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A CROSS SECTIONAL STUDY OF DOURINE IN SELECTED HORSE BREEDING DISTRICTS OF BALE HIGHLANDS OF OROMIA REGIONAL STATE OF ETHIOPIA

By

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LIST OF ABBREVIATIONS

AHS	African Horse Sickness
AI	Artificial Insemination
ARDU	Arsi Rural Development Unit
CATT	Card Agglutination Test for Trypanosomosis
CFT	Complement Fixation Test
DVM	Doctor of Veterinary Medicine
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
IFAT	Indirect Fluorescent Antibody Test
ISMM	Isomethamidium Chloride
IgM	Immunoglobulin M
kDNA	Kinetoplast Diribonuclic Acid
MAECT	Miniature Anion Exchange Centrifugation Test
MAFF	Ministry of Agriculture, Fisheries and Food
MDH	Malate Dehydrogenase
HCT	Haematocrit Test
NTTAT	Non-Tsetse Transmitted Animal Trypanosomes
OIE	World Organization for Animal Health
PCR	Polymerase chain Reaction
rDNA	Ribosomal Deoxyribonucleic Acid
RFLPs	Restriction Fragment Length Polymorphism
RoTat	Rhode Trypanosoma Antigenic Type
VAT	Variable antigen type
VSG	Variable Surface Glycoproteins
µm	Micrometer

ABSTRACT

A cross sectional study comprising serological and questionnaire survey was conducted with the objective of determining the prevalence, extent and distribution of Dourine in selected horse breeding districts of Bale highlands of Oromia Regional State of Ethiopia from September 2007 to May 2008. Parasitological survey in an attempt to determine the parasitological prevalence and to isolate *T. equiperdum* in 71 purposively selected clinical Dourine cases revealed no trypanosomes by Giemsa staining or by Haematocrit Centrifugation Technique (HCT). A total of 880 sera were tested for the detection of antibodies against the causative agent of Dourine in four selected horse breeding districts of the Bale highlands of Ethiopia. Accordingly, the sero prevalence of Dourine was found to be 140 (15.90 %) and 173 (19.66 %) for CATT / *T. evansi* and LATEX / *T. evansi* tests, respectively. Body condition and parity number were found to have significant difference ($p < 0.05$) on the sero prevalence of the disease based on the CATT/ *T. evansi* test only. However, age, sex, history of previous abortion and castration status no significant difference ($p > 0.05$) on the sero prevalence of the disease using CATT/ *T. evansi* test. On the other hand neither of the explanatory variables had significant difference ($p > 0.05$) using LATEX/ *T. evansi* test. Logistic regression analysis revealed that horses without parity number (0) had an OR of 1.80 (1.02, 3.19) positivity compared to horses with parity number (1 and above); and horses with poor body condition (thin and very thin) had an OR of 1.44 (1.00, 2.08); positivity compared to horses with moderate body condition (less thin, less moderate and moderate) scores, on the basis of CATT/ *T. evansi* test. The questionnaire survey indicated that dourine is the major health problem of horses in the selected horse breeding districts of Bale highlands of Ethiopia and locally known as “Derisa” in Oromifa language and “Tigen” in Amharic language that causes high mortality and economic loss in selected horse breeding districts of Bale highlands of Ethiopia. It is a common clinical case throughout the year, however the disease has a seasonal character, which most commonly occurs whenever animals are in a good body condition and following the breeding season from April to late September and during dry season of the year (January to March), which probably associated with the relapse of previously infected and recovered cases due to the stressful condition of feed shortage. Uncontrolled breeding, unrestricted animal movement and absence of officially approved effective trypanocidal drugs against dourine cases are the main factors that play an important role in the distribution and transmission of the disease.

Even though there was no direct parasitological detection of *T. equiperdum*, the result of serological survey conducted by using CATT / *T. evansi* and LATEX / *T. evansi* tests together with the questionnaire survey, provides strong evidence that Dourine is highly prevalent, most important problem in selected horse breeding districts of Bale highlands of Ethiopia, where the problem of dourine is still unsolved. Further more, 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 than is currently realized.

Key words: Bale highlands, CATT/ *T. evansi*, Dourine, Ethiopia, Haematocrit Centrifugation Technique, Horses, LATEX/ *T. evansi*, Sero prevalence.

1. INTRODUCTION

Ethiopia has a very large equine population, approximately 5.2 million donkeys, 2.8 million horses and 0.65 million mules in Africa. Most equines are found in Zones of high human population, with 30 % of all equines being found in only 8 % area of the central highland. Equines are extremely important in the Ethiopian agriculture and for the national economy. Nearly 90 % of agricultural operations depend on human muscle and because of rugged mountainous terrain of this country these animals are still the main method of transporting both people and agricultural products (EARO, 1999; FAOSTAT, 2003).

In developing countries like Ethiopia the contribution of equines is much diversified. The provisions of transport through pack animals, drawing carts, as riding animals or taxi operations almost definitely contributes more to national economy (Feseha, 1993).

African horse sickness, anthrax, dourine, epizootic lymphangitis, glanders, equine piroplasmosis, horse mange, rabies and ulcerative lymphangitis are the major diseases known to affect equines and their contribution, among which dourine is the most complaint of the equine breeders (FAO, 1996). Of the Non-Tsetse Transmitted African Trypanosomsomes (NTTAT), dourine is the only trypanosomosis that is not transmitted by an invertebrates vector and *T. equiperdum* differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood (OIE, 2001).

Local farmers have recognized and indicated the extent and problem of dourine in Arsi-Bale highlands of Ethiopia for many years and it has been found to be a threat to the life and productivity of equine population in the endemic areas. However, the first official report of the disease was made in 1980 when the Arsi Rural Development Unit (ARDU) requested the Tsetse and Trypanosomiasis Survey and Control Department to investigate a persistent disease problem in horses in the administrative regions of Arsi and Bale (Zeleke *et al.*, 1980).

According to this report, the disease was widely spread in Ethaya, Sagure, Bekoji and Kofele districts of Arsi-Bale highlands. In those areas, the disease is known as “Lappesa Hidakta” “Lappessa dugda kuta” which means back bone breaker in Vernacular language or simply “Kuta” which means breaker.

Since then dourine was found to be prevalent in the highlands of Ethiopia particularly in Arsi and Bale zones (Alemu *et al*., 1997). Similarly, multiple cases of serological complement fixation test (CFT) and enzyme linked Immunosorbent assay (ELISA) and Trypanozoon polymerase chain reaction (PCR) positive, yet aparasitemic horses were reported in Arsi and Bale zones of Ethiopia (Clausen *et al*., 2003). Recent study carried out in Arsi Bale highlands, shows the prevalence of dourine 184 (28.35%), 161(24.81%) and 125 (19.26%) for CATT/ *T. evansi*, LATEX/ *T. evansi* and ELISA/ *T. evansi* tests respectively (Hagos, 2005) (Annex 1).

However, it has been reported that the occurrence of dourine outside of the previously known endemic foci in adjacent geographical areas to Arsi-Bale highlands was established and confirmed for the first time in neighboring areas namely in Sidama zone of the Arbegona district, East Shoa zone of the Shashemane district and Guji zone of the Uraga district. Dourine is spreading and becoming a potential threat to equine in the geographically adjacent areas through trade and unrestricted movement of animals outside of the endemic foci. So dourine is known which by default a disease occurring only in the Arsi-Bale highlands is getting spreading and becoming a potential threat to the equines in the geographically adjacent areas through trade and unrestricted movement of animals outside of the endemic foci (Hagos, 2005) (Annex2).

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 (Bishop *et al*., 1995).

The main reason for using serological tests for the diagnosis of trypanosomosis is to overcome the low sensitivity of parasitological tests in detecting chronic infection.

The difficulty in diagnosis of *T. equiperdum* lead to difficulties in achieving reliable data on the prevalence, distribution, implementation, monitoring, treatment and control of the disease programs.

On top of that there are other limitations such as trypanocidal drug shortage, absence of vaccines against dourine, which play an influencing role in controlling, and prevention of the disease in an endemic area (Clausen *et al*., 2003).

In view of the large number of horse population in Ethiopia and lack of adequate facilities for diagnosis and control of the disease in relation to breeding, dourine is potentially a very important disease. Moreover, in many districts of Bale highlands studies on the prevalence of dourine has not yet been conducted. Moreover, there was no evidence of *T. evansi* infection in Agarfa, Dinsho, Goba and Sinana districts. However, adjacent districts in the Bale lowlands such as Barebre, Dello-Mena and Harena-Buluk were known to be endemic for surra (camel trypanosomosis due to *T. evansi*).

Therefore, the present study was intended to meet the following objectives:

- To determine the sero-prevalence of dourine in four selected horse breeding districts of Bale highlands of Oromia regional State Ethiopia based on serological and questionnaire survey
- To determine the parasitological prevalence of the disease as well as to isolate the causative agent of the disease (*T. equiperdum*)
- To determine risk factors affecting for the observed sero-prevalence of the disease (dourine).

2. LITRATURE REVIEW

2.1. Disease description

Dourine is a chronic or acute contagious disease of breeding solipeds that is transmitted directly from animal to animal during coitus. Among the non-tsetse transmitted trypanosomes (NTTAT), dourine is the only trypanosomiasis that is not transmitted by an invertebrate vector. *T. equiperdum* differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood (OIE, 2001).

Dourine is also known under other different names (languages) in different countries. In Arabic: “el Dourine”, in English: “Covering disease”, in Germany: “Beschalseuche”, in French “Mal de coit” and in Russia: “Slucnaja Bolezni” or “Podsedal” (Hoare, 1972).

2.2. Etiology

The name *T. equiperdum* was postulate by Doflein in 1901. *T equiperdum* is a member of non-tsetse-transmitted trypanosome group, which is found outside the tsetse fly (Glosina species) and it is the causal organism of dourine. *T. equiperdum* is a protozoan parasite, morphologically and serologically related to *T. brucei*, *T. rhodensiese T. evansi* and *T. gambiense*. The causative organisms, Trypanosomes, which strictly parasitic are flagellar protozoa that belong to phylum of Sarcomastigophora, the order of Kinetoplastidae, the family of Trypanosomatidae and the genus of Trypanosoma, under the Salivarian group. The subgenus Trypanozoon includes the pathogenic species *T. evansi*, *T. brucei* and *T. equiperdum* as indicated below in the table 1 and 2. *T. brucei* is further divided into three subspecies, the animal pathogen *T. b. brucei* and the trypanosomes responsible for human sleeping sickness, *T. b. gambiense* and *T. b. rhodesiense* (Hoare, 1972).

2.3. Taxonomy

Taxonomy is the classification and orderly arrangement of living organisms according to their structure and shape (morphology), their biological development (life cycles) and, more recently, their molecular structure, particularly that of their genome molecular taxonomy. Nomenclature is the system of naming organisms based on their classification.

Trypanosomes are unicellular organisms (Phylum Protozoa) belonging to the genus *Trypanosoma*, the family Trypanosomatidae and the order Kinetoplastida. Each species is furthermore given two names, the first, always spelled with a capital letter, places it in a relatively small group known as the genus (plural genera) and the last name, spelled with a small letter, indicates the species (Hoare, 1972).

Table 1: Taxonomy of *Trypanosoma equiperdum*

Phylum	Protozoa
Class	Zoomastigophera
Order	Kinetoplastidae
Genus	Trypanosoma
Subgenus	Trypanozoon (Brucei group)
Species	*T. brucei, T.evansi, T.equiperdum*
Subspecies	*T. brucei, T. b. gambiense, T.b. rhodesiense*

Source: Hoare (1972).

Table 2: Classification of the Trypanozoon subgenus based on the biological parameter

Parameters	*T. brucei*	*T. evansi*	*T. equiperdum*
Disease	Nagana	Surra	Dourine
Course	Acute to chronic	Acute to chronic	Acute to chronic
Clinical signs	Anemia,fever, edema, urticarial plaques	Anemia,fever,edema, urticarial plaques	Anemia, fever, edema, paralysis and edematous cutaneous plaques
Transmission	Cyclical (tsetse fly)	Mechanical (biting flies)	Sexual
Host	Multiple	Multiple	Equines
Diagnosis:			
Parasitology	mHCT	MAECT	None
Serology	IFAT, ELISA	CATT /*T. evansi*/ LATEX /*T. evansi*/ ELISA, Trypanolysis RoTat-1.2.	CFT
PCR	PCR /Trypanozoon/	PCR/Trypanozoon/	PCR /Trypanozoon/
Treatment	Various drugs	Various drugs	None

Source: Claes (2003).

