

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

**ASSESSMENT OF PREVALENCE AND HOST- RELATED RISK FACTORS OF
CAMEL TRYPANOSOMOSIS IN SELECTED DISTRICTS OF AFAR PASTORAL
AREA, NORTH EAST ETHIOPIA**

BY
ANDUALEM YIMER

JUNE, 2012
DEBRE ZEIT, ETHIOPIA

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LIST OF ABBREVIATIONS

Ab	Antibody
Ag	Antigen
ANOVA	Analysis of variance
APARDB	Afar Pastoral Agricultural and Rural Development Bureau
BCT	Buffy coat technique
bp	base pair
BSA	Bovine Serum Albumin
CSA	Central Statistics Agency
CATT	Card Agglutination Test for Trypanosomes
CFT	Complement Fixation Test
CI	Confidence interval
DNA	Deoxy Ribonucleic Acid
DNTPS	Deoxynucleotide triphosphates
EDTA	Ethylenediamine-Tetraacetic Acid, Tri Potassium Salt
ELISA	Enzyme Linked Immuno-sorbent Assay
FAO	Food and Agricultural Organization
GSTBS	Geimsa Stained Thin Blood Smear
HCT	Haematocrit centrifugation technique
IFAT	Indirect Fluorescent Antibody Test
ILRAD	International Laboratory for Research on Animal Diseases
IM	Intramuscular
ITS -1	Internal Transcribed Spacer One
kDNA	Kinetoplast Deoxy Ribonucleic Acid
MASL	Meters above sea level
mAECT	Mini Anion Exchange Centrifugation Technique
MoARD	Ministry of Agriculture and Rural Development
MPS	Mononuclear phagocytic system
NTTICC	National Tsetse and Trypanosomiasis Investigation and Control Centre

OIE	Office International des Epizootes
OR	Odds ratio
P	Probability of an event due to chance alone
PA	Pastoral Associations
PATEC	Pan African Trypanosomosis and Tsetse Eradication and Control
PCR	Polymerase Chain Reaction
PCV	Packed cell volume
pH	Negative Log of Hydrogen Ion Concentration
PLITM	Prince Leopold Institute of Tropical Medicine
RBC	Red Blood Cell
rDNA	Ribosomal Deoxy Ribonucleic Acid
RoTat	Rhode Trypanosoma Antigenic Type
RNA	Ribose nucleic acid
SD	Standard deviation
SIT	Sterile Insect Technique
TAE	Tris Acetate Ethylene Diamine Tetra- Acetic Acid
UV	Ultra violet
VSG	Variable Surface Glycoprotein
VAT	Variable Antigenic Type

ABSTRACT

A cross-sectional study was conducted in two districts of Afar region eastern Ethiopia from November 2011 to April 2012 to estimate the prevalence and host-related risk factors for camel trypanosomosis. Accordingly, blood samples were collected from randomly selected 400 camels and examined for parasite detection by Giemsa stained thin blood smear, presence of agglutinating antibodies against *T. evansi* using card agglutination test for trypanosomosis (CATT/*T. evansi*) and ITS-1 PCR test. The overall prevalence of 2 %, 24 %, and 30.5 % using thin blood smear examination, CATT/*T. evansi* and ITS's-1PCR tests, respectively were recorded. *T. evansi* (20%) was found to be the prominent trypanosome affecting camels followed by *T. vivax* (7.5%) as detected by ITS-1 PCR in the study districts. Pregnant camels (OR= 2.74, CI: 1.37- 5.50) and lactating camels (OR= 2.6, CI: 1.34-5.06) were 2.7 and 2.6 times more likely to be infected with trypanosomes compared to non pregnant and non lactating camels, respectively. While camels with poor body condition OR of 2.96 (CI: 1.55- 5.67) were three times more likely to be infected compared to camels with moderate body condition. PCR detected infections in 114 (29%) camels out of 392 camels which were negative based on the parasitological method. Consequently, PCR technique is more sensitive method for diagnosis of trypanosome infected camels than parasitological techniques, and can detect low parasitemic camels in chronic cases. From the results of the present study it can be inferred that camel trypanosomosis is endemic in Chifra and Dewe districts of Afar region, Ethiopia where it demands further study on identification of the principal vector species responsible for transmission and appropriate control and preventive measure should be applied to circumvent the impact of camel trypanosomosis in the surveyed districts.

Keywords: Camel, CATT/*T.evansi*, Ethiopia, ITS1 PCR, Prevalence, Risk factors, Trypanosomes

1. INTRODUCTION

Camels (*Camelus dromedarius*) are vital domestic animal species that are best adapted to harsh environments and fluctuating nutritional conditions of arid and extreme arid zones, as a source of milk, meat and is used for transportation and racing. Thus they are important animals in the socio-economic system of Asia and Africa (Ali *et al.*, 2009). In Ethiopia, as in most dry lands of Africa and Asia, camels are the principal source of income and food for millions of pastoralists. In addition, camels play a central role in providing draught power and determining the wealth and social status of pastoralists. There are approximately 2.3million heads of dromedary camels in Ethiopia, placing it at the third position in camel rearing countries after Somalia and Sudan (FAO, 2008).

In Ethiopia camels inhabit almost all the arid and semi-arid lowlands of eastern and south-eastern part of the country, where pastoral and agro-pastoral production systems predominate. These areas include the major parts of the Somali and Afar National Regional States and some parts of the Oromyia National Regional State (Tezera and Kassa, 2002). The Ethiopian pastoral and agro-pastoral areas are estimated to occupy about 63% of the total area of the country, and are home to 12% of the total population and 100% of the total estimated camel population of the country (MoARD, 2008). The Afar region covers 10% of the total area of the country and 29% of the pastoral lowlands. The regional population is estimated to be 1.2 million of which 90% are pastoralists and 10% agro-pastoralists. The livestock population in Afar national regional state is estimated to reach 2.3 million cattle, 2.5 million sheep, 4.3 million goats and 0.85 million camels (CSA, 2008).

Despite all its ecological and economic importance and significant role in the life of pastoral community, until recently the animals were neglected by researchers to assess the impact of diseases on camel productivity in Ethiopia. This can partly be due to the fact that the production is usually a migratory system in remote areas with harsh living conditions and poor infrastructure; the animals are presumed to be inaccessible for veterinary service delivery (Ali *et al.*, 2009). Although camels are hardy animals to tolerate feed shortages and different infections than most

other domestic animals in general, they do suffer from different parasites and other diseases, the severity of which usually depends on the animal's nutritional status (Richard, 1979; Demeke, 1998).

The main factors of concern for the pastoralists of the camel-rearing areas are the low fertility of camels and the high mortality in young animals. All the camel-rearing areas do not test for trypanosomosis regularly. Consequently, these animals become carriers of the trypanosome organism and thus a potential source of infection which can contribute to the significant health risks existent in these areas (Bekele, 2004). Trypanosomosis, camel pox, contagious skin necrosis, pneumonia, mange mite infections and internal parasites are among the major health problems previously reported in camels in Ethiopia (Demeke, 1998).

Camel trypanosomosis is caused by infection with the protozoal parasites trypanosomes which are mostly transmitted mechanically independent of tsetse flies, is the most important health problem of camels in many countries including Ethiopia (Thewodros and Getachew, 2001). The disease manifests itself in different forms: acute, sub-acute, chronic and unapparent. The chronic form is the most common and is characterised by severe anaemia, general wasting, reduced milk yield, infertility, abortions and death in some animals (Olaho-Mukani *et al.*, 1996). The disease is most important single cause of economic losses in camel rearing areas, causing morbidity of up to 30.0% and mortality of around 3.0% (Njiru *et al.*, 2001). Likewise a number of studies have been conducted on camel trypanosomosis in different parts of the country. For instance epidemiological studies on camel trypanosomosis were conducted by (Richard, 1979; Elias, 2003; Hagos *et al.*, 2009). These studies were carried out using parasitological and serological tests, however these tests are succumbed to low sensitivity and are only conducted in certain parts of the country. To properly understand the epidemiology of the disease and for control and prevention of the disease accurate diagnosis of trypanosome infection in livestock is required (Ali *et al.*, 2009).

Because of the range of agro-ecological zones and the diverse farming systems in which the disease occurs, as well as its debilitating effects on a variety of livestock, the disease has attracted international attention in recent years, with a focus on formulating and implementing effective control strategies aimed at increasing productivity and achieving a decrease in mortality and

morbidity (Obihiro, 1998). Control of camel trypanosomosis depends mainly on the use of curative and prophylactic drugs. Treatment recommendations and strategies for chemotherapeutic control depend on information of trypanosomosis risk and the prevalence of trypanocidal drug resistance in the area (Jones and Davila, 2001).

Parasitological methods used in the diagnosis of *T. evansi* in camels are considered easy, rapid and economic. However, they are not sufficient to detect all trypanosome infected animals, especially in case of low parasitaemia and also in the chronic form of the disease. The serological test such as the card agglutination test (CATT/*T. evansi*) is used for the detection of antibodies circulating in the serum of infected camels, the test could be used under both laboratory and field conditions. It is a quick, simple and easy to perform and sensitive, but with deficiency to distinguish current and past infection (Magnus *et al.*, 2002). To circumvent this problem diagnostic tests based on DNA have been developed. Molecular diagnostic test, Polymerase chain reaction, (PCR) in particular in the widely used means of diagnosis of animal trypanosomosis. Molecular tests are reported to be more sensitive. In recent time camel trypanosomosis was also reported to be caused by other trypanosomes apart from *T. evansi*, thus use of molecular tool could help to assess the possibility of infection by other trypanosome infection such as *T. vivax* (Salim *et al.*, 2011).

Therefore the objectives of this research were

- ☞ To estimate prevalence of camel trypanosomosis in the study area using different diagnostic tools including PCR
- ☞ To compare degree of agreement of three diagnostic tests: parasitological, serological and molecular techniques in the detection of camel trypanosomosis
- ☞ To assess host related risk factors associated with trypanosome infection in camel

2. LITERATURE REVIEW

2.1. Classification of Trypanosomes

Trypanosomes are unicellular flagellated protozoa belonging to the phylum of Sarcomastigophora, the order of Kinetoplastida, the family of Trypanosomatidae and the genus of *Trypanosoma*. They are tissue and blood parasites, which in the vertebrate host occur in the blood and tissue fluid and within that groups are known as haemoflagellates, as they progress activity by the movement of flagellum in the blood (Mehlhorn, 2000).

