evidence that dourine which was previously known to occur only in the horses of Arsi-Bale highlands of Ethiopia [11] is slowly spreading to the adjacent areas of the country.

This seropositivity was in argument with the previous report based on CATT/ *T. evansi*, LATEX/ *T. evansi* and ELISA/ *T. evansi* serological tests in the same area, which so far reported that *T. equiperdum* infection was not present in the area [15]. The result of present serological survey was comparable with that of Hagos *et al.* [25] who reported an overall seropositivity (On CATT/*T. evansi*) of 19.66% in dourine-infected horses of Bale highlands.

Among study districts, the horses of Sululta (Chancho) and Jida (Sirti) showed highest (18.3%) and lowest (5.2%) positive results, respectively in the serological survey. This could be attributed to the variation among the chance of exposure (Sexual contact) to newly introduced infected animals. The animals of good body condition showed a relatively higher (14.3%) serological survey results than that of poor body condition (9.4%). Thus, it is possible to suggest that the animals under good body condition had a relatively higher number of previous mating and genital contacts, which possibly increases chance of acquiring theinfection from an infected or carrier host [12]. The differences in seropositivity among male (13.5%) and female (9.2%), respectively could be associated with the number of animals sampled (Male, n = 111and female, n = 65), which might increase the chance of obtaining seropositive animals.

On the contrary, the overall result of the present serological survey was not affected by sex, age, animal origin and body condition status in general. This revealed that there was no statistically significant variation observed (P>0.05) when the serological occurrence of trypanosomal antibodies on the CATT/*T. evansi* was compared among the examined animals with respect to sex, age, body condition score and study district. Thisis inline withthe report of Hagos *et al*. [12] this might indicate that the animal groups of different sex, age, body condition and along the study districts were equally exposed to the parasite and this can be an indicator for the uniform spread of the disease.The uniform distribution of the infection in this area could be attributed to the unrestricted animal movement from neighboring districts for trade and transport purposes and or to uncontrolled animal breeding and is in agreement with that discussed by Hagos [11].

As the distribution of dourine is not restricted by environmental factors and it is possible through unrestricted movement of infected animals for the infection to be become established almost anywhere. A large number of horses are constantly purchased from the Arsi-Bale highlands and transported into adjacent highlands of Ethiopia by local merchants for trade purpose.This might due to the fact that the Arsi-Bale highlands are known for equine breeding [11]. Therefore, the present serological occurrence of the disease in the study area is possibly through unrestricted movement of infected animals in to the present site.

As there is no concrete information available on the prevalence, extent as well as distribution of either *T. evansi* or *T. brucei* in horses in different parts of the country, it possible that the existence of the present seropositive cases might be cross reactions with other members of the subgenus *Trypanozoon*. However, *T. brucei* is exclusive for this condition due to the fact that its distribution requires the presence of tse-tse flies. In areas where *T. equiperdum* or *T.cruzi* are present, cross-reactions may occur with any serological test employed. In such situations, the exact status of an animal regarding trypanosomosis cannot be established [6].

In horses, the clinical signs common to dourine, such as incoordination, especially of the hindquarters, marked emaciation (Poor body condition) and cutaneous plaques (Swellings), which are regarded as important symptoms [29] were observed during this study. However, in recent dourine infections, edematous plaques may not be observed; but yet in certain surra infections these cutaneous plaques may be observed. Hence, these plaques and other observed clinical findings should not be considered to be pathognomonic for dourine, but rather as an indicative and forewardingsymptoms [30].

So far, 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*. The limitations and difficulties of the serological tests to differentiate *Trypanozoon* infections in solipeds due to*T.equiperdum, T.evansi and T.b.brucei*have beendescribed by Hoare [29] and hence 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) makes a confusion.

Recently, it has also 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).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.

CONCLUSION AND RECOMMENDATIONS

The findings of the present serological survey in association with the observed indicative clinical findings, disclosed that there was a valuable *T.equiperdum* infection (Dourine) in the horses of Selale.

Therefore, the following recommendations are worth mentioning:

- Further detailed studies and diagnostic methods like Latex agglutination test, ELISA should be conducted to clarify the confusion among the infections due to *T. equiperdum* (Dourine) and *T. evansi* (Surra) and to explore the possibility of more sensitive and specific molecular diagnostic techniques of *T. equiperdum* or *T. evansi*.
- The conditions for importation and movement of animals from infected to non-infected areas should be defined; including the status of the exporting farm (Merchants), exported animals, the diagnostic protocol, possibly the preventive administration of curative treatments.
- Education should be provided for the community and animal health Professionals in order to enhance their awareness towards the severity, mode of transmission and economic impact of dourine on working equines.

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