2.4. Morphology

Trypanosoma equiperdum is also morphologically identical to the blood stream slender form of *T. brucei* and *T. evansi*. Like the later species, it is typically monomorphic, being represented by thin (slender and intermediate) trypanomastigotes possessing a free flagellum. Although pleomorphic, stumpy, protonucear forms are known. *T. equiperdum* differs from other mammalian trypanosomes also in the fact that it is primarily a tissue parasite, which rarely invades the blood. Due to these peculiarities the morphology also differs from other trypanosomes except those two trypanosomes subspecies in the Trypanozoon (*T. brucei* and *T. evansi*) are animal pathogens highly virulent in horses and morphologically nearly indistinguishable (Stephen, 1986).

Trypanosoma equiperdum is closely related to *T. evansi* and more distantly to *T. brucei*, because *T. evansi* was originally a parasite of camels and only later spread to horses. In *T. equiperdum* the nucleus lies in the center of the body, the Kinetoplast is more distinct and terminal or sub terminal in position, with well developed undulating membrane and free flagellum as in *T. evansi*. The size of *T. equiperdum* is like wise between the range of *T. evansi*, with the length of different strains varying from 15.6 μm - 31.3 μm and 1.5 μm - 2.2 μm width (Hoare, 1972). *T. equiperdum* also infects equines under natural conditions, and causes a venerally transmitted disease called dourine. Biochemical and molecular biological data indicated that *T. evansi* and *T. equiperdum* are much more closely related to each other than to *T. brucei brucei*. The two species cannot be distinguished morphologically at the light or electron microscope level. However, based on the differences in the mode of transmission, the host range, the pathogenicity, and the location of the parasite in its host, these two trypanosomes were classified as different species (Hoare, 1972).

Table 3: Comparison of the general characteristics of *T. evansi* and *T. equiperdum*

Character	*T. evansi*	*T. equiperdum*
Size	15-36 µm with free flagellum	15.6-31.3 µm
Morphology	Typically monomorphic, pleomorphic forms in some strains.	Typically monomorphic, Pleomorphic forms in some strains.
Natural hosts	Equines, camels, cattle, buffalo, deer, Asian elephant, tigers and vampire bats.	Equines only.
Vectors and transmission	Tabanus species, Stomoxys species (mechanical transmission).	Direct transmission during coitus.
Drugs used for treatment	Diminazine, Suaramin, Quinapyramine and Cymelarsan.	Identical to *T. evansi*, usually not recommended
Isoenzymes	Differences were found in two MDH and ALAT of 16 enzymes between strains of *T. evansi* and *T. equiperdum*.	-
KDNA and hybridization with PTK420	Minicircles only	Minicircles hybridized with PTK420 Maxcircles also present
RFLPs in rDNAs and VSG genes.	No differences were found	-

Source: Brun (1998).

2.5. Epidemiology

2.5.1. Host range and distribution

Dourine mainly affects horses, donkeys and mules. The disease is totally more sever in improved breeds of horses and milder in native ponies, donkeys and mules. Usually male donkeys and mules are thought as carriers. The organism has been adapted to a variety of laboratory animals including rats can also be infected (Theis *et al.*, 1980; Losos, 1986). Zebras have been tested positive by serology, but there is no conclusive evidence of infection. Horses and donkeys appear to be the only natural reservoirs for *T. equiperdum*. Male donkeys can be asymptomatic carriers (OIE, 2001). Host range is limited to equines and because the transmission depends on sexual compatibility. As dourine does not depend on vectors and their ecology, its epidemiology is simpler than the cases of insect borne trypanosomiasis. The spread of dourine depend primary on the conditions convenient for the transfer of *T. equiperdum* from one animal to another (Luckins, 1994).

Trypanosoma equiperdum infection as a venereal disease is even less restricted by climate and in the past has spread as far as Canada and Russia in the northern hemisphere, and as far to the south as Chile and South Africa. Its present distribution is not very well known; *T. equiperdum* is sometimes difficult to distinguish from *T. evansi*. It has been eradicated from North America and most of Europe. It is certainly present in northern and southern Africa and in tropical Africa at least in Ethiopia and probably in Sudan. It has made a comeback or perhaps has been rediscovered in Europe (Italy, Russia, possibly other countries), and is still present in parts of Asia, including Uzbekistan and China. It is also believed to be still present in parts of South America, but there is little reliable information. Dourine was once spread, but has been eradicated from a number of countries. Currently the disease is endemic in most Asia, northern and southern Africa (Caporale *et al*., 1980). The latest official reports of dourine (i.e. CFT positive cases) were in China, Kazakhstan, Kyrgyzstan, Pakistan, Ethiopia, Botswana, South Africa, Brazil, Italy and Germany (OIE, 2000, Zablotskij *et al*., 2003) (Annex 4).

In Ethiopia particularly in Arsi-Bale highlands dourine was found to be prevalent and a great threat to the equine population in these areas. However the first official report of the disease was made in the 1980 when the Arsi rural development unit (ARDU) requested the tsetse and trypanosomosis survey and control department to investigate a persistent disease problem in horses in the administrative region of Arsi-Bale. According to this report, the disease was widely spread in Itaya, Sagure, Bekoji and Kofele districts of Arsi-Bale highland. In these areas the disease is known as "Lappessa Hida kuta", "Lappessa Dugda kuta" which means back bone breaker vernacularly or simply "Kuta" which means back bone breaker, whilst in Bale region known as "Derissa," (Zeleke *et al*., 1980; Hagos, 2005). According to recent study carried out in Arsi-Bale highlands, out of 649 horse sera tested for the detection of antibodies against *T. equiperdum* 184 (28.35%), 161 (24.81%) and 125 (19.26%) samples were found to be seropositive for CATT/ *T. evansi*, LATEX/ *T. evansi* and ELISA tests, respectively (Hagos, 2005) (Annex 1).

In view of the large number of horses in Ethiopia, the un restricted movement of animals through out the country for trade and transport purpose, lack of adequate facilities for diagnosis and control of the disease in relation to breeding, it is likely that dourine may have much wider distribution than the mentioned areas, especially in places, where there are high equine population. The occurrence of dourine for the first time also has been established in areas outside of the endemic foci, in Uraga, Arbegona and Shashamane districts through purchase and unrestricted movement of animals from Arsi-Bale highlands for trade purpose (Hagos, 2005).

2.5.2. Mode of transmission

Since the transmission of dourine does not require insect vectors that are influenced by climatic factors, the disease may, in principle, been found anywhere. Almost exclusively coitus spreads this venereal disease. Organisms are present in the urethra of infected stallions and in vaginal discharges of infected mares. The organism may pass through intact mucous membranes to infect the new host. Infected animals do not transmit the infection with every sexual encounter.

As the disease progresses, trypanosomes periodically disappear from the urethra or vagina. During these periods the animals are non- infective. Non-infective periods may last for weeks or months and are more likely to occur in the later stages of the disease. Thus, transmission is most likely early in the disease process. It is possible for mares to become infected and pregnant after mating with an infected stallion. Foals born to infected mares may be infected. But it is unclear if this occurs in uterus or during birth. Because of trypanosomes may occur in the milk of infected mares, these foals may be infected per os during birth or by ingestion of infected milk. Foals infected in this way may transmit the disease when mature and develop a lifelong positive CF titer. However, this method of disease transmission is rare. Some foals may acquire passive immunity from colostrum of infected mares without becoming actively infected. In such foals the CF titer declines and the animal becomes sero negative by 4 to 7 months of age. Although the possibility of non-coital transmission remains uncertain, it is supported by sporadic infections in sexually immature equids (Barrowman, 1976; Caporale *et al.,* 1980; Wang, 1988).

Human carelessness may also be responsible for conveying the infection, for instance when contaminated utensils are used for grooming the horses or contaminated instruments are used for artificial insemination. Other means of transmission may also be possible, but there is no evidence that arthropod vectors play any role in transmission. Animal other than equines can be infected experimentally (OIE, 2001).

2.5.3. Pathogenesis and pathology

Dourine is the only form of trypanosomiasis, which is transmitted directly from one animal host to another of the same species without the intervention of an insect vector. *T. equiperdum* differs from other mammalian trypanosomes also in the fact that is primarily a tissue parasite. Therefore most of its clinical manifestations are the result of the histoparasitism of *T. equiperdum*, especially for the mucosa of the genital organs and for cutaneous tissue. It is thought that, when the parasites invade the tissue they cause vasomotor disturbances with exudation of the plasma and an inflammatory reaction at the site of irritation, giving rise to the edematous swelling and plaques (Hoare, 1972).

The pathological effect has been attributed to the secretion of the toxin. The toxin elaborated in these lesions is carried away through the bloodstream, causing inflammation and degeneration of the peripheral nerves. Likewise, the sudden death of infected rodents at the height of parasitaemia was due to the release of toxins into the circulation. According to this view, the motor and sensory disturbances in the later phases of the disease are the direct result of these changes, while the atrophy of the muscles due to the damaged of nerves serve the emaciation of the animals (Watson, 1920).

Generally the first symptoms of dourine may appear in the genitalia between one week and 3-4 months after infection, when the parasites are localized in the mucous membrane and edematous of equines of both sexes. About 30 days later the trypanosomes enter to the bloodstream, which they are carried into the various parts of the body and invade the skin giving rise about 40-60 days after the onset of the infection to the characteristic urticarial plaques, which may appear and disappear at irregular intervals (Hoare, 1972).

The second stage of the dourine is characterized by anemia and nervous disorders, manifested chiefly by paralysis of the hind limbs (Hoare, 1972). Though as a rule dourine is a fatal disease with an average mortality of 50 %, especially in the stallions. Infected animals some times recover spontaneously (OIE, 2001). Finally dourine is characterized by progressive anemia, disorders of the nervous system, mainly paralysis of the hind legs and paraplegia and at the end death (Stephen, 1986).

2.5.4. Immunity

Dourine may run a symptomless course in native free-range horses, and that horses of different breeds, as well as individual animals, vary in their susceptibility to the disease. Donkey and mules are more resistant to the infection than horses. Infected animals produce antibodies to successive antigenic variants (OIE, 2001). It would therefore seem that in addition to certain degree of individual immunity, equines might possess some racial (ethnic) immunity probably acquired naturally through prolonged contact with the disease.

The chronic and often prolonged courses of the disease as well as cases of spontaneous recovery provide evidence of acquired immunity in dourine. In addition to the humoral factors, local phagocytosis plays a part in the immune reaction to infection with *T. equiperdum* (Watson, 1920). In general, Immunity to trypanosomiasis is complicated as *T. equiperdum* has the ability periodically to replace major surface glycoprotein antigens, which is a strategy supporting chronic infections (Buck *et al*., 1984). No method of immunization against dourine exists at present (OIE, 2001).

2.6. Clinical signs

The incubation period is highly variable. Clinical signs usually appear within a few weeks of infection but may not be evident until after several years (Barrowman, 1976 and Mcentee, 1990). The disease is marked by stages of exacerbation, tolerance or relapse, which varies in duration and which may occur once or several times before death or recovery. Clinical signs vary considerably depending on the virulence of the infecting strains, the nutritional status of the infected animal and the presence of other stress factors. Most of the clinical manifestations of dourine are the result of the histoparasitism of the *T. equiperdum* especially for the mucosa of the genital organs and cutaneous tissues. Dourine is characterized mainly by swelling of the genetalia, cutaneous plaques and nervous signs. The earliest signs usually consist of swelling and local oedema of the genital organs. Later, major clinical manifestations such as fever, anemia and neurological symptoms and abortion are observed, especially in the late stage of infection (Stephen, 1986). The symptoms of the disease are variable. Classically, there are three stages.