Table 1: General classification of trypanosomes

Sub kingdom	Protozoa
Phylum	Sarcomastigophora
Subphylum	Mastigophora
Class	Zoomastigophorea
Order	Kinetoplastida
Sub order	Trypanosomatina
Family	Trypanosomatidae
Genus	<i>Trypanosoma</i>

Source: Desquesnes and Davilla, 2002

The genus *Trypanosoma* is further divided into two sections on the basis of their invertebrate cycle and preferred host species: The Salivaria; which contains the subgenera, *Duttonella*, *Nannomonas*, *Pycnomonas* and *Trypanozoon*. Salivarian trypanosomes complete their cyclical development in the 'anterior station' of the vector and infective stages are transmitted to the mammalian host through the bite of an infected fly. The other section is the *Stercoraria* which

include three subgenera, *Megatrypanum*, *Herpetsoma* and *Schizotrypanum*. The Stercoraria contain species in which the entire development is confined to the gut of the vector and infective meta-trypanosomes can be found in the faeces of vectors (Stephen, 1986).

Table 2: Classification of trypanosomes of veterinary importance, development and transmission

Subgenus	Species/group	Development/transmission
Duttonella	Vivax group	In tsetse: Proboscis
	<i>T. vivax</i>	Also by mechanical transmission
	<i>T. uniforme</i>	
Nannomonas	Congolense group	In tsetse: midgut and proboscis
	<i>T. congolense</i>	Not known to maintain itself by mechanical transmission
	<i>T. simiae</i>	
	<i>T. godfreyi</i>	
Trypanozoon	Brucei group:	In tsetse: midgut and salivary glands
	<i>T. brucei brucei</i>	Oral transmission in carnivores
	<i>T. brucei rhodesiense</i>	
	<i>T. brucei gambiense</i>	
	<i>T. evansi</i>	Mechanical transmission
	<i>T. equiperdum</i>	Venereal transmission

Online Source: [http:// www.fao.org/ docrep/006/x0413e/ xo413E02.htm](http://www.fao.org/docrep/006/x0413e/xo413E02.htm)

2.2. Pathogenic trypanosomes

All trypanosomes are heteroxenous which means they require more than one host to complete a life cycle. There are numerous different trypanosomes and the effect of an infection varies greatly based on the trypanosome specie. There are some species that hardly seem to be virulent at all, as for instance *T. theileri*, where effects only can be seen if the host is already sick. Other species are highly virulent and cause severe diseases in their mammalian hosts. Trypanosomes cause a large

number of diseases in different species of vertebrate hosts. Some trypanosomes can infect several vertebrate hosts whereas others are host-specific (d'Ieteren *et al.*, 1998).

In humans there are two diseases that are caused by trypanosomes; Human African Trypanosomosis (HAT), also called sleeping sickness, which is caused by *T. brucei gambiense* and *T. brucei rhodesiense*, and Chagas disease in America which is caused by *T. cruzi*. Other examples of diseases caused by trypanosomes are Dourine in horses caused by *T. equiperdum*, and Surra in horses and camels caused by *T. evansi*. Cattle are infected by the virulent *T. brucei brucei*, *T. congolense* and *T. vivax* as well as the avirulent *T. theileri*. Often *T. vivax* is found to be responsible for the first infections in cattle, whereas *T. congolense* often is considered to be the most virulent of the trypanosomes in cattle (d'Ieteren *et al.*, 1998).

2.3. Camel trypanosomosis

2.3.1. History and origin of the disease

Most people generally associate trypanosomosis with tsetse flies (*Glossina* spp.) and tropical Africa, however camel trypanosomosis proves an exception. Since most camels live outside of Africa's tsetse belt (NTTICC, 2004, Sinshaw *et al.*, 2006), majority of camels suffer from trypanosomosis caused by *T. evansi* and less likely by mechanically transmitted *T. vivax* which are spread mechanically independent of tsetse flies. Camels are also affected to a lesser extent by the tsetse-transmitted trypanosome species *T. brucei* and *T. congolense* (Evans *et al.*, 2005).

Although various scientists had observed 'motile elongated microorganisms' in the blood of fish and amphibians it was Gruby who in 1843 created the generic name *Trypanosoma*. The first pathogenic mammalian species of *Trypanosoma*: *T. evansi* was discovered in 1880 by Evans in India, when investigating a disease of horses known by the local Indians as 'Surra' (meaning low and rotten) (Evans, 1882). In the same work he also demonstrated experimentally that *T. evansi* was most likely transmitted by haematophagous flies. Since then, studies have shown that the

parasite can infect all species of domesticated livestock, although the principal host varies geographically (Al-Rawashdeh *et al.*, 2000).

Many different scientific names for the parasite were used, until it was found that all these non-tsetse transmitted organisms of the subgenus *Trypanozoon* (except *equiperdum*) were *evansi* (Indrakamhang, 1998). *T. evansi* is related to the African trypanosomes and is thought to have evolved from *T. brucei*, the cause of nagana in animals in Africa. It is thought that *T. evansi* evolved from its ancestors along the edges of the tsetse fly belt in Africa and from there was spread via infected camels used for trade with India. Continuous mechanical transmission by blood-sucking flies in the absence of *Glossina* caused the loss of cyclic transmissibility and gave rise to a predominance of slender parasite forms (Hoare, 1996). Lick wise *T. vivax* is geographically the most extensive pathogenic trypanosome, it has been found over an enormous area of Africa with livestock movements and mechanical transmission extending its range outside the tsetse belts (Gardiner and Wilson, 1987). The hosts of *T. vivax* include cattle, water buffalo, sheep, goats, camels, horses and donkeys (Stephen, 1986; Gardiner, 1989; Urquhart *et al.*, 1996).

2.3.2. The parasite and its biology

2.3.2.1. Taxonomy and identification

The subgenus *Trypanozoon*, the brucei group, comprises five members: *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, *T. evansi* and *T. equiperdum*, which exactly similar in morphology, but only *T. brucei gambiense* and *T. brucei rhodesiense* are the cause of human sleeping sickness (FAO, 2000). *T. evansi* is morphologically identical with, and indistinguishable from, slender forms of other members of the subgenus *Trypanozoon* and is described as **monomorphic with length of 15 to 34 µm**. Leaf-like slender forms are characterized by a long free flagellum, which may be up to one half of the length of the organism with narrow and drawn out posterior end (Figure 1). Kinetoplast is terminal or sub terminal in position and the nucleus is central (Queiroz *et al.*, 2000).

The kinetoplast is a complex network of mitochondrial DNA which is concentrated in part of the parasites single, elongated mitochondrion and is attached to the basal body of the flagellum

(Robinson and Gull, 1994). The kinetoplast DNA which consists of mitochondrial DNA is organised in structures called maxicircles and minicircles (Simpson, 1986). Maxicircles which play an important role in cyclic transmission in tsetse flies, are absent in mechanically transmitted *T. evansi*, while the sequence of the minicircle kDNA of various isolates of *T. evansi* is consistent. Likewise *T. vivax* as seen in the blood of mammals is essentially monomorphic, with a free flagellum. Its length, including the free flagellum, varies from 18 μm to at least 26 μm . The kinetoplast is large and terminal or almost so. It is much larger than any of the other pathogenic species, and this is a distinguishing feature (Borst *et al.*, 1987).



Figure 1: *T. evansi* isolated from the blood of an infected camel (Giemsa stain).

Source: (Kirsten, 2007)

2.3.2.2. Life cycle and transmission

Based on mode of transmission, trypanosomes could be classified as cyclical, mechanical and venereally transmitted. Thus, blood stream forms (trypomastigotes) ingested by the fly undergo considerable changes, in morphology as well as in their metabolism. They change into long slender forms called epimastigotes, which multiply and finally give rise to the infective metatrypanosomes. The infective metatrypanosomes undergo development and multiplication at the site of infection and finally the mature blood trypanosomes (trypomastigotes) are released via lymph vessels and lymph nodes into the blood circulation (Liu-Liu *et al.*, 2005).

Replication of the trypanosome occurs by longitudinal binary fission both in the host and in the vector with the flagellum and kinetoplast dividing together (Liu-Liu *et al.*, 2005), but in the non cyclically transmyted trypanosomes developmental stages were not observed in any of the mechanical vectors. Consequently a procyclic or insect stage (epimastigotes) does not exist in *T. evansi* which is attributed to the lack of maxi circles in the kinetoplast DNA (Ellie *et al.*, 1999). The non-cyclical transmission of thus trypanosomes is aided by biting flies and thus, in the absence of *Glossina*, the transmission is maintained in the ecosystem. Biting flies, such as *Tabanids* (horse flies), *Stomoxys* and *Hippoboscids* (camel flies), transmit mechanically through their mouthparts when they feed on more than one host within a short interval between interrupted blood meals (Evans *et al.*, 2005).

The importance of this mode of transmission is variable from place to place, depending on the numbers of hosts and biting insects present, and also on the species of trypanosome. The parasitemia of the donor most often determines the number and types of vectors required to transfer infection. Certainly, the number of insects and the quantity of blood transferred between hosts is important (Rogers, 2003).

2.3.2.3. Antigenic variation and immune evasion

Metacyclic and blood stream trypanosomes possess a glycoprotein (Variable Surface glycoprotein, VSG) coat, which is antigenic and provokes the formation of antibodies that cause opsonization, and lysis of the trypanosomes. It is likely that the VSG repertoire of *T. evansi* is smaller than that of trypanosomes with a tsetse fly intermediate host because exchange of genetic information and rearrangement of VSG repertoires occurs in this vector. Perhaps the most important aspect of trypanosomosis, which accounts for the persistent parasitaemia, is the way in which the parasite evades the immune response of the host (Engstler *et al.*, 2007).

During the ascending phase of the parasitemia, the majority of parasites are of the same antigenic type called homotype (Figure 2a). The host immune system recognizes this homotype and makes antibodies against it. As the parasites of the major variable antigenic type (VAT) are eliminated

the parasitemia goes in descending phase but at the same time, the parasites expressing the heterotype (Figure 2b) or the minor VATs are multiplying and one of them overgrows others. As a result this one becomes the new homotype (Figure 2c), leading to a new wave of parasitemia and resulting in a long-lasting chronic infection. So expression of the VSG is central in the antigenic variation process and eventually for exhausting the host immune system in the benefit of the parasite, which helps the parasite to survive attack by the host's immune system (Field *et al.*, 2009).