2.6.1. Genital swelling (edema)

It is the primary phase that occurs in horses infected *with T. equiperdum* and during this stage the genitalia become swollen and in mares there is a discharge from the vagina, and loss of pigment in the mucosa of the vulva or penis; this depigmentation occurs in patches. Slight fever and a loss of appetite may be noticeable. Swelling and edema of the vulva develop later and extend along the perineum to the udder and ventral abdomen. There may be vulvitis and vaginitis with polyuria and other signs of discomfort such as an elevated tail. Abortion is not the

feature of the infection with mild strains, but significant abortion losses may accompany infection with more virulent strains. In stallion, the initial signs are variable.

Edema of the prepuce and glans penis, that spreads to the scrotum, perineum, ventral part of the abdomen and thorax. Paraphymosis may be observed. The swelling may resolve and reappear periodically. Vesicles and ulcers on the genetalia may heal and leave permanent white scars Leukodermic patches (OIE, 2001).

2.6.2. Second phase (Coetaneous plaques)

After a month or so the second stage starts with round urticarial eruptions, plaques (patches), and a pathognomonic sign is the edematous plaques consisting of an elevated lesion in the skin as if a coin has been inserted under the skin up to 5-8 cm in diameter and 1 cm thick. The plaques usually appear over the ribs in both sexes, although they may occur anywhere on the body, and usually persist for between 3 and 7days (OIE, 2001). They are not a constant feature. Watson (1920) stated that skin plaques are rear symptoms and they can be observed in comparatively few cases. These patches do not occur with all strains and have also been observed sporadically in animals infected with *T. evansi*. Conjunctivitis and keratitis are often observed in outbreaks of dourine and may be the first signs noted in some infected horses (Brun *et. al.*, 1998). Plaques on the skin are characteristic of dourine. However, these urticarial plaques do also occur sporadically in animals infected with *T. evansi*. The major difference between the two species is that *T. evansi* is a blood parasite that spreads throughout the whole body, while *T. equiperdum* usually parasitises the tissue and causes local clinical symptoms of the genital organs at the beginning of infection. However, the pathogenicity of the two species is similar, and pathogenesis cannot be used alone to distinguish the two species (Stephen, 1986).

2.6.3. Tertiary phase (Nervous signs)

In the third stage a paralysis sets involving various muscles and spreading to the hind legs causing incoordination. Complete paralysis of the four legs may finally occur. It is the final phase of the disease characterized by nervous disorders and may be seen after the genital edema or may follow by weeks or months.

Initially these signs consist of restlessness and the tendency to shift weight from one leg to another followed by progressive weakness and in coordination and ultimately by paralysis with spasmodic contraction of the hind limbs, recumbency and partial facial paralysis may be seen (Personal observation). Beside these other clinical signs may include anemia, conjunctivitis, keratitis, intermittent fever and emaciation.

Dourine also results in a progressive loss of condition, predisposing animals to other disease (Hoare 1972; Stephen 1986). In general, the third phase of dourine is characterized by progressive anemia, nervous system disorders mainly paralysis of the hind legs and paraplegia and finally death (Stephen, 1986).

2.7. Diagnosis

The diagnosis of dourine is not always easy. A definitive diagnosis depends on the recognition of the clinical signs and the demonstration of the parasite. This is rarely possible because: (a) although the clinical signs and gross lesions in the developed disease may be pathognomonic, they cannot always be identified with certainty, especially in the early stages or in latent cases. They can be confused with other conditions, such as coital exanthema, moreover in some countries (e.g. in South America), *T. evansi* infections give rise to similar clinical signs); (b) the trypanosomes are only sparsely present and are extremely difficult to find, even in edematous areas; and (c) the trypanosomes are only fleetingly present in the blood, and in small numbers that defy detection. For unknown reasons, no parasite strain of *T. equiperdum* has been isolated in any country of the world since 1982 and most of the strains currently available in national veterinary diagnostic laboratories are related to *T. evansi (*Claes *et. al*, 2003). In practice, diagnosis is based on clinical evidence supported by serology. Recently, other approaches have been studied and reported on *(*Claes *et al.*, 2003). In infected animals trypanosomes are present in low numbers only in lymph and edematous fluids of the external genitalia, in the vaginal mucus and fluid contents of plaques. They are usually undetectable in the blood, but may be found in the urethral or vaginal mucus collected from preputial or vaginal washings or scrapings 4-5 days after infection. Later, parasites may be found in the fluid contents of edemas and plaques, especially shortly after their eruption. The skin of the area over the plaque should be washed, shaved and dried, and the fluid contents aspirated by syringe. Blood vessels should be

avoided. The fresh aspirate is examined microscopically for motile trypanosomes. These are present for a few days only so that lesions should be examined at intervals. The parasite is rarely found in thick blood films, but is sometimes detectable after centrifuging blood and examining the re-centrifuged plasma. As dourine is the only trypanosome to affect horses in temperate climates, the observation of trypanosomes in thick blood films is sufficient for a positive diagnosis.

In countries where nagana or surra occur, it is difficult to distinguish *T. equiperdum* microscopically (morphology, motility) from other members of the subgenus Trypanozoon (*T. evansi, T. brucei*). In particular, *T. equiperdum* and *T. evansi* cannot be differentiated on the basis of morphological criteria. Because, both are monomorphic, slender trypomastigotes with a free flagellum, although pleomorphic, stumpy, proteonuclear forms are recognized (Stephen, 1986).

2.7.1. Clinical diagnosis

Clinical symptoms, if typical, can be of great help in an area where the disease is known to occur. In area where dourine is endemic and susceptible animals are exposed to the disease, the clinical signs of dourine such as the edematous swellings, developing of plaques and the nervous signs may enable to suspect the disease. Meanwhile, dourine should be differentiated in the early stages and latent cases (Hoare, 1972) from other diseases such as coital exanthema, which is manifested by the appearance of vesicles, ulcers and depigmented spots on vaginal mucous membrane. These signs are not important in dourine. The differential diagnosis includes Surra, Anthrax, Equine infectious anemia, Equine viral arthritis and purulent endometritis such as contagious metritis should be also considered, as their signs are resembled with dourine signs. In some countries (e.g. in South America) *T. evansi* infection can give rise similar signs (OIE, 2001).

2.7.2. Parasitology

Trypanosomes are not normally found in the circulating blood, but can often be detected in fresh preparations or stained smears from the mucous discharge of vagina or tissue fluid taken from the swollen genitalia or the urticarial plaques. Parasitological diagnosis of dourine in chronically infected horses or donkeys is difficult due to the uncertainty of finding parasites in the tissues and its fleeting presence in the bloodstream. In practice, diagnosis is based on clinical evidence supported by serology (Hoare, 1972).

In the early stages of the disease the parasites are sought for in the vagina of the mare, by microscopical examination of the vaginal washings or exude after scarification of the mucosa with the aid of spatula, and in the scraping of the urethra in the stallion obtained with the aid of a probe.

In the urethral and vaginal mucus trypanosomes are sometimes detectable 4-5 days after the onset of the infection. In the later stages of the disease the parasites may be found in the fluid contents of the edema and plaques especially, shortly after the eruption of the skin over the swellings is washed, saved and dried, after which punctures are made through its border and the exuding fluid is examined microscopically in fresh preparations. In the blood the parasites are practically undetectable even in thick films, but they are sometimes revealed by centrifugation of the blood (Hoare, 1972; Barrowman, 1976; Alemu *et al*., 1997).

2.7.3. Serology

Diagnosis of *Trypanosoma equiperdum,* the causative agent of dourine in horses by standard parasitological techniques is difficult owing to the low numbers of parasites present in blood or tissue fluids and the frequent absence of clinical signs of disease. Consequently, the demonstration of trypanosomal antibodies in the serum has become the most important parameter in determining the disease status of individual animals and serological testing by the complement fixation test (CFT) is widely used in the health certification of horses for export (Wassal *et al.,* 1991).

Humoral antibodies are present in infected animals whether they display clinical signs or not. The complement fixation (CF) test Ministry of Agriculture, Fisheries and Food (MAFF) (1986) is used to confirm clinical evidence and to detect latent infections. Uninfected equids, particularly donkeys and mules, often give inconsistent or nonspecific reactions because of the anti complementary effects of their sera. In the case of anti complementary sera, the indirect fluorescent antibody (IFA) test is of advantage. There is no internationally adopted protocol. Cross-reactions are possible due to the presence in some countries of other trypanosomes for example *T. cruzi* and *T. evansi*. Enzyme-linked immunosorbent assays (ELISAs) are also used. *T. equiperdum* is closely related to other Old World trypanosomes, including *T. brucei* and *T. evansi*. Members of this genus all share conserved cytoskeletal elements that provoke a strong and cross-reactive serological response.

All diagnostic antigens and antisera (monoclonal and polyclonal) currently available for use in sero diagnostic testing contain these conserved elements or antibodies to them and therefore none of the serological procedures described is specific for dourine. The diagnosis of dourine must include history, clinical, and pathological findings as well as serology.

Significant improvements in dourine sero diagnosis will require development of more trypanosome-specific subunit antigens and antibodies to them. Therefore, the principal reason of using serological tests for the diagnosis of trypanosomiasis is to overcome the low level of sensitivity of parasitological tests in detection of chronic infections. Serological tests relying on antibody detection are more sensitive, however they fail to distinguish between an active and cured one. So dourine is usually diagnosed by serology combined with clinical signs (Nantulya, 1990; Luckins, 1992).

Consequently the demonstration of trypanosomal antibodies in the serum has become the most important parameter to determine the status of the disease of individual animals and Complement Fixation Test (CFT) is widely used in the health certification of horses for export (Wassal *et al.*, 1991).

Other serological tests including the Radioimmunoassay and Card Agglutination Test for trypanosomosis (CATT/*T. evansi*, Ro Tat 1.2 and LATEX/*T.evansi*) can be used for the purpose (Bajayna and Hamers, 1988; Wassal *et al.*, 1991; Bishop *et al*., 1995; Touratier, 2001; Claes, 2003).

Complement fixation test (CFT)

Complement fixation test (CFT) is the most commonly used OIE-prescribed sero diagnostic test for international trade for dourine. Despite the usefulness and universal acceptance of the CFT for diagnosing dourine, some discrepancies have been recorded. The disadvantages of the CFT are that it requires careful continuous titration of numerous labile agents and that it does not function with sera having anti complementary activity (OIE, 2001). CFT is not species specific, but only specific for the genus Trypanosoma. The diagnostic significance of this test is therefore doubtful in countries where both *T. equiperdum* and *T. evansi* infection occur in equines.

The test interpretation is often subjective, test sensitivity is relatively low compared with more modern assay methods and the sensitivity of the CFT declines as the serologic responses of exposed animal shifts from initial Ig M based reactions to those of other immunoglobulin classes and subclasses (Katz *et al.*, 1999).

Enzyme Linked Immunosorbent Assay (ELISA)

Although the CFT has been in use many years for diagnosis of dourine it is considered to be less sensitive than ELISA and it has been suggested ELISA could replace the CFT for health certification. Enzyme Linked Immunosorbent Assay (ELISA) is a very sensitive technique and the use of ELISA for routine diagnostic serology of dourine would provide a significant advantage over current serological tests if a defined antigen were used, since it would permit test standardization and more readily allow comparison of tests among laboratories. It additionally, lends itself to a considerable degree of automation, which makes it suitable for large number of samples (Wassal *et al.,* 1991; Bishop *et al.,* 1995). Different workers have stated that the ELISA has a satisfactory concordance ratio with CFT and can be used to supplement CFT (Williamson *et al.,* 1988; Alemu *et al.,* 1997).

Trypanozoon group- specific trypanosomal antigen could be of use in an antibody assay for the diagnosis of *T. equiperdum* infections. Characterization of a group- specific antigen would allow identification of the peptide sequence in the epitope, which could be synthesized and used in a standard ELISA for serological testing of dourine. In addition, an antigen detecting ELISA using monoclonal antibody directed against the same group- specific antigen might also be of use in identifying animals with active infections. To ascertain what is the likelihood than an infection with *T. equiperdum* is present when a test result is positive or that *T. equiperdum* is absent when a test result is negative, requires information on predictive values of the assays (Bishop *et al*., 1995).

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

The monolayer of variable surface glycol proteins (VSG) determines the variable antigenic type (VAT) of an individual trypanosome is highly immunogenic and elicits VAT specific antibodies with agglutinating and lytic activities (Van Meirvenne *et al.,* 1995).