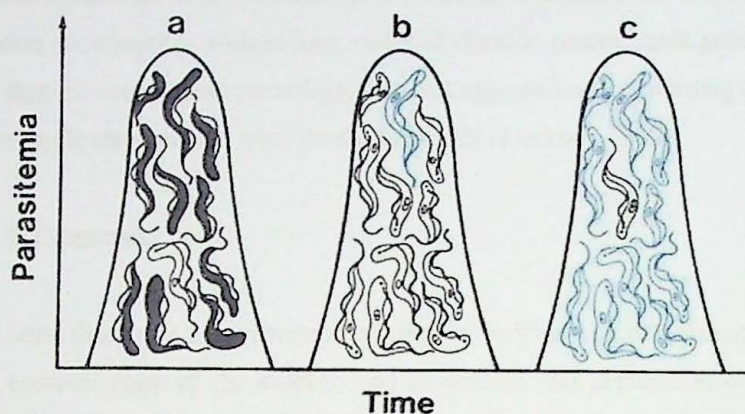


Figure 2: Schematic diagrams illustrating the process of antigenic variation in trypanosome population.

Online source: <http://www.ilri.org/InfoServ/Webpub/fulldocs/ilrad89/image/FIG20.jpg>

2.3.3. Clinical manifestations

T. evansi can infect a variety of hosts and causes a species-specific pathology. In camels trypanosomosis occurs both in chronic and acute forms (Payne *et al.*, 1990). The acute form of the disease in camel may last for up to three months and is characterized by irregular fever, reduced appetite and water intake, as the disease progresses hump disappear (FAO, 2000), recurrent keratoconjunctivitis and urticarial plaques on the neck and flank, dependent oedema under the belly, marked depression, dullness, loss of condition, the hair coat become dull and rough with loss of hair at the tail and often rapid death (Luckins, 1998). There is also pallor of mucous

membranes of the eye, the urine usually has a characteristic smell and a fluctuating temperature with initial peaks of up to 41°C and increases in body temperature correspond with peaks of parasitaemia. Anaemia is observed to be a major clinical finding in camel trypanosomosis (Rami *et al.*, 2003).

Chronic cases develop recurrent episodes of fever. Some camels develop oedema in their dependent parts of the body, urticaria plaques and petechial haemorrhages in serous membranes. Death finally ensues if untreated however; some may harbour trypanosomes for 2-3 years thus constituting reservoirs of infection to susceptible camels and hosts. Other well documented field reports are abortion, weight loss, reduced draught power, milk production and nervous signs like circling movement and trembling, unusual aggressiveness, running aimlessly and sudden collapse in severely stressed and over worked animals (Luckins, 1998).

2.3.4. Diagnosis

Accurate diagnosis of trypanosome infection in livestock is required for a proper understanding of the epidemiology of the disease and in control and prevention of the disease. Besides clinical diagnosis, direct (parasitological) and indirect (serological tests) diagnostic methods with varying degrees of sensitivity and specificity are available for diagnosis of trypanosomosis (Gardiner, 1989). Other diagnostic methods including capillary concentration technique, biochemical tests, serological tests, and the molecular tests DNA – Probes and PCR techniques could be used which are discussed in short below.

2.3.4.1. Clinical diagnosis

A tentative diagnosis can be reached without microscopy, by taking into account the owner's observations and clinical examination of camels in the field as dromedaries are usually far away from laboratory facilities. Chronic form is most common and may present an association with secondary infections due to immunosuppression caused by *T. evansi* infection, which complicates clinical diagnosis. Fever and/or anaemia are the clinical indicators of camel trypanosomosis, but these signs are not pathognomonic (Dargantes *et al.*, 2005).

2.3.4.2. Parasitological diagnosis

Several techniques are available depending on the type of diagnostic approach followed. Parasitological diagnosis is the direct demonstration of the parasite in blood or less commonly in other body fluids using a microscope. Trypanosomes can be detected in blood 13 to 16 days after an infective fly has had a meal to confirm infection. For parasitological tests, a thick or thin wet blood smear can be performed and examined under light microscopy (OIE, 2004). For infections with a low parasitaemia, concentration techniques can be carried out, where the trypanosomes are concentrated into a specific region and then observed under light microscopy. However, the test has a poor sensitivity, often less than 50%. The implication of this is that in most situations *T. evansi* is under-diagnosed and the level of infection is greater than frequently reported (Luckins, 1992; Yadvendra *et al.*, 1998).

Direct examination techniques

The simplest techniques are examination of wet, thin or thick films of fresh blood, usually obtained from the ear vein, jugular vein or tail or from peripheral lymph node aspirations.

Thick blood films

They are made by placing a droplet of blood on a clean microscope slide and covering with a cover slip (22 to 24mm). The blood is examined microscopically at total magnification of x400 with condenser aperture, phase contrast. Trypanosomes can be recognized by their movement among the red blood cells (RBC). The advantage of this method is: it is simple, inexpensive and gives immediate results. Depending on the trypanosome size and movements a presumptive diagnosis can be made of the trypanosome species. This method is relatively less sensitive, with a detection limit of about 8300 trypanosomes per ml of blood, and finding the parasite may also depend on the examiners experience (Paris *et al.*, 1982).

Thin blood smear

These are made by placing a drop of blood (5-10 μ l) on a clean microscope slide and spreading it over an area of approximately 2 cm in diameter, using the corner of another slide. The film is dried thoroughly by rapidly waving in the air. It is stained for 30 minutes with diluted Giemsa stain. Slides are then washed in tap water and dried. Examine at a magnification of 40–100 $\times$ with oil immersion. This technique permits detailed morphological studies and identification of the *Trypanosoma* species, but may be damaged during the staining process and it is of a very low sensitivity (it can detect parasitaemia >500,000 trypanosomes/ml of blood) (OIE, 2010).

Concentration methods: Buffy coat examination (also called the Woo method).

In this techniques blood collected in heparinized tube will be transferred to capillary tubes. Blood is taken up at one end, until about three-quarters of the length of the tube is filled and centrifuged at 12000 rpm for five minutes after sealing the other end of the tube by special wax or plasticine. Upon centrifugation the blood will be separated in to three components: a liquid, the plasma, the red cells (erythrocytes), white cells and thrombocytes (Woo, 1970). This concentration technique has been standardized by the use of microhaematocrit capillary tubes and a specially designed centrifuge. The packed cell volume (PCV) value can be read directly on a special reader. The tube is then examined for the presence of trypanosomes, either by direct examination of the Buffy coat/plasma junction, or after making a smear of this area. For direct examination the capillary tubes are placed in special viewing chamber, to which clean water is added, covered with cover slip and examined at x40 magnification power. Woo method has higher sensitivity than that of the direct methods, but this also depends on the species of trypanosome. The fresher the sample, the better is the sensitivity as strong parasitic movements make trypanosomes more visible. This technique can detect around 50– 200 trypanosomes/ml of blood (Reid *et al.*, 2001).

Mini-anion exchange centrifugation technique (mAECT)

This is the most sensitive method for trypanosome detection in blood and is based on a purification technique and adapted for diagnosis of animal infections with *T. brucei* and *T. evansi*

(Gutierrez *et al.*, 2004). In mAECT, trypanosomes are separated from 350 µl of blood by anion exchange chromatography on diethylaminoethyl cellulose (DEAE). When a blood sample from infected animal with trypanosomes is passed through an appropriate anion-exchange column, the host blood cells, being more negatively charged than trypanosomes, are absorbed into the anion-exchanger, while the trypanosomes are eluted, retaining viability and infectivity (Waitumbi *et al.*, 1994). The sensitivity of this technique can be increased by some approximately tenfold by the use of buffy coat preparations rather than whole blood eluted. Trypanosomes are then concentrated by low speed centrifugation followed by direct microscopic examination of the sediment in a transparent collector tube. The large volume of blood examined allows detection of fewer than 50 trypanosomes/ml (Gutierrez *et al.*, 2005).

Animal inoculation

Laboratory animals may be used to reveal subclinical (non-patent) infections in domesticated animals. *T. evansi* has a broad spectrum of infectivity for small rodents, and so rats and mice are often used. Rodent inoculation is not 100% sensitive (Monzon *et al.*, 1990) but further improvement in its efficacy can be obtained by the use of buffy coat material. Such a procedure was able to detect as few as 1.25 *T. evansi*/ml blood (Reid *et al.*, 2001). This technique is suitable when highly sensitive detection is required by inoculating heparinised blood intraperitoneally into rats (1–2 ml) or mice (0.25–0.5 ml) and sensitivity of this *in vivo* culture system may be increased by use of immunosuppressed laboratory animal (Monzon *et al.*, 1990). However, lab animals are resistant to *T. vivax* (Hoare, 1972).

2.3.4.3. Molecular Tests

The principle of molecular tests is the demonstration of the occurrence of sequences of nucleotides, which are specific for a trypanosome subgenus, species or even type or strain. A positive result indicates active infection with the trypanosome for which the sequences are specific. As parasite DNA will not persist for long in the host after all live parasites have been eliminated. These tests are not only suitable for detecting parasites in the mammalian host, but also in the insect vector (Holland *et al.*, 2001). There are a number of molecular techniques used

as diagnostic methods which depend on detection and identification of DNA of infectious organism. But recently detection of DNA based on polymerase chain reaction takes the lion share.

PCR tends to be highly specific and sensitive and may constitute as the gold standard test for many infections. The PCR process allows a small amount or a specific region of DNA to be amplified, using precise temperature conditions and ingredients like primers, which read the specific region of DNA, a DNA polymerase, and deoxynucleotide triphosphates (dNTPs), which build up the new DNA copy (Holland *et al.*, 2001). However, most molecular tests usually require well-equipped laboratories, expensive reagents and well-trained personnel. Due to such features, these techniques remain primarily as research tools and are not currently utilized as routine diagnostic tests.

PCR allows the amplification of a specific DNA segment for which, at least, 18–30 bases of the extremity sequences are known. Following 30–40 PCR cycles, the DNA will theoretically have been amplified approximately 1 billion times, and the resulting PCR product can be visualised on an agarose or polyacrilamide gel, after staining with ethidium bromide and exposing it under ultraviolet light. The specific size of the PCR product is evaluated by simultaneous migration of molecular size markers and a positive control. A negative control is run together to evidence any DNA contamination. In some instances, primers can amplify more than one tandem repeat which gives several specific bands however, a sample is positive when, at least, the single product is observed. Generally, once the specificity of the primers has been established, the size of the PCR product is sufficiently characteristic for diagnostic purposes (Desquesnes and Dávila, 2002).