The variable antigen type (VAT) Rhode Trypanosoma Antigenic Type (Ro Tat 1.2) has been cloned from a *T. evansi* strain isolated in 1982 from a water buffalo in Indonesia.

Based on the Ro Tat 1.2 VAT different diagnostic antibody detection tests for *T. evansi* have been developed namely CATT/ *T. evansi*, a direct agglutination test (Bajyana and Hamers, 1988), an indirect agglutination test LATEX/ *T. evansi* (Verloo *et al.,* 2001) and Immune Trypanolysis (Van Meirvenne *et al.,* 1995).

However, based on encoded evidence it appears that *T. equiperdum* infected laboratory animals and horses suspected of dourine also positively react in the CATT/ *T. evansi* and ELISA/ *T. evansi* prepared with fixed whole trypanosomes of the Ro Tat 1.2 VAT. The CATT/ *T. evansi* developed for *T. evansi* infection is also recommended as a field-screening test for *T. equiperdum* diagnosis (Toutaier, 2000). The CATT/ *T. evansi* antigen is a freeze-dried purified suspension of purified fixed and stained blood stream from trypanosomes expressing a predominant variable antigen type of *T. evansi* (Rot Tat 1.2) (Bajyana and Hamers, 1988). The CATT/ *T. evansi* has its advantage in its simplicity, although the test interpretation is subjective.

It was observed that the CATT/ *T. evansi* is not as sensitive as ELISA, CFT and IFAT, but able to detect all animals with clinical signs of dourine. It could be usefully employed especially as a field test (Williamson *et al.,* 1988).

LATEX Agglutination / *T. evansi* Test

LATEX Agglutination / *T. evansi* test is a rapid antibody detecting agglutination test, in which the antigen consists of purified variable surface glycol proteins (VSG) of *T. evansi* Vat Ro Tat 1.2 covalently coupled to latex particles (0.9 micron in diameter). The reagent is realized by lyophilisation and dehydrates with deionized water before use. Serum dilutions are prepared both for CATT// *T. evansi* and LATEX/ *T. evansi* as two-fold dilution with PBS.

Twenty micro liters of diluted sera are mixed with twenty micro liters of reagent on a test card. This method is more specific in testing for *T. evansi* than the CATT/ *T. evansi* method (Verloo *et al*., 2001).

Other serological tests

Other serological tests have been used including radioimmunoassay, counter immunoelectrophoresis and agar gel immunodiffusion (AGID) tests (Caporale *et al.,* 1981; Hagebock *et al.,* 1993). The AGID has been used to confirm positive tests and to test anti complementary sera. A seven-well pattern in 0.8% agarose in Tris buffer is used with the CF test antigen in the center well and positive control sera and unknown sera in alternate peripheral wells. A method has been published for diagnosing equine piroplasmosis, glanders and dourine at the same time, using immunoblotting (Katz *et al.,* 1999).

The IFAT is frequently used for the diagnosis of dourine as a confirmatory test for CFT results since immunofluorescence provides a reliable and sensitive technique. But its interpretation is both subjective and labor intensive and it is therefore more suited to the testing of small members of sera (Wiliamson *et al*., 1988).

2.7.4. Animal inoculation

Mice, rats, rabbits and dogs are susceptible to infection with *T. equiperdum* once it is established in laboratory animals, but animal inoculation is of little use as a routine method of diagnosis, because it is very difficult and often impossible to obtain a first passage. Blood from suspected animals can also be inoculated into laboratory rodents. Under laboratory condition dogs can develop dourine. Different routes of infection such as subcutaneous, intra peritoneal, intravenous, intra urethral and intera vaginal transmission were tested and all gave rise to clinical signs of dourine. In dogs inoculation of *T. equiperdum* produces the typical picture of dourine with trypanosomes present in the lesions, but not in the blood, the infection may last from one to several months (Stephen, 1986; Claes, 2003).

The susceptibility of dogs to *T. equiperdum* is generally high and this means that strains can be sent from remote countries after the animals have been experimentally infected. Strains of *T. equiperdum* were successfully isolated by the intra testicular injection of rabbit with blood or material from infected horses (Claes, 2003).

However, in a recent report blood and genital washes from antigenaemic horses did not lead to infections when inoculated into mice (Alemu *et al*., 1997).

2.7.5. Post-mortem examination

Post-mortem lesions include gelatinous exudates under the skin. In the stallion, the scrotum sheath and testicular tunica are thickened and infiltrated. In some cases the testes are embedded in a tough mass of sclerotic tissue and may be unrecognizable. In the mare, the vulva, vaginal mucosa, uterus, bladder and mammary glands may be thickened with gelatinous infiltration. The lymph nodes particularly in the abdominal cavity are hypertrophied, softened and in some cases hemorrhagic. The spinal cord of animals with paraplegia is often soft, pulpy and discolored particularly in the lumbar and sacral regions (Barrowman, 1976).

The most consistent postmortem pathological lesions include lymphadenitis particularly in lymph nodes draining the areas of edema in the pelvic and inguinal regions. There is also marked thickening of vulva, vagina and mammary glands and in the stallion the scrotum and tests are embedded in scar tissue. Microscopic lesions are present in the lumbar and sacral regions of the spinal cord, involving cellular infiltrations and degenerative changes in spinal nerves and large peripheral nerves, especially in sciatic nerve (Hoare, 1972).

2.8. Prevention and control

2.8.1. Treatment (Chemotherapy and chemoprophylaxis)

There are no officially approved drugs to treat horses suffering from dourine, although some older publications mention experimental treatment of horses with naganol and neoarsphenamine (Ciuca, 1933) or quinapyramine sulfate. International regulations currently impose the slaughtering of CFT-positive horses.

Nevertheless, in vitro sensitivity of different *T. equiperdum* strains to suramin, diminazene, quinapyramine and melarsomine has been reported (Zhang *et al.,* 1992).

Although there are reports of successful treatment with trypanocidal drugs like Suramin at dose of 10mg/kg IV and Quinapyramine dimethylsulphate at dose of 3-5mg/kg S.C), treatment is more successful when the disease is caused by the more virulent (European) strains of the parasite. In general treatment is not recommended for fear of continued dissemination of the disease by treated animals. Treatment may result in apparent disease carriers and is not recommended in dourine free territory (Barrowman, 1976; Losos, 1986; OIE, 2000).

For the treatment of *T. equiperdum* infection Diminazene and Cymelarsan can also be used. Evidence from in vitro drug sensitivity determination of *T. equiperdum* (Zhang *et al*., 1992; Brun and Lun, 1994) indicates that Suramin, Diminazene, Quinapyramine and Cymelarsan are effective against this trypanosome species, although no reports on clinical efficacy have been published.

However, only neoarsphenamine and suramine have been used in large dourine eradication programme. It is recommended that neoarsphenamine be administrated twice in high doses of 40 grams to 50 grams per adult horse. According to the recent study carried out by Hagos (2005), in the endemic area of dourine of Arsi Bale highland of Ethiopia, the therapeutic effect of some trypanocidal drugs such as Isometamidium chloride and Diminazene aceturate in dourine clinical cases have been shown very effective result.

2.8.2. Test and slaughtering policy

There are no available biological products. Control of the disease depends on compulsory notification and slaughter of infected animals. The most successful prevention and eradication programs have focused on serological identification of infected animals. Infected animals should be humanly destroyed to prevent further transmission. The CFT is very reliable and an essential tool in identifying positive animals. International regulations currently impose the slaughtering of CFT positive horses (Zhang *et al*. 1992). These measures can include the segregation and quarantine of reactors, treatment with high doses of trypanocides followed by close surveillance for several months or the slaughtering of the reactors.

Eradication of dourine can be achieved by following of the program of diagnosis followed by Sterilization or slaughter of infected animals (OIE, 2001).

2.8.3. Animal management

In prevention and controlling of the dourine strict control of breeding is very essential. Good hygiene at assisted matting is also important. Serological testing ensures that infected animals should not be used for breeding and legislations required for export prevent the movement of the infected animals. Restriction the movement of infected animals limits further transmission of the disease. Serologically positive as well as negative animals can be castrated, but it is important to note that castrating adult stallions dose not always change the copulatory ability of such animals and it should be performed with caution when attempting an eradication program. In case of dourine quarantine is an essential method for controlling and eradication program and breeding should be stopped for 1-2 months while testing should be continuous in areas where dourine is found. This may be supported by proper fencing farms and by taking necessary precautions. Sanitation and disinfections are ineffective means of dourine control, because the disease normally spreads by coitus (OIE, 2001).

3. MATERIALS AND METHODS

3.1. Study area

The present study was conducted in Bale highlands of Oromia Regional State of Ethiopia, mainly in four selected horse breeding districts namely Agarfa, Dinsho, Goba and Sinana. Bale zone is found in the Oromia Regional state southeast of the country, where Robe the capital city of Bale Zone is located 430kms away from Addis Ababa. Topographically, the altitude ranges from 500 to 4377 m.a.s.l. Three climate zones, including arid (63.53 %), mid altitude (21.54 %) and highland (14.93 %) forms are known to exist. The area experiences a bimodal rainfall occurring from March to the beginning of June and July to October. An average annual temperature of 28.8^{o}C and rainfall of 400 mm in the lowlands and 3.5^{o}C with a rainfall of 1200 mm in highlands are recorded. Vegetation of the area change with an altitude and rainfall ranges from scattered trees and bushes to dense shrubs and forest in different altitude and from thorny and fibrous grass of dry season to bushy and soft grass of rainy season. The total area of Bale is 621,113.31 hectares, and from this area 216,765.62 hectares (34.89 %) are used for cultivation, 60,891.25 hectares (9.80 %) for grazing, 110,036.92 (17.72 %) for forest land, 158,983.64 hectares (25.60 %) are productive and 74,405.88 hectares (11.98 %) unproductive, and unutilized land (Bale Zone Agricultural and Rural Development Office).