PCR applied to trypanosomal detection

The first developments of PCR for trypanosome identification were published by Moser *et al.* (1989). Dially *et al.* (1994) followed by Ijaz *et al.* (1998), have described oligonucleotides that recognize *T. evansi* but do not react with *T. brucei* spp. The preferred PCR targets are those which are present in a high copy number in the genome of trypanosomatids; the more copies of the target, the greater the chances of amplifying it by PCR. Single copy genes are more difficult to amplify and are rarely targeted since low parasitaemia is a characteristic of trypanosome infection

and the sensitivity would be too low. *T. evansi*, do not present maxi-circles in their kDNA network (Masiga *et al.*, 1992). Kinetoplastid mini-circle DNA sequences have been a useful target for development of specific primers for detection of *T. evansi* and *T. cruzi*. Internal transcribed spacers (ITSs) of ribosomal DNA are suitable targets for PCR-based trypanosome diagnosis which are widely used (Desquesnes *et al.*, 2001).

ITS1 PCR, Ribosomal DNA Spacer

Internal transcribed spacer (ITS) region of the ribosomal RNA locus of eukaryotes have a high copy number (100-200 copies), having a small size (300 – 800bp), and vary both in sequence and length between species, but highly conserved in size in a given taxon, making it a preferred diagnostic target for universal test. An identification system using ITS-1 was proposed to replace the multiple species- specific tests for identification of African trypanosome species (Desquesnes *et al.*, 2001). There is sufficient interspecies length variation in the ITS-1 region to enable identification of trypanosome species by the size of the PCR- amplified product. It is quicker and three to five times cheaper than the species- specific technique, because the number of PCRs required per samples is greatly reduced, and therefore larger numbers of samples can be processed. It is also able to detect mixed infections in a single PCR, which can be identified by the presence of multiple bands (Desquesnes and Davila, 2002).

2.3.4.4. Serological Tests

The aim of serological tests is to detect specific antibodies, developed by the host against the infection or, inversely, to demonstrate the occurrence of circulating parasitic antigens in the blood by the use of characterized specific antibodies. The detection of antibodies indicates that there has been infection, but as antibodies persist for some time (weeks, sometimes months) after all trypanosomes have disappeared from the organism (either by drug treatment or self-cure) a positive result is no proof of active infection. Ab-detection tests are sensitive, cannot distinguish current from cured infections (Luckins, 1988). However, serological techniques; immunofluorescent antibody test (IFAT), antibody detection enzyme Linked Immunosorbent Assay (*ab*-ELISA) and the Card Agglutination Test for Trypanosomosis (CATT) have been

developed for immunodiagnosis of trypanosomosis based on the detection of immune responses of the animal to the infection (OIE, 2010).

On the other hand, circulating trypanosomal antigens are eliminated quickly after the disappearance of the trypanosomes, and their presence therefore shows almost always that live trypanosomes are present in the animal (Luckins, 1992). Demonstration of trypanosomal antigens in the blood of the infected animal would be synonymous with parasitological diagnosis (Voller and Desavigny, 1981). Recent tests like latex agglutination test (LAT) or Surratex and antigen detection ELISA (ag-ELISA) based on trypanosome- antigen detection in blood or serum, are more reliable and have shown a high correlation with patent or sub-patent disease in camels (Olaho-mukani *et al.*, 1996).

Card agglutination test for trypanosomosis/T. evansi (CATT/T. evansi)

The CATT is a rapid agglutination test, originally developed to detect infection with *T. brucei gambiense* in humans (CATT, Testryp®) that was later applied to the diagnosis of animal trypanosomosis. The test uses a freeze-dried, Comassie-stained whole trypanosome antigen representing a single VAT. AnTat 1.8 and LiTat 1.3 of *T. b. gambiense* are used to diagnose sleeping sickness in humans (Kageruka *et al.*, 1996) and Rode Trypanozoon antigen Type (RoTat 1.2) is used for surra (Bajyana and Hamers, 1988). It is able to detect anti-*T. evansi* antibodies in livestock 7 to 9 days after experimental infection with *T. evansi* (Wernery *et al.*, 2001; Hilali *et al.*, 2004). The VAT RoTat 1.2 antigen was originally cloned from an isolate of *T. evansi* collected from a buffalo in Indonesia in 1982. It is known to be specific (Plitm, 2001; Claes *et al.*, 2002) and predominant among *T. evansi* worldwide (Verloo *et al.*, 2001).

Recent studies have demonstrated that a small number of isolates of *T. evansi* from camels in Kenya did not possess the RoTat 1.2 VSG gene and that animals infected by these isolates were negative when tested using the CATT (Ngaira *et al.*, 2004; Ngaira *et al.*, 2005). However, RoTat 1.2 antigen has been demonstrated in two isolates of *T. evansi* from Mindanao (Verloo *et al.*, 2001; Njiru *et al.*, 2006). Consequently, the CATT sometimes gives false negative results, particularly in other trypanozoal infections, since such prevalent VATs do not occur in all species

of trypanosomes. Moreover, the CATT also provides false positives, as it is based on agglutinating antibody detection. So CATT alone cannot differentiate between infected individuals and some treated former patients (Kashiwasaki *et al.*, 2000; Zeyed *et al.*, 2010). The test is performed on a plastic card with 10 reaction zones. The reconstituted antigen suspension is mixed with blood or diluted serum/plasma and rotated for five minutes at 60 to 70 revolutions per minute on a card test rotator (Plitm, 2001). Blue clumping indicates the presence of antibodies in the test sample. The test is not strictly species specific. This may complicate the interpretation of positive results in areas where other species of Salivarian trypanosomes occur (Plitm, 2001). The sensitivity of CATT relayed to early expression of the antigen VAT RoTat 1.2 after infections, thus anti *Trypanosoma* spp. antibodies are first detected at day 4 to 7 from infection and persist long after (Ngaira *et al.*, 2004).

2.3.5. Treatment and control

The aim of control and prevention is to break the vector transmission cycle in camels and elimination of trypanosomes from the blood of animals or elimination of the vectors from the environment (Luckins, 2000). However there is no obvious way of developing exclusion zones for animals on grazing land and limited likelihood that vector control will be successful compared to tsetse-transmitted trypanosomes as the population of biting flies are extremely numerous, widely distributed, and difficult to deal with (Jones and Davila, 2001) .

Currently the strategy for treatment and control depends mainly on chemotherapy and chemoprophylaxis. The major problems are availability of few drugs, development of drug resistance, high price of drugs and toxicity to the drugs narrow therapeutic index. Treatment of surra depends largely on four drugs: suramin, diminazene aceturate (Berenil[®]), melarsomine (cymelarsan[®]) and quinapyramine. Suramin and quinapyramine have been used for the treatment of *T.evansi* infection in camels, and only recently melarsomine (cymelarsan[®]) was introduced for the treatment of surra in camels because of the problem of drug resistance. Most drugs are either not curative such as homidium bromide, or are too toxic for camels such as diminazene aceturate (Bourdichon, 1998). Treatment of *T. evansi* infected camels in Morocco with melarsomine (cymelarsan) reduced the sero prevalence level from 58 to 19% within a year (Rami *et al.*, 2003).

Various options proposed to limit the impact of biting flies are application of chemicals to stable walls or if necessary on the animals and managing the grazing periods for stock at peak period of biting activity of the flies since they are most active in the middle of the day in sunlight, housing animals during the day would offer them protection allowing animals to have access to field shelters could also help separate hosts from vectors (Coetzer *et al.*, 1994). The application of synthetic pyrethroids for the control of *Tabanidae* and *Muscidae* has been effective in cattle. Sheep and goats, which are often kept close to camels, act as asymptomatic carriers of *T. evansi* from where camels can get the infection (Evans *et al.*, 2005). There is no vaccine available for use due to the trypanosome antigenic variation (Taylor *et al.*, 1999).

2.4. Epidemiology

The epidemiology of vector-borne diseases is complex due to variability in the ecology of the different actors involved, i.e. parasites, vectors and hosts.

2.4.1. Host range and geographic distribution

T. evansi, the causative agent of surra occurs not only in Africa, but also in Central and South America, the Middle East, and Asia. Surra has a wide host spectrum, the main host species varies with the geographical region. In Africa, beyond the northern most limits of the tsetse fly belt, and in parts of East Africa, dromedary camels are the most important host, whilst in Central and South America the horse is principally affected (Dia *et al.*, 1997). The ability to be transmitted by blood-sucking insects other than *Glossina*, has enabled *T. evansi* to extend its range into African areas north of the Sahara desert, into Asia Minor, Pakistan, India, the USSR, China, Sumatra, Java, the Philippines, Mauritius, Madagascar, and South and Central America (Table 2). It was introduced by camels into Australia, North America and South-West Africa (Elamin *et al.*, 1999). In Asia, a much wider range of hosts is involved, including the Bactrian camel and dromedaries, cattle, buffalo, horses and pigs (Pacholek *et al.*, 2001). This is contrary to observations in Africa and South America, where there is little evidence to suggest that domesticated livestock other than camels and horses, respectively, are clinically affected or infected with *T. evansi* (El-Sawalhy and

Seed, 1999). The disease is most severe in horse, donkeys, mules, camels, dogs and cats. Camels, horses, dogs and Asian elephants are more susceptible than sheep and goat, which are more susceptible than bovines and pigs. Rats and mice are highly susceptible as experimental hosts for detecting sub clinical (non-patent) infection (Reid and Husein, 2001). It has been suggested that, unlike in tsetse-transmitted trypanosomosis, wildlife reservoirs of infection are unimportant with *T.evansi*, although it is possible that South American coatis and capybaras are an exception to this (Herrera and Dvila, 2004).

Table 3: Prevalence of camel Trypanosomosis in some countries

Country	Prevalence	Type of diagnostic test	Source
Sudan	38.3	ITS-1 PCR	Salim <i>et al.</i> , 2011
Kenya	26.6	ITS-1 PCR	Njiru <i>et al.</i> , 2004
Ethiopia	24.9	CATT/ <i>T.evansi</i> test	Hagos <i>et al.</i> , 2009
Nigeria	14.63	BCT	Mbaya <i>et al.</i> , 2010
Chad	30.5	CATT/ <i>T.evansi</i> test	Arnaud and Abdesalam, 2004
Morocco	14.1	CATT/ <i>T.evansi</i> test	Atarhouch <i>et al.</i> , 2003
Pakistan	11.25	GSTBS	Bhutto <i>et al.</i> , 2010
India	17.05	ITS-1PCR	Singh <i>et al.</i> , 2004
Spain	7.5	Ab-ELISA	Molina <i>et al.</i> , 2000

2.4.2. Camel trypanosome in Ethiopia

In Ethiopia, the distribution of *T. evansi* causing surra in camel coincides with the distribution of camels in the semi-desert environment of southern and eastern regions of the country (Figur 3). This trypanosome also occurs in the dry country of the North West near the Sudan border. In Southern Ethiopia (Borena), the disease caused by *T. evansi* is well known to the breeders by the local name “Dhukane” and is given the first priority in its order of importance among camel diseases (Demeke, 1998; Tekle and Abebe, 2001).