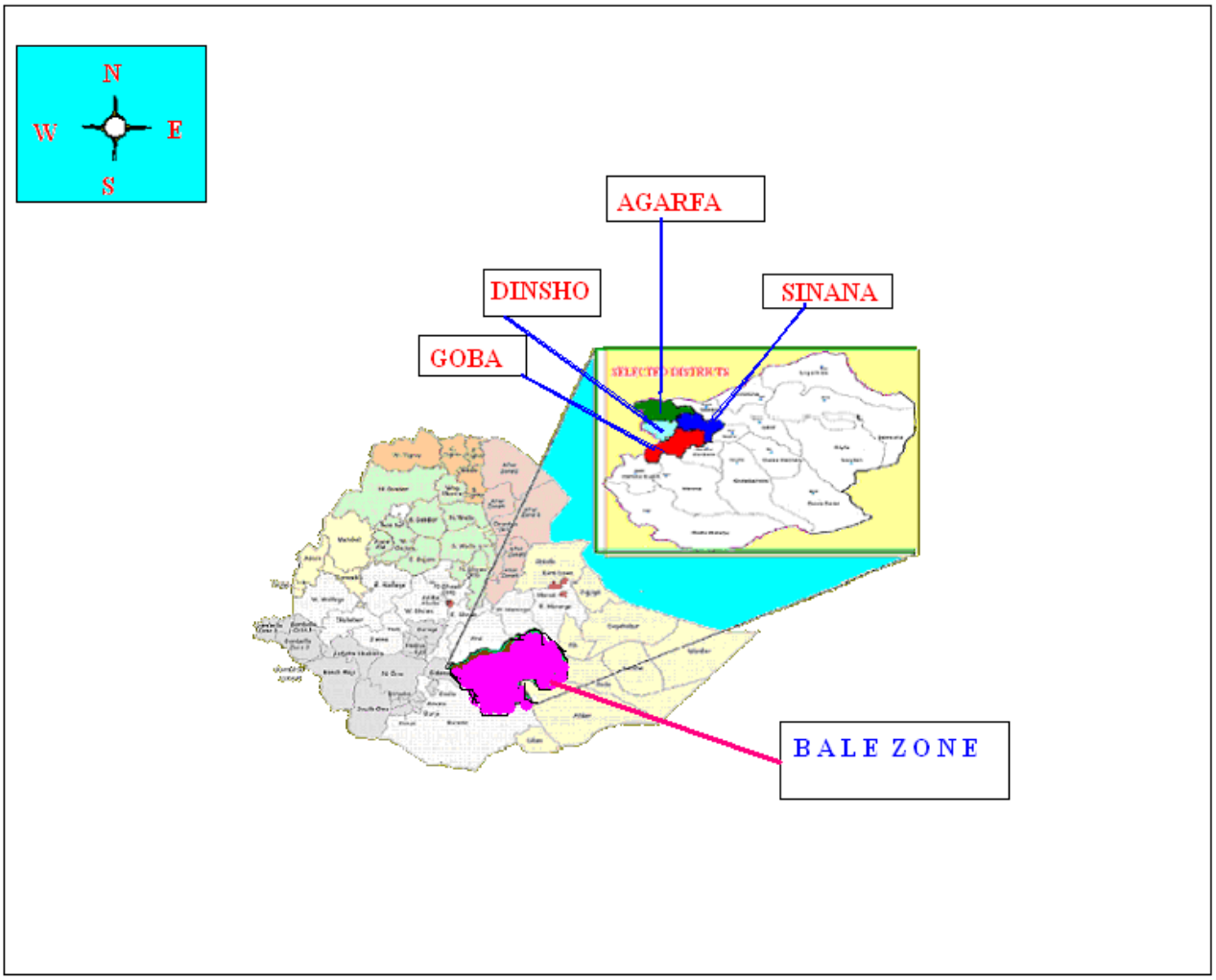

Figure 1: Map of study districts: Agarfa, Dinsho, Goba and Sinana

3.2. Livestock population and production system

The major land cover is thus used for cultivation. Agriculture is the mainstay of livelihood of peoples and the leading economic activity of the area with a mixed farming system covering the highest percentage of the total agricultural activities with crop-livestock production. Cattle, sheep, goats, horses, donkeys, mules, camels and poultry are the main livestock species reared in the Zone.

It is eminent that livestock products and by-products in the form of meat, milk, eggs and others supply the needed protein that contributes to the improvement of the nutritional status of the people. Livestock also plays an important role in providing export commodities such as live animals, hide and skins to earn foreign exchanges to the country. Draught animals provide power for cultivation and crop threshing, and especially horses are serves as essential modes of transport. The contribution of equine population has the prominent position in the agricultural and transport system as draft, pack and riding animals (Bale Zone Agricultural and Rural Development Office).

Bale Zone has large equine population and it is about 53,119 in number. Out of this population, 46,310 (87.18 %) horses are found in the highlands. Among 18 districts found in the Bale Zone, 8 districts represent highland and mid altitude. These districts are well known in horse breeding practice in the Zone. A significant number of horses are also purchased from the area for breeding and other purposes (transportation and trade purpose) by neighboring adjacent districts (Bale Zone Agricultural and Rural Development Office).

3.3. Study design

A cross sectional study design was employed based on parasitological, serological and questionnaire survey in four selected horse breeding districts of Bale highlands.

For the parasitological survey conducted to determine parasitological prevalence and to isolate the causative agent of the disease (*T. equiperdum*), a purposive sampling was carried out on a total of 71 selected dourine clinical cases (48male and 23 female horses).

Table 4: Study animals (n = 71) considered for parasitological investigation of Dourine

District	Sex		Total
	Male	Female	
Agarfa	12	5	17
Dinsho	13	7	20
Goba	15	6	21
Sinana	8	5	13
Total	48	23	71

For serological survey a combination of multistage stratified and simple random sampling methods were applied according to Thrusfield, (1995). First four discrete study districts were selected from Bale Zone (first stage) to represent Bale highlands. Then selected peasant associations (PAs) based on the convenience (second stage) and sampling peasant associations were selected based on representation of respective districts and accessibility. Villages were selected in collaboration with respective district's animal health personnel and selected by purposive sampling on the basis of farmers' cooperation, logistics, share of communal grazing land, accessibility and suspecting of dourine by the veterinary professionals in all study sites.

All of the study districts (Agarfa, Dinsho, Goba and Sinana) are located between 2400 to 4377 m.a.s.l. There was no evidence of *T. evansi* infection in the selected study area. However, adjacent districts in the Bale lowlands such as Barebre, Dello-Mena and Harena-Buluk were known to be endemic for surra (camel trypanosomosis due to *T. evansi*).

Taking an average prevalence of 31.39 % based on the previous reports (Hagos, 2005), absolute desired precision of 5 % and confidence level of 95 % for estimating prevalence in simple random sampling according to Thrusfield (1995), the sample size was determined as follows: -

$$n = \frac{1.96^2 P_{exp} (1- P_{exp})}{d^2}$$

Where:

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

Accordingly, the calculated sample size for estimating prevalence in simple random sampling was 331. In order to adjust the sample size required for the present multistage random sampling method and to make a prevalence estimate more precise, the sample size was inflated 2.65 times than in simple random sampling and was set to be 880. Hence, a total of 880 horses were sampled equally from each selected districts of Bale highlands, 220 horses from Agarfa (25 %), 220 horses from Dinsho (25 %), 220 horses from Goba (25 %) and 220 horses from Sinana (25 %) districts.

All horses used in this study were sexually matured above 4 years old of both sexes living under a traditional management system of free grazing. Animals were selected by systematic random sampling method by taking every fifth horse for blood sample collection at each study sites. Important study variables including age, sex, parity number, history of previous abortion, castration status and body condition score were considered and recorded in the pre designed data collection format (Annex 7).

Aging of horses was made based on the description provided by Coombs, (2002). 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, (2002) Details are attached in (Annex 5).

Table 5: Study animals (n = 880) considered for the serological investigation of Dourine

District	Sex		Age		Previous abortion history		Castration status		Parity number		Body Condition	
	M	F	Y (4-6)	A > 6	Yes	No	Castrated	Un castrated	0	1 and > 1	Poor	Moderate
Agarfa	113	107	44	176	22	85	66	47	21	86	90	130
Dinsho	133	87	68	152	24	63	87	46	14	73	77	143
Goba	136	84	74	146	23	61	70	66	27	57	92	128
Sinana	146	74	86	134	22	52	100	46	34	40	59	161
Total	528	352	272	608	91	261	323	205	96	256	318	572

3.3.1. Parasitological survey

Blood was collected from the jugular vein of horses in heparinised Venojects. Subsequently, Haematocrit Centrifugation Technique (HCT) was conducted for parasitological diagnosis of dourine (Woo, 1970). Vaginal and preputial discharges from clinically suspected cases of dourine were collected in sterile tubes. Washings were centrifuged at 3000 rpm and the deposit examined as a wet smear preparation under a cover slip with a 300x magnification. At the same time thin smears were prepared (dried, fixed, stained with Giemsa) and microscopically examined under oil immersion. Swollen clitoris or prepuce as well as glans penis were cleaned and incised with a sterile scalpel and blood/oedematous fluid thin smears were prepared and microscopically examined under oil immersion.

3.3.2. Serological Survey

Blood samples were collected from jugular vein of 880 horses (528 males and 352 females) using plain vacutainer tubes and needles, after the site is wiped with cotton wool soaked in alcohol. The vacutainer tubes were labeled and the blood was allowed to clot overnight at room temperature and the serum was separated by centrifugation. Then sera were filled into serum storage (polypropylene strile cryogenic vials and stored at -20^{o} C until tested by card agglutination for trypanosomosis test (CATT/ *T. evansi*) and LATEX/ *T. evansi*. The test was conducted at Debre Zeit Faculty of Veterinary Medicine Molecular Biology Laboratory, which established by Ethio-Belgium Dourine Project. For both tests (CATT/ *T. evansi* and LATEX/ *T. evansi*) positive results were determined at cut-off point dilutions 1:4.

The tests were checked with positive and negative controls before the whole samples are tested. 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 (Annex 9, A and B).

3.3.3. Questionnaire survey

A questionnaire was used to gather information from horse owners and veterinary professionals on their perception about the occurrence of dourine in the study area. Owners of individual horses were selected randomly and interviewed to provide details of their horse's health status in the past and present. Veterinary professionals were also interviewed regarding the disease status and epidemiological risk factors attributing for the occurrence and transmission of dourine. A pre tested structured questionnaire designed to include questions about risk factors either known or thought to influence the spread of dourine was administered. The questionnaire was pre tested before the actual survey for time, resource and relevance of type of questions included. The questionnaire format was filled by directly interviewing randomly selected animal owners (50 farmers from each district a total of 200 farmers) (Annex 8) and veterinary professionals working in the area. The effect of interpreter was totally avoided, as the researcher was able to speak, read and write the regional language (Afan Oromo).

3.4. Data Analysis

All obtained Data inserted into MS Excel spread sheets (Microsoft crop) and transferred to the SPSS 15.00, 2006 software program. Descriptive statistics and logistic regression were used to determine prevalence, to assess major risk factors and analyze questionnaire survey results. The impact of the explanatory variables (sex, age, parity number, history of previous abortion, effect of castration status and body condition) on sero prevalence result using CATT/ *T. evansi* and LATEX */T. evansi* were also assessed using descriptive statistics and logistic regression models. The exponentiated estimate of the coefficients of the models was interpreted as odds ratio (OR) and Chi Square.

Univariable logistic regression analysis was employed to determine the association of risk factors with the sero-prevalence of dourine in the present study area. Only risk factors with p-value <0.05 in CATT/ *T. evansi* on univariate analysis were subjected to multivariate analysis to determine major risk factor.

4. RESULTS

4.1. Parasitological data

No trypanosomes were detected in all examined Giemsa stained smears (thin blood, genital discharge and tissue fluids) as well as in blood samples by HCT.

Different characteristic signs of dourine were observed in clinically sick horses of both sexes. In females, vaginal discharge mainly of muco purulent type with foul odour, oedema of the external genitalia and presence of depigmented scars over the external genitalia were the prominent signs observed. In males, oedema of the scrotum and prepuce accompanied by prepucial as well as urethral discharge and ulceration of the genital mucosae mainly of the penile tissue were the frequently observed signs. In both sexes, lameness in one or both legs, restlessness, partial dragging of the hind legs, incoordination, asymmetrical posture and tendency to shift weight from one leg to another were the dominant signs observed as nervous form of the disease.

The cutaneous form of the disease, which mainly characterised by “urticarial plaques”, marked by distinct, raised round or oval shaped patchy eruptions that appear on the skin in both sexes, was not observed.

4.2. Serological data

The present serological survey of dourine was conducted on a total of 880 sera tested for the detection of antibodies against *T. equiperdum* in four randomly selected horse breeding districts of Bale highlands of Ethiopia. The results revealed a seroprevalence of 140 (15.90 %) and 173 (19.66 %) for CATT /*T. evansi* and LATEX /*T. evansi* tests, respectively (Figure 2).