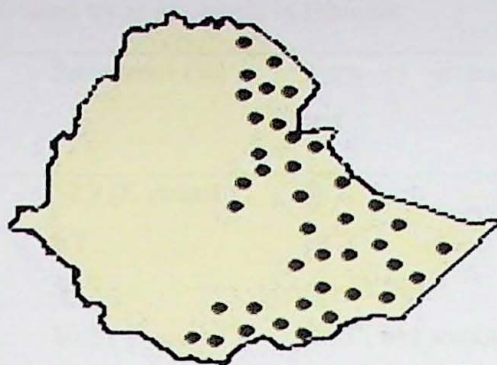


Figure 3: Distribution of *Trypanosoma evansi* in Ethiopia

Source: Getachew, 2005

Similarly *T. vivax* is found in the entire country except in the highlands, which are 2500 meters above sea level. The highlands include: The north central and the Arsi-bale massifs; the Tigrean plateau, the Showan plateau, the south-western plateau, and the Harar plateau. In Ethiopia, it is indicated this trypanosome species is most prevalent in cattle in tsetse free areas even the livestock were not in contact with tsetse (Figure 4) (Bekele, 1980; Abebe and Jobre, 1996; Sinshaw *et al.*, 2006). The prevalence of camel trypanosomosis in different part of Ethiopia is presented in table below (Table 3).

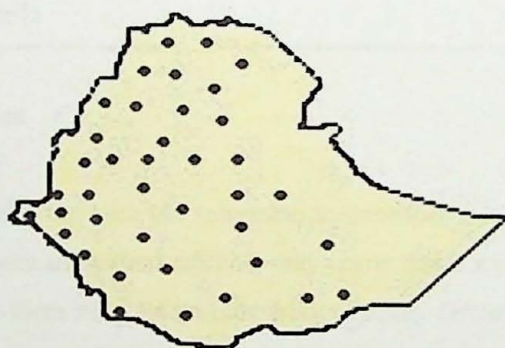


Figure 4: Distribution of *Trypanosoma vivax* in Ethiopia

Source: Getachew, 2005

Table 4 : Prevalence of camel trypanosomosis in Ethiopia

Region	Location	Prevalence (%)	Type of diagnostic test	Source
Oromia	Borena	10.9 (<i>T. evansi</i>)	BCT	Tekle and Abebe, 2001
		6.7	BCT	Demeke, 1998
		56.5	Ab-ELISA	
		10.2 (<i>T. evansi</i>)	BCT , wet season	
	Leben,Borena	2.8 (<i>T. evansi</i> + <i>T. vivax</i>)	BCT , dry season	Getahun, 1998
	Dello-Mena and Sawena	12.12 (<i>T. evansi</i>) 24.9	BCT CATT/ <i>T.evansi</i>	Hagos <i>et al.</i> , 2009
Tigrai- Afar salt convoy	Beracaale	5 (<i>T. evansi</i>)	BCT	Hailu, 2000
Somali	Somali	7.7 (<i>T. evansi</i>)	BCT	Issa, 1998
	Ogaden	6.54 (<i>T. evansi</i>)	Thin blood smear	Abebe, 1991
	Jijiga	3.9 (<i>T. evansi</i>)	BCT	Tadesse <i>et al.</i> , 2012
Afar	Gowani and Fantalle	5 (<i>T. evansi</i>)	Thin blood smear	Hailemariam <i>et al.</i> , 2008

2.4.3. Risk factors

The sequel to infection with the salivarian trypanosomes is not always a disease some may affect self-cure, but some individual animals may come down with the disease of different stage. For a disease to result there must be an inter-play of many factors that relate to the vectors, wild hosts, livestock and their management, the trypanosomes and the climatic conditions (Whiteside, 1985).

2.4.3.1. Host factors

In natural hosts and some domestic animals, the salivarian trypanosomes do not evoke clinical signs. This suggests that there is host-parasite equilibrium. Some breeds of indigenous cattle tolerate light to moderate challenges by limiting the multiplication of trypanosomes in blood (Mwangi *et al.*, 1998). This phenomenon is called trypanotolerance and is both genetically and environmentally determined (Murray *et al.*, 1990). In trypanotolerant animals, it has been found that some may effect self-cure, but some individual animals may come down with the disease due to stress. Stress occurs in late pregnancy and early in lactation in animals that are more susceptible (Ogwu and Njoku, 2007). Inter-current infections (e.g. helminthosis), also stressful, may accentuate the severity of the disease.

Trypanotolerance may also be reduced by low plane of nutrition (MacLennan, 2004) and when animals have to trek for long distances in search of water and pasture in the dry season. This is especially common in the pastoral communities. Age has some effect on resistance to trypanosomosis; camel born in an infested area do not immediately succumb to infection, though they acquire trypanosomal infection when young (Evans *et al.*, 2005).

2.4.3.2. Pathogen factors

Trypanosome infected animals commonly survive for prolonged periods, thus there are ample opportunities for fly transmission, especially of *T. brucei* and *T. congolense*. In contrast some strains of *T. vivax* in cattle and *T. simiae* in domestic pigs kill their hosts within 1-2 weeks so that the chances of fly infection are more limited. The developmental cycle of trypanosomes in the fly varies from species of parasite. For instance, *T. vivax* has adapted very well, with a relatively simple complete cycle in a fly of 5 to 13 days, compared to one of between 17 to 45 days for *T. brucei*. Thus, more flies are found infected by *T. vivax* parasite (FAO, 2000). *T. evansi* has adapted to an entirely mechanical, non-cyclical mode of transmission by blood-sucking flies other than tsetse and infects a wide range of animal hosts (Connor, 2004).

2.4.3.3. Environmental factors

There is usually a build-up of fly vector populations (*Tabanids*, *Hippoboscids*, *Stomoxys*) during the rains due to a good humid environment for breeding hence resulting in increase of new infections. Thus, rainfall, suitable moisture retaining clay soil and surface water pools also support the development of suitable camel browsing conditions, where *Acacia senegal* shrubs grow in abundance (Luckins, 2004). Towards the end of a dry season, pastoralists usually take their animals to riverine or swampy areas, which are also favourable grounds for these flies (Evans *et al.*, 2005). The degree of risk depends on the challenge, i.e., the number of infective tsetse/vector fly bites that an animal experiences in a given time. But the interaction of infective tsetse/vectors, host preference, host susceptibility and the virulence of the parasite determine the true challenge or risk (Rogers, 2003).

2.4.4. Vectors of mechanically transmitted trypanosomes

Mechanically transmitted trypanosomosis of animals are transmitted from host to host mainly by hematophagous insects including *Tabanidae* (mainly the genus *Tabanus*), *Culicidae*, *Muscidae*, *Hematopoda*, *Leperosia*, *Stomoxys*, and *Chrysops* (Seifert, 1986; Clair, 1988; Urquhart *et al.*, 1996; Desquesnes and Dia, 2004). Similarly, (Itard, 1989) explained that the most frequent vectors of mechanical transmission of trypanosomes are *Tabanidae* and *Stomoxynae*. *Hipposcidae* are also involved some times. In Africa, *Tabanidae* and *Hippoboscidae* flies principally transmit *T. vivax* whereas, the vector of *T. vivax* in the western hemisphere remains unknown but several species of hematophagous flies such as *Tabanidae* and *Hippoboscidae* are believed to serve as mechanical vectors (Luckins, 1992; Mare, 1998; Molyneux and Ashford, 2003).

More than 20 different species of *Tabanus* have been shown experimentally to transmit *T. evansi* (Luckins, 1998). Furthermore, surveys of *Tabanus* in the various tropical areas have shown a definite correlation between the seasonal outbreaks of *T. evansi* infections and the increase in number of *Tabanus* during the rains (Mahmoud and Gray, 2000; Njiru *et al.*, 2002). Thus, rainfall, suitable moisture-retaining clay soil and surface water pools also support the development of suitable camel browsing conditions, where *Acacia senegal* shrubs grow in abundance. The

prevalence of some *Tabanus* spp. all year round ensures that transmission of the parasite occurs wherever reservoir hosts, vectors and susceptible hosts co-exist. This finding may explain the sporadic occurrence of the disease during the dry season and outbreaks during the rainy season (Njiru *et al.*, 2002).

Transmission by biting flies is not the sole means by which infection is perpetuated; ingestion of meat from infected carcasses by carnivores can result in infections, and in South America, vampire bats are said to be of importance both as reservoirs of infection and as vectors (Luckins, 1998). In rare cases mechanical transmission can occur by iatrogenic means for instance by using the same needle or surgical instrument on more than one animal, at sufficiently short intervals that the blood on the needle or instrument does not dry (Luckins, 1998)

2.5. Pathology and pathogenesis

Anaemia is a major component of the pathology of surra and of African animal trypanosomosis. Generally the degree of anemia might be considered as an indicator of the disease severity. The parasitaemia causes a large number of red blood cells (RBCs) to be removed from circulation by cells of the mononuclear phagocytic system (MPS) in the spleen, bone marrow, and haemal lymph nodes. The removal of a large number of RBCs leads to a fall in packed red cell volume (PCV) to below 24% or even to as low as 10% (Evans *et al.*, 2005).

In the late stages, anaemia continues to be a major factor, with probably additional causes. However, irrespective of the cause of anaemia the primary abnormality of function are the anoxic conditions created by the persistent anaemia tissue anoxia, which results in a fall in tissue PH and vascular damage (Connor, 2004). Following this are signs of dysfunction which appear in the various organs. An increase in cardiac output due to increases in stroke volume and heart rate and a decrease in circulation time are obvious manifestations. The central nervous system is reported to be most susceptible to anoxia with consequent development of cerebral anoxia (FAO, 2000).

2.6. Immune response

Although the immune system is designed to protect a host from pathogens it can sometimes be overwhelmed, respond inappropriately or result in immune mediated disease with clinical signs (Stijlemans *et al.*, 2007). Circulating trypanosomes are rarely observed in individuals suffering from chronic disease and studies have failed to show a correlation between the intensity of inflammation and the level of parasitaemia (Olivares-Villagomez *et al.*, 2008). This may be because the immune response is directed against both parasites and self antigens. The parasites might achieve this through molecular mimicry or inflammation and tissue damage leading to the release of tissue proteins which stimulates formation of self antigens (Soares and Santos, 2006). An increase in gamma-globulin (IgM) during both acute and chronic *T. evansi* infections in camels has been reported but this is not protective, as the majority of the antibodies are auto antibodies (Boid *et al.*, 2001).