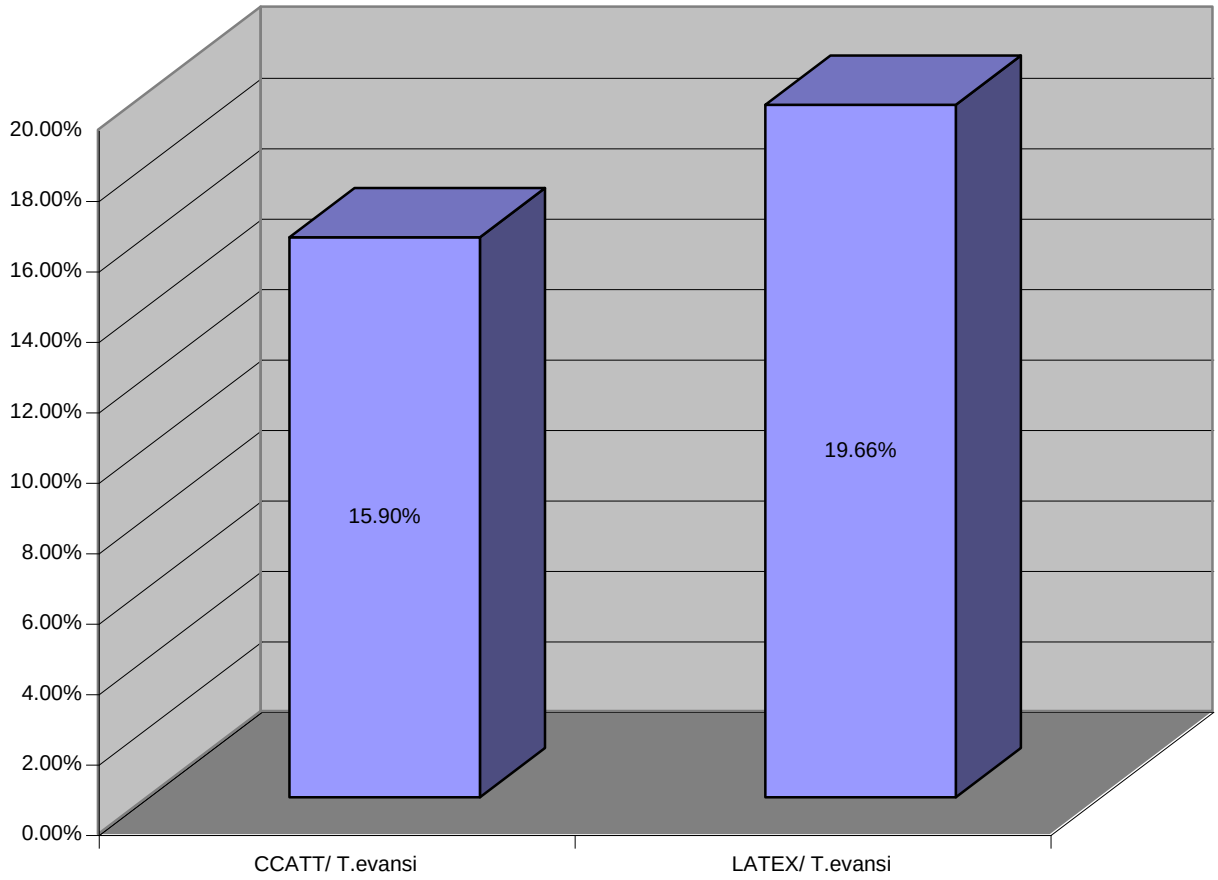

Figure 2: Sero prevalence of Dourine on the basis of CATT/*T. evansi* and LATEX/ *T. evansi* in four selected districts of Bale highlands

Of the 880 horses sera tested for the detection of anti *T. equiperdum* antibody using CATT/ *T. evansi* and LATEX/ *T. evansi* tests, 140 samples had positive result out of which 76 (8.64 %) were males and 64 (7.27 %) were females and 173 samples had positive result of which 98 (11.14 %) were males 75 (8.52 %) were female for CATT/ *T. evansi* and LATEX/ *T. evansi* tests, respectively (Table 6 and 7).

Table 6: Seroprevalence of Dourine (*T. equiperdum*) of horses in selected districts of Bale highlands on the basis of CATT/ *T. evansi* test

Factors		Animal tested	Prevalence	P- value	Chi square
Sex	F	352	64/352 (18.20 %)	0.132	2.265
	M	528	76/528 (14.40 %)		
Age	4-6	272	52/272 (19.10 %)	0.082	3.030
	>6	608	88/608 (14.50 %)		
Body condition	Poor	325	62/325 (19.10 %)	0.049	3.865
	Moderate	555	78/555 (14.10 %)		
Parity number	No	96	24/96 (25.00 %)	0.042	4.125
	Yes	256	40/256 (15.60 %)		
Previous abortion history	No	261	49/261 (18.80 %)	0.626	0.238
	Yes	91	15/91 (16.50 %)		
Castration status	Uncastrated	205	24/205 (11.70 %)	0.161	1.963
	Castrated	323	52/323 (16.10 %)		
Overall prevalence		880	140/880 (15.90%)		

Table 7: Seroprevalence of Dourine (*T. equiperdum*) of horses of selected districts of Bale highlands of Ethiopia on the basis of LATEX / *T. evansi* tests

Factors		No. tested	Prevalence	P- value	Chi square
Sex	F	352	75/352 (21.30 %)	0.315	1.008
	M	528	98/528 (18.60 %)		
Age	4-6	272	53/272 (19.50 %)	0.931	0.008
	>6	608	120/608 (19.70 %)		
Body condition	Poor	325	72/325 (22.20 %)	0.154	2.031
	Moderate	555	101/555 (18.20 %)		
Parity number	No	96	18/96 (18.80 %)	0.473	0.515
	Yes	256	57/256 (22.30 %)		
Previous abortion history	No	261	61/261(23.40 %)	0.109	2.567
	Yes	91	14/91(15.40 %)		
Castration status	Uncastrated	205	40/205 (19.50 %)	0.654	0.201
	Castrated	323	58/323 (18.00 %)		
Overall Prevalence		880	173/880 (19.70%)		

Major aspects of the findings are summarized in Table 6 and 7 above. Accordingly, explanatory variables such as age, sex, history of previous abortion and castration status had no significant difference ($p > 0.05$) on the sero prevalence of the disease using CATT/*T. evansi* test. However, body condition and parity number had significant difference ($p < 0.05$) on the sero prevalence of the disease based on the CATT/ *T. evansi* test. On the other hand, neither of the major risk factors had a significant difference ($p > 0.05$) on the sero prevalence of the disease based on LATEX/ *T. evansi* test.

Logistic regression analysis revealed that horses without parity number (=0) had an OR of 1.80 (1.02, 3.19) positivity compared to horses with parity number (1 and above 1); and horses with poor body condition (very thin and thin) had an OR of 1.44 (1.00, 2.08) positivity compared to horses with moderate body condition (less thin, less moderate and moderate) scores, on the basis of CATT/*T.evansi* test. Therefore, the major risks of seropositivity in horses include parity number and body condition, which could only be identified by CATT/*T. evansi* test, (Table 8).

Table 8: Odds ratio comparison of the sero-prevalence of dourine based on CATT /*T. evansi* test of major risk factors in selected horse breeding districts of Bale highlands

Major risk factors	CATT/*T evansi*	
	OR	95 % C.I and P-Value
Parity number 0 *Versus* 1 and above	1.80	(1.02, 3.19) 0.04
Body condition Poor *Versus* Moderate	1.44	(1.00, 2.08) 0.05

NB: Poor refers to very thin and thin, while moderate refers to less thin, less moderate and moderate body scales.

4.3. Questionnaire survey

Animal owners and veterinary professionals working in the study area reported the presence of major diseases of horses such as Dourine, Epizootic Lymphangitis, African Horse Sickness (AHS), Anthrax and Ecto and Endo-parasites in the selected districts of Bale highlands of Ethiopia. They also reported that dourine is a major health problem of horses causing considerable high economic loss.

According to the present questionnaire survey, dourine is well known by farmers as they explained by describing the various clinical signs attributed to the disease. About 190 (95 %) of the respondents replied that they know the disease very well and can indicate its main clinical manifestations (Table 9). Some of the most important clinical signs of the disease include in coordination especially of the hindquarters and swelling of external genitalia, emaciation, lameness and weakness and finally paralysis. In females mucoid and purulent vaginal discharges were quite common.

Farmers also disclosed the occurrence of death in untreated cases. The disease is known by the farmers almost for a long period of time and locally known as “Derisa” in Oromifa language and “Tigen” in Amharic language by people of the study districts of Bale highlands, which refer to the paralysis of hindquarters (starting from Lumbal region to hind legs). Farmers and professionals also emphasized that the problem of dourine is the constant problem in the area. Majority of the respondents 173 (86.5 %) replied that dourine is a common clinical case throughout the year. On the other hand, some respondents 27 (13.5 %) have reported that the disease has a seasonal character, which commonly occurs whenever, animals are in a good body condition and following the breeding season from April to late September, and during dry season of the year (January to March), which probably associated with the relapse of previously infected and recovered cases due to the stressful condition of feed shortage. Farmers as well as professionals emphasized that the problem of dourine is getting worse and increasing its extent and magnitude as there are no effective drugs for treatment and prophylactic purposes (Table 9).

In other hand, veterinary professionals working in the study area indicated that the main factors that play an important role in the distribution and transmission of the disease are uncontrolled breeding, unrestricted animal movement for the purpose of trade and transportation and absence of effective drugs against dourine cases.

Table 9: Farmers perceptions and knowledge about dourine (n = 200)

Interview (Points of focus)	No. of respondents	Proportion (response rate)
Knowledge of the disease	200	
Yes:		190 (95 %)
No:		10 (10 %)
Dourine a major problem of horses	200	
Yes:		163 (81.5 %)
No:		37 (18.5 %)
Seasonality of the disease	200	
Yes:		27 (13.5 %)
No:		173 (86.5 %)
Status of the disease	200	
Getting Worse:		131 (65.5 %)
Getting better:		9 (4.5 %)
No change:		56 (28 %)
I do not know:		12 (6 %)
Mares with previous abortion history	200	
Yes:		17 (8.5 %)
No:		173 (91.5 %)
Personnel involved in the treatment	200	
of dourine		
Professionals:		125 (62 %)
Drug smugglers:		53 (27 %)
Farmers:		22 (11%)

However, based on personal communication with professionals and observation, trypanocidal drugs commonly used for the treatment of dourine cases were Dminazene aceturate (Verbien), Isomethamidium chloride (Trypamidium) and Quinapyramine sulphate (Triquin-S®, Wockhardt Veterinary Ltd., India). Regional government, NGOs and private drug smugglers often supply these drugs. Moreover, professionals, private drug smugglers and even some farmers were involved in the treatment of dourine-infected animals (Figure 3).

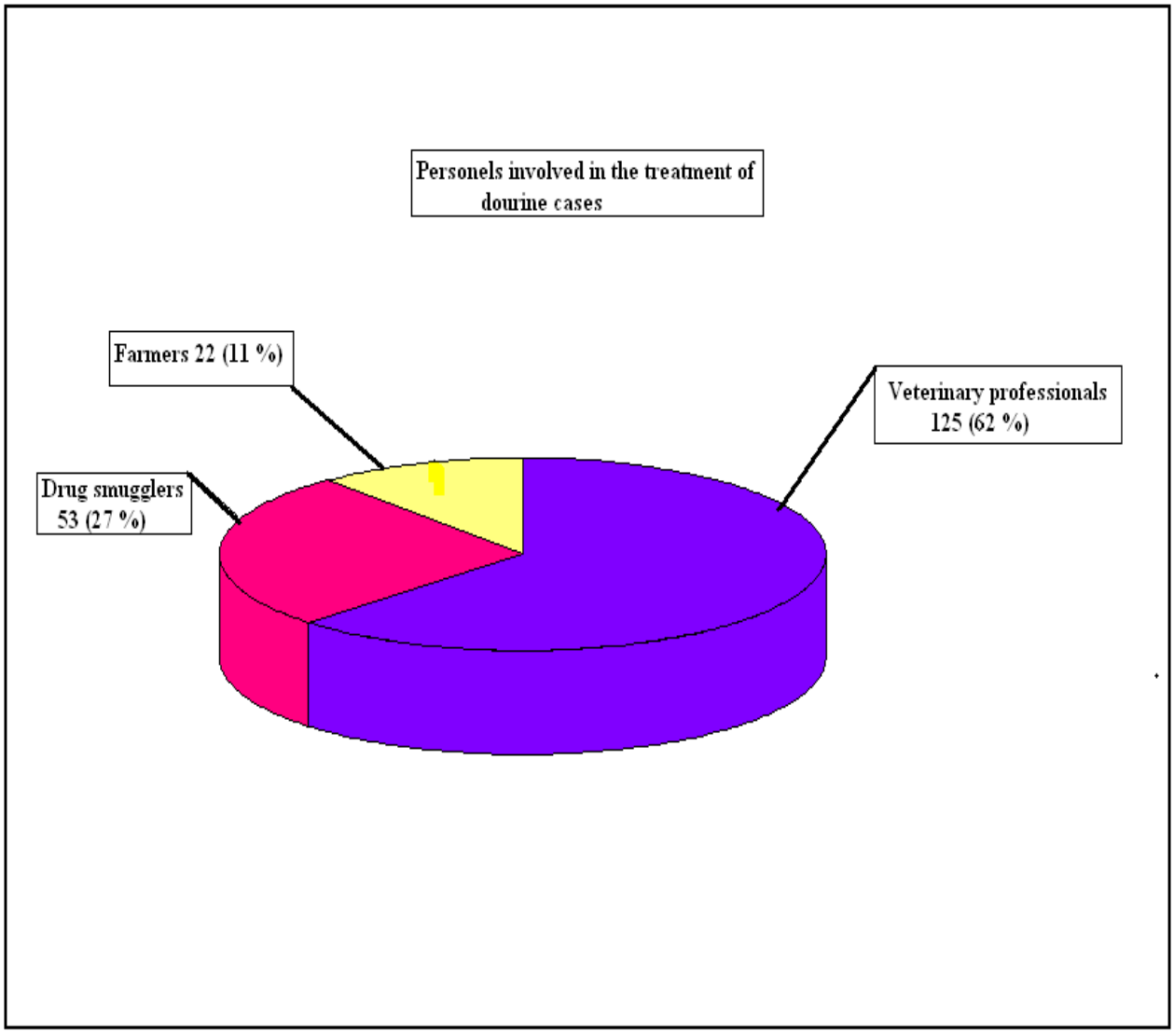

Figure 3: Personnel involved in the treatment of dourine in selected horse breeding districts of the Bale highlands

5. DICUSSION

Dourine is a chronic or acute contagious disease of breeding solipeds that is transmitted directly from animal to animal during coitus. Among the non-tsetse transmitted trypanosomes (NTTAT), dourine is the only trypanosomiasis that is not transmitted by an invertebrate vector. *T. equiperdum* differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood (OIE, 2001).