3. MATERIALS AND METHODS

3.1. Study area

The present study was conducted in Chifra and Dewe district of Afar Region, located in the North-Eastern part of the country between 39°34' and 42°28'E longitude and 8°49' and 14°30'N latitude in the rift valley (Figure 5).

Chifra is located about 557 km away from Addis Ababa, about 162 km from the regional capital city (Semera), part of the Administrative Zone 1. Mille and Logiya rivers defines part of the boundary with Zone 4 of the region. The average elevation in this district is 825 meters above sea level with annual temperature ranges from 25 °C to 33 °C. Chifra is climatically characterized as arid and semi-arid agro-ecological area, where livestock production is the main occupation of the community (APARDB, 2006). The rainfall is bimodal with erratic distribution, with the long rainy season (Kerma) between Mid-June to Mid-September and short rainy season (Sugum) occurs between March and April, and the average annual rainfall is between 400 and 600 mm. The dominate soil types in these areas are black, sandy, vertisoils and deposits of silt and fine sand particles occur in the plain flat areas where cultivation is practiced. The district covered by sparse *Accacia* species and extensive grazing land (Hailu, 2009).

Dewe is about 508 kms away from Addis Ababa, part of the Administrative Zone 5. The district is located in south part of Afar region bordering Telalak in the north, Dalifage in the South, Gewane in the west and Dewa-Chefa of Oromo zone in the east (Figure 5). The total area of the woreda is 1450 square kilometer. The other agro-ecological conditions of this district are very similar with Chifra district (Hailu, 2009).

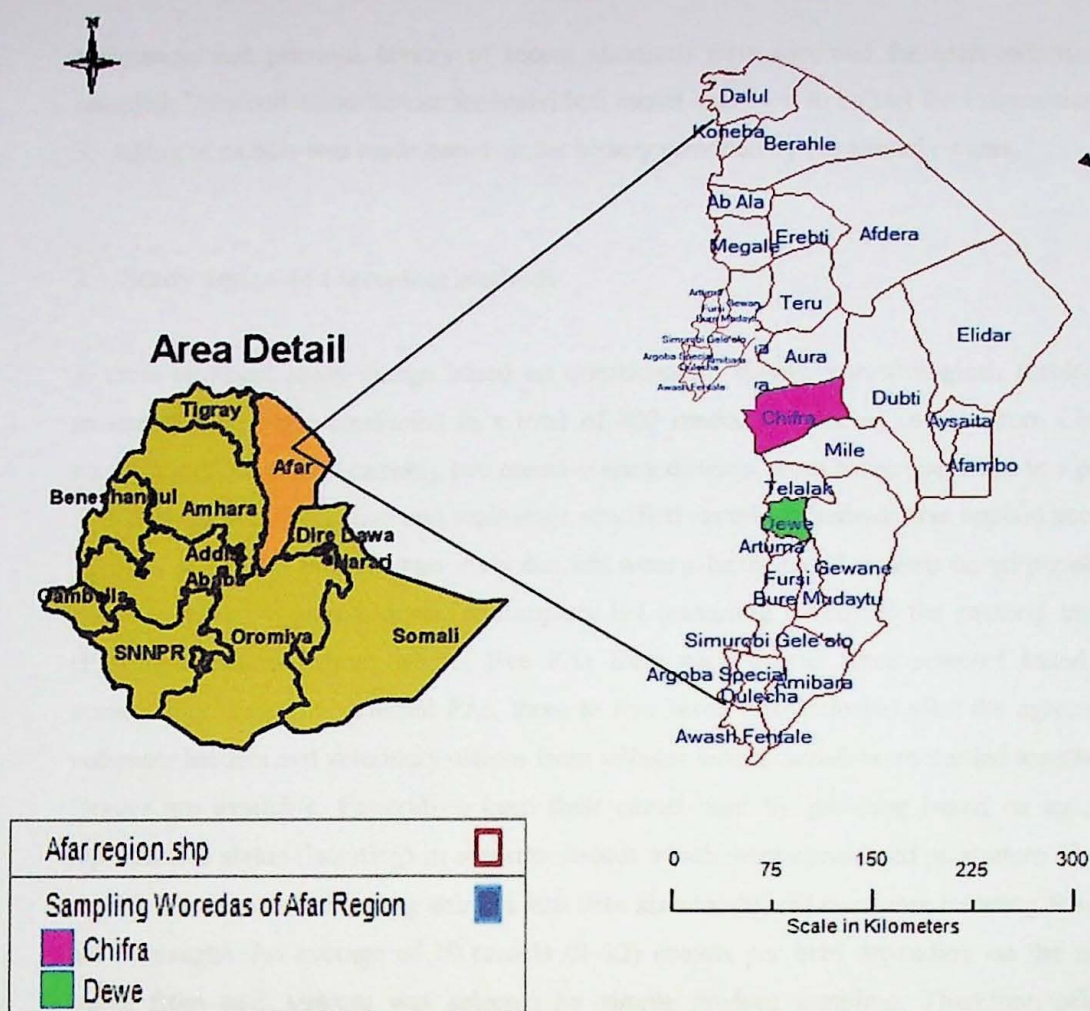


Figure 5: Administrative location of Afar Region and map of the study districts

3.2. Study population

The camel population of Chifra and Dewe Districts is about 135,367 (126,349 camels in Chifra district and 17,105 camels in Dewe). Individual camels having an age of six months and above were considered for blood sampling. To categorize the data in a defined age group as those 6 months -5 years (young), 5-10years (adult) and above 10 years of age (old). During data collection parameters like age, sex, body condition scores, and reproductive status (lactation,

pregnancy, and previous history of recent abortion) were recorded for each individual camel sampled. Data collection format for individual camel was used to collect the information (Annex 2). Aging of camels was made based on the history provided by the animal owners.

3.3. Study design and sampling methods

A cross-sectional study design based on questionnaire survey, parasitological, serological and molecular tests were conducted in a total of 400 randomly selected camels from Chifra (200 camels) and Dewe (200 camels), two camel-rearing districts, from November 2011 to April 2012. A combination of purposive and multistage stratified sampling methods was applied according to Toma *et al.* (1999). First the two study districts were selected based on their camel population and claim of camel trypanosomosis. A complete list (sampling frame) of the pastoral associations (PAs) was obtained from where; five PAs from each district were selected based on their accessibility. From the selected PAs, three to five herds were selected after the agreement with volunteer herders and veterinary offices from villages where camels were stabled together around forages are available. Pastoralists keep their camel herd by grouping based on age, sex and reproductive status (lactating) in separate stables which were considered as stratum (Annex 5, 6 and 7). However, some young animals less than six months and dominant lactating female could not be caught. An average of 10 camels (8–12) camels per herd depending on the number of camel from each stratum was selected by simple random sampling. Therefore, selection of individual camels for the study purpose was executed on basis of stratified simple random sampling technique.

3.4. Sample size

The number of animals required for the study was calculated based on the formula given by Thrusfield (2005). A precision level of 5% and a 95% confidence interval was used to calculate the sample size using the formula.

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

Where: n = required sample size, 1.96 = the value of Z at 95% confidence interval, P_{exp} = Expected prevalence of camel trypanosomosis (50%), in the study area D = Desired absolute precision level at 95% confidence interval

Accordingly, the calculated sample size for estimating prevalence in simple random sampling was 384. In order to make the prevalence estimate more precise, the sample size was inflated and was set to be 400. Hence, a total of 400 camels were sampled from Chifra and Dewe districts of Afar region, and equally divided for the two districts (200 for each district).

Blood samples were collected from each randomly selected camel through jugular vein into 10 ml heparinized vacutainer tubes after the site is wiped with cotton wool soaked in alcohol. The vacutainer tubes were labelled and thin smears were prepared at the field from the collected blood for parasitological examination. For molecular tests about 250 μ l bloods was preserved in equal volume of AS-1 buffer. The remaining blood was centrifuged and plasma was collected and preserved at -20°C until serological test was performed. Molecular and serological test were conducted at Debre Zeit, College of Veterinary Medicine and Agriculture, Molecular Biology Laboratory.

3.5. Methodology

3.5.1. Questionnaire survey

Field investigations were carried out by interviewing volunteer herd owner pastoralists in a village during sample collection individually with the aid of a questionnaire about disease status, usage of trypanocidal drugs, and physiological status. Both open-ended and closed type questionnaires were applied to interview individual pastoralists. A questionnaire survey format was used to collect relevant information related to trypanosomosis (Annex 1)

3.5.2. Parasitological test

3.5.2.1. Giemsa stained thin blood smear

A drop of blood was placed on one end of a clean microscope slide and a thin film (smear) was drawn out at the field after the sample collected and ladled. The blood smears were air-dried, fixed in methyl alcohol for 2 minutes and allowed to dry, then stained by 10% Giemsa stain. Trypanosomes were detected by microscopic examination of the slides at magnification 100 xs by help of oil immersion lens, at each study districts veterinary clinic. For PCV determination, blood samples were drawn into heparinized microhaematocrit capillary tube up to $\frac{3}{4}$ of their length. One end of the tubes was then sealed with critoseal. The tubes were symmetrically loaded in the haematocrit centrifuge, with the sealed end outwards, centrifuged at 12,000 rpm for 5 minutes. PCV levels of individual samples were determined on haematocrit reader (Hawaksly, England) and the values were expressed in percentages (OIE, 2010).

3.5.3. Serological test

3.5.3.1. Card agglutination test for trypanosomes (CATT/*T. evansi*)

The test was performed as per the manufacturer's instructions (Laboratory of Serology, Institute of Tropical Medicine, Antwerp, Belgium). Briefly, 25 μ l of camel plasma, diluted 1:4 in CATT-buffer, was pipetted onto a reaction zone of a plastic coated test card. After adding one drop (about 45 μ l) of CATT reagent, the reaction mixture was spread out by means of a stirring rod and allowed to react on a Card Test Rotator for 5 min at 70 rpm. A sample was considered positive when blue agglutinates were visible (Annex 4). The agglutinate formed was compared with the agglutination formed in case of the positive control included in the test kit (Verloo *et al.*, 2000).

3.5.4. Molecular test (ITS-1PCR)

3.5.4.1. DNA extraction

To perform molecular test, the DNA was extracted from all the samples collected. The DNA extraction was carried out in molecular biology laboratory at Addis Ababa University College of Veterinary Medicine and Agriculture, Debre Zeit, Ethiopia. The DNA extraction was carried out according to the manufacturer QIAGEN, DNA extraction protocol (QIAamp®, 2010). Briefly, 200µl of whole blood, 20 µl of proteinase K stock solution, and 200 µl of lysing buffer (AL) were pipetted into 1.5 ml eppendorf tube mixed thoroughly by vortexing for 15 second followed by incubation at 56 °C in water bath for 10 minute and brief spinning. 200 µl of absolute ethanol was added to the sample and the mixture was mixed by vortexing and spinning. The mixture was transferred to the QIAspin column, and centrifuged for 1 minute at 8000rpm and the filtrate was removed while the DNA remains bound to the Silicium membrane of the spin Column. Then the spin column was put back in new collection tube and 500 µl of AW 1 buffer was added without wetting the rim. Close the cap and centrifuged removed AW1 buffer. The spin column was again put in new collection tube and 500 µl of AW 2 buffer was added and centrifuged at 13000 rpm for 4 minute and a labeled eppendorf tube 1.5 µl was used for further collection of the purified DNA after adding 200 µl of AE buffer (elution buffer) incubated for 1 minute and centrifugation at 8000 rpm for 1 minute and the collected DNA was stored at -20°C.

3.5.4.2. ITS-1 PCR

ITS-1 PCR was conducted according to Claes *et al.* (2003) , the reaction mixture for this PCR was prepared in 25 µl volume containing 14.65 µl of water, 2.5 µl of buffer, 2 µl of dNTPs, 1 µl of MgCl₂, ITS-1 forward and reverse primer, 0.25 µl of BSA , 0.1 µl of Hot start Taq polymerase and 2.5 µl template. The reaction mixture was placed in a thermal cycler (Eppendorf Master Cycler Gradient, Hamburg, and Germany). The primer sequence, cycling condition and amplification product for different species of animal trypanosome is as presented in Table 5.

Table 5: ITS-1 PCR Primer sequence cycling condition and Amplification product for the different animal trypanosomes

PCR	Primer sequence	Cycling condition	Species	Amplification product
ITS-1	ITS-1F: 5'TGTAGGTGAACCTGCAGCTGGATC-3'	94°C for 15' 40 cycles of 94°C 30", 60°C 30", 72 ° C 30" and final extension at	<i>T. congolense</i>	650
	ITS-1R: 5' CCAAGTCATCCATCGCGACACGTT-3'	72°C for 5'	<i>Trypanozoon</i>	450
			<i>T. theileri</i>	350
			<i>T. vivax</i>	150

Source: (QIAGEN®, USA)

For detection of amplified product, 10 µl of the PCR product was mixed with 4 µl of loading dye and electrophoresed on a 2% agarose gel in 1X TAE buffer for 30 min at 100 V. The gels were stained with Ethidium bromide for 30 min and visualized on a UV transilluminator (Transilluminator Fisher Biotec, Australia). A 100 bp DNA ladder (QIAGEN®, USA) was included in each gel as a marker for molecular weight Identification was made based on the size of the amplicon and the positive controls included.

3.6. Data analysis

3.6.1. Data management and Statistical analysis

Raw data of questionnaire and on individual animals, parasitological examination, serological and molecular tests results were stored into MS excel spread sheets to create a database and imported to SPSS (Statistical Package for Social Sciences, version 16) was used for data analysis. Prevalence of the disease was calculated by dividing the number of animal positive for the tests to total animals examined. The degree of test agreement among the diagnostic tests was assessed using kappa statistics as described in Viera and Garret (2005). Furthermore, Chi-square (χ^2) test was applied to compare the prevalence of trypanosome infection in different risk factors. Binary logistic regression was used to calculate odds ratios to estimate the risk of camel trypanosomosis in both a univariate and multivariate analysis. For the latter, variables were selected using a backward stepwise selection procedure, retaining only variables with a $P < 0.2$ for the final model and those significantly associated factors were assessed by using univariable and then multivariate logistic regression analysis to determine the association of each risk factor with positive result using Odds Ratio (OR). ANOVA was used to assess the difference in mean PCV of the animals in trypanosomes infected and non-infected camels. 95% confidence interval and $P < 0.05$ was set as statistically significance level.

4. RESULTS

4.1. Questionnaire survey

The results of the questionnaire survey revealed the presence of major diseases of camels such as trypanosomosis ('Garamolle'), mange mite infection ('Agare'), tick infestation ('Silmi'), pneumonia ('Gublo') and unspecified diarrhea ('Gechita') in the study districts. All the pastoralists interviewed have agreed that trypanosomosis is present in their village and they ranked trypanosomosis to be the first in causing high mortality and affecting camel productivity of the study districts. Among the domestic livestock species camels were affected by trypanosomosis. However 5/60 (8.3%) pastoralists respond that cattle are also affected. According to the pastoralists trypanosomosis in camel was manifested by symptoms of hair loss at the base of the tail, emaciation, diarrhea, inappetance, loss of milk production and abortion. The herders also reported that, they noticed a characteristic 'benzen-like' odour of the camel's urine and identify infected camels by this sign. Since surra is chronic disease and causes loss of hair at the tail pastoralists of the study area used to call it 'Garamolle'.

The season that camels were commonly seen sick of trypanosomosis was at the late rainy and early dry seasons (August to January) and 49/60 (81.7%) of pastoralists claimed the months of September to December as the peak disease time. Surprisingly the entire interviewed pastoralists did not know that flies are transmitters of trypanosomosis. All of the respondent pastoralists claimed that the problem of surra is expanding from time to time and riverine areas are mostly affected. Camels were not treated against trypanosomosis due to unavailability of trypanocidal drugs in the study districts. Thus the pastoralists emphasized that the problem of trypanosomosis was becoming more and more severe as there was no curative or prophylactic therapeutic agents in the study districts.

4.2. The prevalence (Parasitological, Serological and ITS-1 test results)

The prevalence study carried out by three diagnostic tests in present study indicated that the overall prevalence of camel trypanosomosis was 2%, 24%, and 30.5% by thin smear examination, serological and ITS-1 PCR test, respectively (Figure 6). There was significant

difference ($P < 0.05$) in prevalence of trypanosomosis in camel using the three tests. Significantly higher prevalence of camel trypanosomosis was detected by ITS-1 PCR followed by CATT/ *T. evansi* (Table 13 and 14). No statistically significant ($P > 0.05$) difference in prevalence of trypanosomosis was recorded among the study districts by all diagnostic tests (Table 6). The parasites observed by thin blood smear examination had a morphology characteristic of *T. evansi* with sub terminal small kinetoplast at the posterior end (Figure 7) and free flagellum. The species of trypanosomes detected by ITS-1 PCR were *T. evansi*, *T. vivax*, and mixed infections of *T. evansi* and *T. vivax* at prevalence of 20 %, 7.5%, and 3 %, respectively (Table 7).

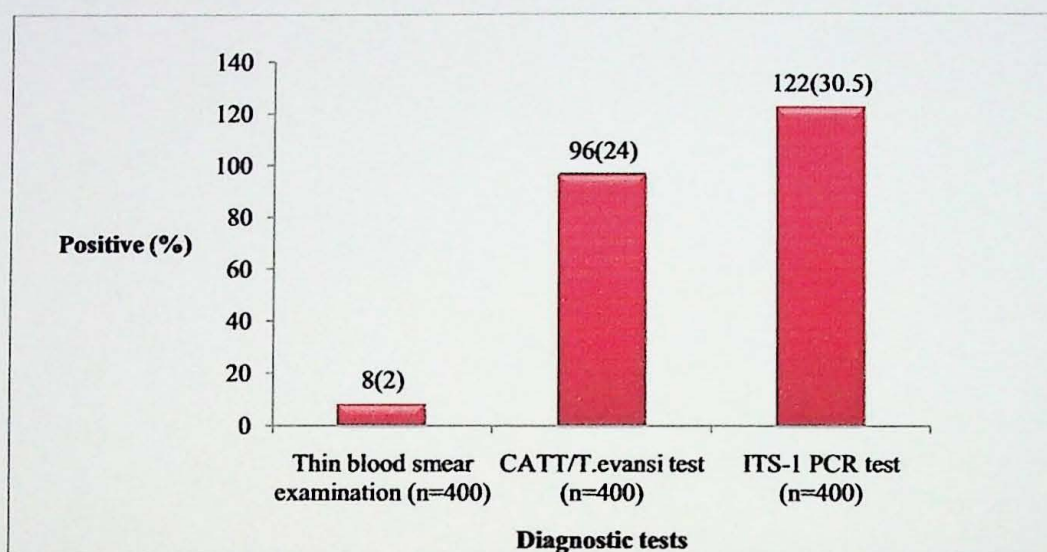


Figure 6: Over all prevalence of camel trypanosomosis in study districts of Afar region



Figure 7: *T. evansi* in camel blood, Giemsa stained smear (100 x) from Chifra district

The prevalence of camel trypanosomosis by using thin blood smear examination, CATT/ *T. evansi* and ITS-1PCR tests on the basis of explanatory variables such as sex, age, body condition and their reproductive status was indicated. Results obtained with different age groups showed that infections were observed in camels of all ages, but statistical significant differences ($P < 0.05$) between age group were observed in all diagnostic tests especially the prevalence was higher in old camels (> 10 yr age group). Likewise camels with poor body condition were significantly ($P < 0.05$) susceptible to trypanosome infection than camels with moderate body condition (Table 6). However, there was no significant difference in the prevalence between sexes in the parasitological examination, but both serological and molecular tests showed a significant difference ($P < 0.05$) where prevalence of trypanosome was higher in females (Table 6).