The present cross sectional study conducted using parasitological, serological and questionnaire survey in selected horse breeding districts of Bale highlands of Oromia Regional State of Ethiopia disclosed that dourine is a major health problem and a potential threat to the equine population in the area.

No trypanosomes could be detected in all examined Giemsa stained smears (thin blood, genital discharge and tissue fluids) as well as in blood samples by HCT. This result is consistent with those of previous reports (Watson, 1920; Alemu *et al*., 1997; Clausen *et al*., 1999; Hagos, 2005). Also several attempts to isolate *T. equiperdum* in Eastern Europe were unsuccessful (Touratier, 2001). As *T. equiperdum* is primarily a tissue parasite, its establishment in the blood of laboratory animals presents the greatest difficulty (Hoare, 1972). Unfortunately, parasitological techniques cannot always detect ongoing infections as level of parasitaemia is often low and fluctuating, particularly during chronic stage of the disease (Nantulya, 1990).

In horse, the clinical signs common to dourine, such as incoordination, especially of the hindquarters, and oedematous swelling of the external genitalia (Hoare, 1972) were observed in this study. Skin plaques, which are regarded as important symptoms in cases of dourine, were not observed during this study, although skin eruptions and poor body conditions were prominent. However, in recent dourine infections oedematous plaques were not observed (Alemu *et al*., 1997), yet in certain surra infections these cutaneous plaques may be observed (Brun *et al*., 1998); hence these plaques should not be considered to be pathognomonic for dourine.

The sero prevalence result is almost consistent with the previous reports based on indirect methods (antibody and antigen detection) in the Arsi Bale highlands of Ethiopia (Zeleke *et al*., 1980; Alemu *et al*., 1997; Clausen *et al*., 1999 Hagos, 2005).

Logistic regression models result suggested that the serological test employed by CATT/ *T. evansi* results were affected by two explanatory variables specifically zero parity number and poor body condition. The possible explanation for the marked correlation between highest seroprevalence and independent variables like zero parity number and poor body condition is associated with the number of previous sexual contacts with infected or carrier animals which can increase the chance of acquiring the infection. CATT/ *T. evansi* serological results were not affected by sex, age, history of previous abortion and castration status. This might be attributed to the fact that the animals in all sex, age, history of previous abortion and castration status were equally exposed to the disease. Therefore, the major risk factors of seropositivity could be identified as zero parity number and poor body condition.

On the other hand one serological test results of LATEX/*T. evansi* were not affected by all explanatory variables namely sex, age, body condition score, parity number, history of previous abortion and castration status. This might be due to the fact that animals in all sex, age, body condition, parity level, history of previous abortion and castration status were equally exposed to the infection. More specifically LATEX/*T. evansi* test is a more specific test compared to CATT/ *T. evansi* test.

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 (Clausen, 1999; Clausen, 2003) allow a subspecies identification within the subgenus Trypanozoon and therefore no definitive diagnosis can be given for *T. equiperdum*.

In a recent study conducted by Hagos (2005) a very high concordance was observed between the serological results in both CATT/ *T. evansi* and ELISA/ RoTat 1.2 and the clinical (dourine) status of the examined animals. Hence it appears that these tests can be valuable for the detection of dourine although initially designed to detect surra infections in camel. A similar result was obtained in a previous study in Kazakhstan, where a high concordance was found between CFT and CATT/ *T. evansi* (Claes *et al*., 2005).

The results of the questionnaire survey revealed the presence of major diseases of horses such as: Dourine, Epizootic Lymphangitis, African Horse Sickness, Anthrax and Ecto-Endo parasitism in selected districts of Bale highlands of Ethiopia. Questionnaire survey confirmed that, the disease is well known by professionals as well as farmers in terms of clinical signs in selected horse breeding districts (Agarfa, Dinsho, Goba and Sinana) of Bale highlands. The disease is locally known as "Derisa" in Oromifa and "Tigen" in Amharic language, which refers to the paralysis of hind legs and incoordination.

Both animal owners and professionals interviewed reported that dourine is a major health problem of horses causing high mortality and economic loss. Farmers reported that the first sign of the disease in clinically affected horses is incoordination, especially of the hindquarters and swelling of the external genitalia. In both sexes can be observed vaginal and urethral discharges.

It was also emphasized by the farmers as well as professionals that the problem of dourine is becoming more and more severe and increasing its extent and magnitude, as there are no effective curative or prophylactic therapeutic agents. Though, dourine is a common clinical case through out the year.

On the other hand, some respondents 27 (13.5 %) have emphasized that the disease has a seasonal character, which commonly occurs whenever, animals are in a good body condition and following the breeding season from April to late September, and during dry season of the year (January to March), which probably associated with the relapse of previously infected and recovered cases due to the stressful condition of feed shortage.

This fact is not consistent with those of previous studies, which showed that dourine has a seasonal character that mainly coincides with breeding season (Zeleke *et al*., 1980, Alemu *et al*., 1997; Hagos, 2005).

Some of the most important clinical signs of the disease include in coordination especially of the hindquarters and swelling of external genitalia, lameness and weakness and finally paralysis. In other hand farmers and professionals emphasized the occurrence of nervous symptoms in the clinical dourine infected horses, which might be associated with the presence of the parasite (*T. equiperdum*) in the cerebrospinal fluids (Barrowman, 1976). Farmers also disclosed the occurrence of death in untreated dourine clinical cases. However, skin plaques, which are regarded as pathognomonic symptoms in dourine, were not indicated by the farmers as well as by professionals. Similarly study conducted in Arsi-Bale highlands (Alemu *et al.,* 1997) confirmed that edematous plaques were absent in recent infections, while in *T. evansi* infections cutaneous plaques have been observed (Brun *et al*., 1998). This seems that skin plaques cannot necessarily considered to be pathognomonic symptoms for dourine, as these plaques can be observed in animals with *T. evansi*, although in early stages of the infection edema of genital organs and fever are the rule.

Horses are treated against dourine only irregularly, when trypanocidal drugs are available and even such treated animals show frequent relapse and generally treatment is not able to cure clinical cases. However previous studies showed that a prominent feature of trypanosomosis is the relapsing nature of the disease where there is periodical expression of surface coat glycoproteines of a differing antigenic nature (Hoare, 1972).

Some of the trypanocidal drugs used in the area, whenever available include Veriben (Diminazene aceturate), Isomethamidium chloride (Trypamidium) and quinapyramine sulphate (Triquin- S®, Wockhardt Veterinary Ltd., India). Interviewed professionals underlined that the presence of drug smugglers and the practice of self-treatment by the farmers, as the main issue which has raised the fear of drug resistance for dourine, as it has been in other type of trypanosomosis, which caused by different species of trypanosomes.

Apart from this, there is the lack of sufficient and regular trypanocidal drugs supply to the study area. This may arise from the lack of awareness of the government (Oromia Rural and Agricultural Bureau) as dourine is an important disease and due to this more attention and emphasis is given to supply trypanocidal drugs to the other parts of Oromia (Western Oromia and low land of Oromia), where tsetse and other trypanosomes are well known by the government.

Some professionals based on several years of work experience recommended a treatment schedule. In view of the frequent occurrence of relapses where one time treatment of clinical cases is not curative enough it can possibly be recommended that an animal should get on average two treatments per year and treatment should be repeated. A treatment schedule that they found to be effective as providing double dose in the first day and then after 48 hours of treatment.

However, as no method of immunization against dourine exist at present and moreover, treatment of clinical cases with the available trypanocidal drugs may result in apparent disease carriers and is not recommended in dourine free territories (OIE, 2001). On the other hand, treatment of clinical cases in such endemic areas seems to be beneficial taking into account the indispensable role played by horses in the Bale highlands due to the rugged mountainous terrain of the area where these animals are still the main method of transporting both people and agricultural products, as treatment reduces mortality and results in marked improvement of clinical signs. This is due to the fact that whether clinical cases are treated or not, the animal will tend to be carrier. Therefore, in such an endemic area where it is difficult to effectively control the disease, it will be worth treating clinical cases to alleviate the disease, enable the animal to perform well and thereby reduce mortality.

Study conducted on the drug therapy (Abebe *et al*., 1996) gave a good witness of that drug therapy has been the main strategy used in the past to control trypanosomiasis throughout Ethiopia.

Moreover, since horses travel long distances for trade and transport purposes in Bale highlands of Ethiopia it can be believed that the disease may have a much wider distribution than the study area and specially in places where there is large equine population.

The occurrence of dourine was established and confirmed for the first time in those selected horse breeding districts of Bale highlands namely Agarfa, Dinsho and Sinana districts through unrestricted animal movement from neighboring districts for the trade and transport purpose.

On the other hand, as other environmental factor does not restrict the distribution of dourine it can easily be established almost anywhere where there is large equine population (Luckins, 1994).

Therefore, this study provides strong evidence that Dourine is highly prevalent endemic disease causing a catastrophic damage to the existing horse population and potential threat to equine population of the region at large requiring an immediate professional intervention.

6. CONCLUSIONS AND RECOMMENDATIONS

Dourine is among highly prevalent equine diseases in Arsi Bale highlands of Oromia regional State of Ethiopia. Local farmers have recognized this problem for many years. 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. Accordingly, serological as well as questionnaire based survey used in the present study disclosed the occurrence and establishment of dourine for the first time in the selected horse breeding districts of Bale highlands, namely Agarfa, Dinsho and Sinana districts. Moreover, dourine can be easily established anywhere where there is equine population and may have much wider distribution than previously realized areas, as other environmental factors does not restrict the distribution of it.

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 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.
- We propose further studies to determine the socio economic impact of the disease.

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8. ANNEXES

Annex 1: Seroprevalence of dourine (*T. equiperdum)* in horses of Arsi-Bale highlands based on CATT/ *T.evansi*, LATEX / *T.evansi* and ELISA tests

Study Districts	N^{o}. of animals examined	Seroprevalence		
		CATT/ *T.evansi*	LATEX/ *T.evansi*	ELISA
Arsi-Robe	128	20 (15.63 %)	17 (13.28 %)	13 (10.16 %)
Asassa	130	36 (27.69 %)	34 (26.15 %)	26 (20 %)
Kofalle	128	49 (38.28 %)	46 (35.94 %)	36 (28.13 %)
Dodolla	91	30 (32.97 %)	26 (28.57 %)	22 (24.18 %)
Goba	86	27 (31.39 %)	18 (20.93 %)	14 (16.28 %)
Kokosa	86	22 (25.58 %)	20 (23.26 %)	14 (16.28 %)
Overall prevalence	649	184 (28.35 %)	161 (24.81%)	125(19.26%)

Source: Hagos (2005).

Annex 2: Results of dourine investigation on the basis serological and questionnaire survey in horses from different selective parts of Ethiopia

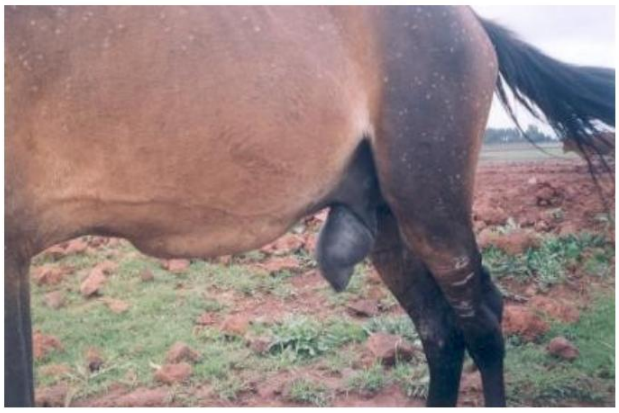
Region	Zone/district	No. of sero positive animals			Serological survey	Questionnaire survey
		CATT	LATEX	ELISA		
Amhara	Awi /Enjebara	1	2	2	Suspected	Absent
Amhara	S. Wollo /Kutaber	4	3	3	Suspected	Absent
Oromiya	Guji / Uraga	18	12	14	Present	Present
Oromiya	Jimma /Dedoo	4	2	2	Suspected	Absent
Oromiya	E. Shoa /Shashemane	6	5	1	Present	Present
Oromiya	Selale /Fitche	0	0	0	Absent	Absent
Somali	Shinelle	3	2	0	Suspected	Absent
SNNPRS	Sidama /Arbegona	9	6	1	Present	Present

Source: Hagos (2005).

Annex 3: Clinical signs of dourine in mare and stallion from Bale highlands of Ethiopia

A

B

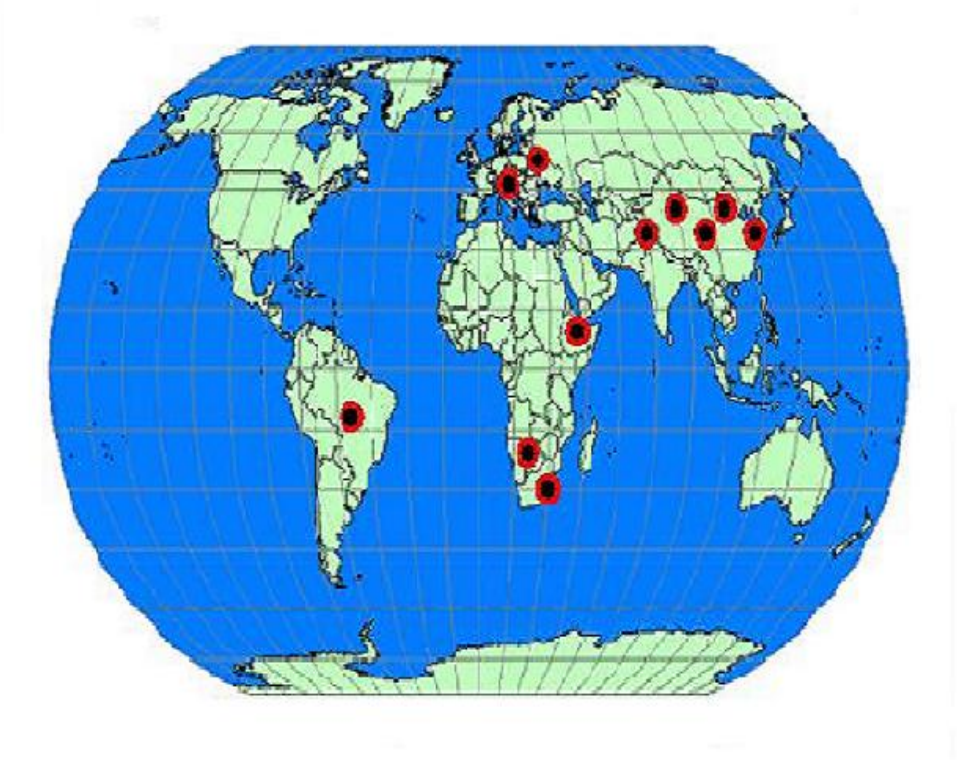


A: Photo showing edematous swelling over the external genitalia

B: Photo showing edematous swelling over the ventral abdomen and penis

Source: Hagos (2005).

Annex 4: Distribution of Dourine based on CFT results



Source: OIE, 2003

Annex 5: Body Condition Scoring Guidance for Horses (According to Coombs, 2002)

Body condition Score	Description of the animal
1. Very thin (Emaciated)	Emaciated animal, weak and lethargic with very little muscle covering bones. Bony structure easily seen over entire body.
2. Thin	Individual ribs, dorsal spinous processes of vertebrae and spine of scapula are sharply defined. Thin neck, sharply angled shoulders. Very little muscle.
3. Less thin	Vertebral column prominent and individual spinous processes can be felt, but some muscle-covering spine. Ribs and bony prominences of rump prominent. Lion and rump areas concave. Little fat or muscle over withers and shoulder.
4. Less than moderate	Body prominences are palpable but less obvious. Vertebral column visible. Rump flat but not concave. Some muscle and fat over withers shoulder and neck.
5. Moderate	Muscle over dorsal of spine developed, can still palpate spinal column. Rump rounded with tuber coxae and tuber ischii no longer visible. Ribs can be felt but not visible. Some fat at base of neck and front of chest.

Annex 6: Questionnaire survey to assess the overall situation of dourine in Bale highlands of Ethiopia

Contact details: -

Date of interview ----------

1. Owners name ----------.

Address: -District ----------PA /Clinic/----------

2. Animal information: - Species----------, Sex----------, Age----------

3. What are the major diseases of equine (horse)? ----------

4. Does the animal have clinical signs of dourine? -Yes/No,

If yes which signs: - ----------

5. Had the animal a problem of dourine previously? Yes/No,

If yes, - When ----------

- Has the animal been treated? ----------

If yes, -when ----------

- Which drug was used? ----------
- Who treated the animal? ----------
- Was the animal cured? Yes, /No, /relapse ----------

6. Had the animals have previously abortion case? Yes/no ----------

If yes, how many times? ----------

7. What do you call dourine by the local name?

8. What is the importance of this disease compared to other diseases? ----------

9. In which season does the disease occur commonly? ----------

10. When did you know the problem of dourine in the area? ----------

11. What is the status of the disease once you know in this area? It is getting better/worse/Nothing is changed/I do not know ----------

12. What are the major factors playing the major roles for transmission and distribution of the disease (dourine) in the area?

Annex 7: Data collection sheet: Serological survey of dourine in four selected horse breeding districts of Bale highlands of Ethiopia

District ----------

Date ----------

N^{o}	Owners name	PA	Animal code	SPP	Sex	Age	Parity number	Previous abortion Yes/No,	Castration status	Body condition

Annex 8: Data collection sheet: Parasitological survey of dourine in four selected horse breeding districts of Bale highlands of Ethiopia

District ----------

Date ----------

N^{o}	Owners name	PA	Animal code	SPP	Sex	Age	Clinical signs	Sample collected		
								Blood	Vaginal discharges	Preputial discharges

Annex 9: Clinical Signs Recording Codes

Code	Parameters
A =	Fever
B =	Oedema of the genitalia
C =	Oedema of the prepuce
D =	Oedema of the scrotum
E =	Swelling of the vulva
F =	Swelling of the udder
G =	Urticarial plaques on the flanks
H =	Weakness
I =	Oedema of the ventral abdomen
J =	Mucoid vaginal or urethral discharge
K =	Ulceration of the genital mucosa,
L =	Incoordination
M =	Lameness of one or both limbs
N =	Muscular atrophy in the gluteal region
O =	Depigmented scars
P =	Ataxia
Q =	Paralysis

Annex 10: Details of the serological tests and laboratory procedures followed in the present dourine study area

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

The CATT/*T. evansi* is direct card agglutination test which uses formaldehyde fixed, freeze dried trypanosomes expressing a predominant variable antigen type of *T.evansi* (Ro Tat 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 homogeneous suspension
- Put a dropper on the vial. The antigen suspension is ready for use.

Reconstitution of the controls

- Using a 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 dilution on a test area of the card
- Add one drop (about 45 µl) of the well homogenised 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 the test area. Wipe off the stirring rod after each use

- Rotate the test card on a flat bed orbital for five minutes at 70 rpm

Reading and interpretation

Evaluate the agglutination reaction as indicated below in the table X as follows:

Result interpretation recording format for CATT/*T.evansi* test

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

B. 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.

Reconstitution of Lyophilized latex reagent

- Resuspend the latex reagent with 1 ml of buffer (PBS). Mix gently for 30 second. 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 diluton is needed. If not used the same day, store at -20^{o}c.

Dilution of the test samples

- Prepare serum dilution 1:2, 1:4, 1: 8, 1:16, 1:32 and 1:64 in buffer (PBS) in a micro plate 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 micro plate, from column 6, to dilute 8 other samples.

Execution of the test

- The test on serum is performed with 20 µl of dilutions 1:2, 1:4, 1:8 and 1:16.
- 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 on the card.

Reading the test result

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

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

Annex 11: Format for describing the distance/ location/ of the different sites of the study area

Study Site (Zone/District)	Distance from A.A (km)	Round Trip distance from A.A
Robe (capital of Bale Zone)	430	860
Dinsho	400	800
Agarfa	425	850
Sinana	430	860
Goba	445	990

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9. CURRICULUM VITAE

Personal Information

Name:	Degefa Guta Tullu
Nationality:	Ethiopian
Sex:	Male
Place of birth:	Sinana, Bale
Date of Birth:	26th August1964
Martial Status:	Married
Academic qualification:	Doctor of Veterinary Medicine (DVM)
Scientific membership:	Member of the Ethiopian Veterinary Association (EVA)

Proficiency

Language skill:

- ✓ Oromifa: Speaking, reading and writing,
- ✓ Amharic: Speaking, reading and writing.
- ✓ English: Speaking, reading and writing,
- ✓ Russian: Speaking, reading and writing,

Educational Background

- 1972-1979: Grade 1-8. Robe Elementary and junior secondary school, Bale Robe.
- 1980-1983: Grade 9-12. Battu Terarra Comprehensive secondary school, Bale Goba.Award: Ethiopian School leaving certificate Examination,
- 1984 Robe Teachers Training Institute Candidate teacher for elementary school Bale Robe.
- 1988-1993. X USSR Moldovian state, Moldovian Agricultural University, Faculty of Veterinary Medicine, undergraduate student in Veterinary Medicine.

- 2005 to the present: Addis Ababa University, Faculty of Veterinary Medicine in tropical veterinary parasitology

Work Experience

- From July 1985-Aug 1988 – Bale Zone Goba, Elementary school teaching at Goba 03 kebele Elementary and junior secondary school.
- From March 10/1994- July 8/2001, Ministry of Agriculture Field veterinarian at Goro and Veterinary Service Team leader at Goba Agricultural and Rural development Office.
- From July 9/2001- February 2005 Ministry of Agriculture Bale Zone Goba head for Agricultural and Rural development Office at Goba Agricultural and Rural development Office.
- Participate on training of Trainers (TOT) for Community based Animal Workers held at Adama that organized by Oromia Pastoralist Area Development Commission (OPADC) and Food and Agricultural Organization from 19-31 March 2007.
- Participate on Refresher training entitled "Veterinary epidemiology and disease control" from 11- 27 April 2007 at Addis Ababa University, Faculty of Veterinary Medicine Debre Zeit, which organized and supported by National Livestock Development Project (NLDP), Federal Ministry of Agricultural and Rural Development, Ethiopia.
- From February 2005 to the present, Bale Zone Agricultural and Rural Development Veterinary Service Team Leader.

10. SIGNED DECLARATION SHEET

I, the undersigned, declare, that this thesis is my original work and has not been presented for a degree in any other university and that all source of materials used for the thesis have been duly acknowledged.

Name: Degefa Guta Tullu

Signature: _____

Date of submission: June 25, 2008

This thesis has been submitted for examination with our approval as University advisors.

Academic Advisors: Signature

Dr. Hagos Ashenafi (D.V.M, MSc $^{+}$, Assistant Professor) _____

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