Table 6: Prevalence of camel trypanosomosis based on sex, age and body condition as revealed by thin blood smear examination, CATT /*T. evansi* and ITS-1PCR tests

Factors	No. of camels examined	Thin blood smear examination		CATT / <i>T. evansi</i> test		ITS-1 PCR test	
		Positive (%)	P= value	Positive (%)	P= value	Positive (%)	P= value
District			1.000		0.101		0.193
Chifra	200(50)	4(2)		41(20.5)		67(33.5)	
Dewe	200(50)	4(2)		55(27.5)		55(27.5)	
Sex			0.144		0.000		0.001
Male	149(37.3)	1(0.7)		26(17.4)		30(20.1)	
Female	251(62.8)	7(2.8)		70(27.9)		92(36.7)	
Age			0.026		0.027		0.003
Young (6 mon-5 years)	123(31.0)	0(0.0)		21(17.1)		32(26.0)	
Adult (5-10 years)	252(62.8)	6(2.4)		65(25.8)		76(30.2)	
Old (> 10 years)	25(6.3)	2(8)		10(40.0)		14(56.0)	
Body condition			0.000		0.000		0.000
Poor	88(22)	6(6.8)		41(46.6)		42(47.7)	
Moderate	312(78.0)	2(0.6)		55(17.6)		80(25.6)	
Total	400	8(2)		96(24)		122(30.5)	

Assessment of association of the prevalence of trypanosomosis with history of abortion ($P = 0.000$) using chi-square indicated significant association with trypanosome infection ($P < 0.05$). On the contrary, pregnancy ($P = 0.637$) and lactation ($P = 0.267$) were found to have no significant association ($P > 0.05$) with camel trypanosomosis as revealed by thin blood smear examination (Figure 8). On the other hand, pregnancy ($P = 0.017$) and history of abortion ($P = 0.010$) were significantly associated ($P < 0.05$) with trypanosomosis seropositive camels, however, lactation ($P = 0.27$) was found to have no significant association ($P < 0.05$) with seropositivity (Figure 8).

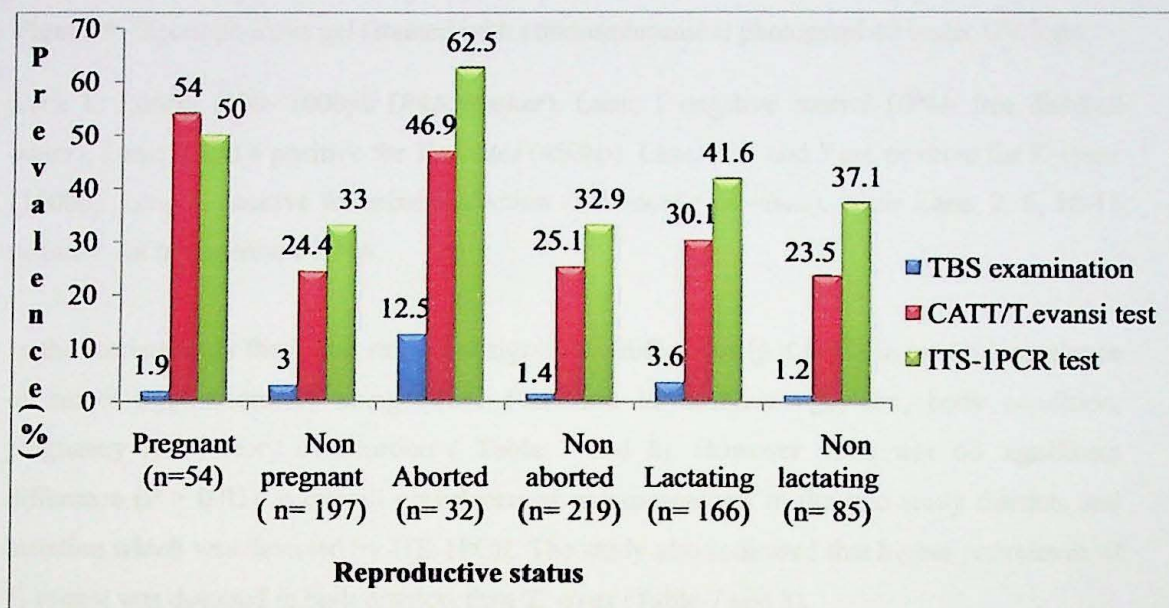


Figure 8: Prevalence of camel trypanosomosis based on reproductive status as revealed by thin blood smear examination, CATT /*T. evansi* and ITS-1PCR test

4.2.1. Molecular test (ITS-1 PCR)

PCR amplification was carried out targeting ITS-1 region of the rDNA of trypanosomes. All DNA samples ($n=400$) were tested by PCR with ITS-1 primer, giving a PCR product of the 450bp in the band for *T. evansi* and 150bp band was for *T. vivax* while the common band was a PCR artefact resulting from nondescript primer dimer bonding (Figure 9).



Figure 9: Electrophoresis gel (stained with ethidiumbromide) photographed under UV light

Lane L: Ladder (100- 1000pb DNA marker), Lane; 1 negative control (DNA free distilled water), Lane; 3 and 8 positive for *T. evansi* (450bp), Lane; 4, 5 and 7 are positive for *T. vivax* (150bp), Lane 9; positive for mixed infection (*T. evansi* + *T. vivax*), while Lane; 2, 6, 10-15 negative for trypanosome DNA.

In the current study there was statistical significant difference ($p < 0.05$) in overall prevalence of camel trypanosomosis using ITS-1 PCR test in different age, sex, body condition, pregnancy and history of abortion (Table 7 and 8). However there was no significant difference ($P > 0.05$) in overall prevalence of trypanosomosis in the two study districts and lactation which was detected by ITS-1PCR. The study also indicated that higher prevalence of *T. evansi* was detected in both districts than *T. vivax* (Table 7 and 8).

Table 7: Association of ITS-1 PCR prevalence with sex, age, body condition and district

Factors	No of camels examined	ITS1 PCR test result				P-value
		<i>T. evansi</i>	<i>T. vivax</i>	Mixed T.e+T.v	Total positive	
District						0.376
Chifra	200(50.0)	41(20.5)	18(9.0)	8(4.0)	67(33.5)	
Dewe	200(50.0)	39(19.5)	12(6.0)	4(2.0)	55(27.5)	
Age Category						0.000
Young	123(30.8)	16(13.0)	13(10.6)	3(2.4)	32(26.0)	
Adult	252(63.0)	55(21.8)	15(6.0)	5(2.0)	75(29.8)	
Old	25(6.3)	9(36.0)	2(8.0)	4(16.0)	15(60.0)	
Sex						0.003
Female	251(62.8)	61(24.3)	19(7.6)	11(4.4)	91(36.3)	
Male	149(37.3)	19(12.8)	11(7.4)	1(0.7)	31(20.9)	
Body condition						0.000
Moderate	312(78.0)	48(15.4)	23(7.4)	8(2.6)	79(25.4)	
Poor	88(22.0)	32(36.4)	7(8.0)	4(4.5)	43(48.9)	
Total	400	80(20)	30(7.5)	12(3)	122(30.5)	

T.e : *T. evansi* T.v : *T. vivax*

Table 8: Association of ITS-1 PCR prevalence with reproductive status

Reproductive status	No. animals examined	<i>T. evansi</i>	<i>T. vivax</i>	Mixed T.e+T.v	Total positive	P-value
Pregnant	54(21.5)	22(40.7)	3(5.6)	1(1.9)	26(48.2)	0.014
Non pregnant	197(78.5)	39(19.8)	16(8.1)	10(5)	65(33)	
Previous abortion						
Yes	32(12.7)	12(37.5)	5(15.6)	2(6.3)	19(59.4)	0.027
No	219(87.3)	49(22.4)	14(6.4)	9(4.1)	72(32.9)	
Lactating	166(66.1)	45(27.1)	15(9.0)	9(5.4)	69(41.5)	0.095
Non lactating	85(33.9)	16(18.8)	4(4.7)	2(2.4)	22(25.9)	
Total	251(62.75)	61(24.3)	19(4.8)	11(2.8)	91(22.8)	

4.3. Host related risk analysis

4.3.1. Univariable analysis

After univariate analysis, it was found that significant risk factors for camel trypanosomosis in study districts were, having a poor body condition (OR= 4.076, CI: 2.447- 6.789), old age (OR= 3.238, CI: 1.280- 8.189) compared to young age groups and females (OR= 1.83, CI: 1.104- 3.032) were showed a higher risk for having seropositive camels based on CATT/*T.evansi* test (Table 9). The chance of seropositivity for camel with poor body condition was about more than four times the chance of camel with moderate body condition likewise pregnant (OR= 2.134, CI: 1.133- 4.018) and camels with an abortion history (OR= 2.631, CI: 1.232- 5.617) had about two times higher chances of seropositivity compared to non-pregnant and camels without history of abortion. Risk factor like lactation (OR= 1.401, CI: 0.768- 2.555) and adults compared to young camels (OR= 1.688, CI: 0.976- 2.92) showed weak association with the outcome even though not reached statistically significant (Table 9).

Age, sex, body condition and reproductive status of the animal were found to be significantly associated with ITS-1PCR test positive camels. Likewise, poor body condition (OR = 26.877, CI: 3.260- 221.575) and abortion (OR= 10.286, CI: 2.188- 48.357) showed strong association with camel trypanosomosis in thin blood smear examination (Table. 9). Other risk factors like pregnancy (OR= 0.601, CI: 0.071- 5.099), age and lactation showed either no or only a weak association with the outcome and none of them are statistical significant.

Table 9: Summary of the univariable analysis of risk factors based on thin blood smear examination, CATT/ *T. evansi* and ITS-1 PCR tests

Risk factors	Thin blood smear examination			CATT/ <i>T. evansi</i> test			ITS-1 PCR test		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
Age Category									
Young*						0.031			0.006
Adult				1.688	0.976- 2.920	0.061	1.21	0.742- 1.957	0.451
Old	4.3	0.789- 23.293	0.093	3.238	1.280- 8.189	0.013	4.27	1.742-10.448	0.002
Sex									
Male*									
Female	4.25	0.517- 34.854	0.178	1.830	1.104- 3.032	0.019	2.16	1.351-3.470	0.001
Body condition									
Moderate*									
Poor	26.88	3.26- 221.57	0.002	4.076	2.447- 6.789	0.000	2.82	1.727-4.599	0.000
Reproductive status									
Non pregnant*									
Pregnant	1.663	0.196- 14.084	0.640	2.134	1.133- 4.018	0.019	1.886	1.024-3.473	0.042
Previous abortion									
No*									
Yes	10.286	2.188- 48.357	0.003	2.631	1.232- 5.617	0.012	2.984	1.396- 6.379	0.005
Non lactating*									
Lactating	3.150	0.373- 26.597	0.292	1.401	0.768- 2.555	0.272	2.037	1.146- 3.621	0.015

*Reference category

4.3.2. Multivariable analysis

Multivariate logistic regression analysis was applied for controlling confounders and for evaluating the effects of risk variables on camel trypanosomosis. Sex of the animal was excluded for the multivariable model due to co-linearity with the variable reproductive status. Other variables found to be associated with trypanosomosis infection in the univariate analysis ($P < 0.2$) were entered into the logistic regression model. Multivariate analysis for CATT/*T.evansi* test positive camels showed that major predictive factors for camel trypanosomosis infection were poor body condition (OR= 7.263, CI: 3.680- 14.332) and pregnancy (OR= 2.364, CI: 1.170- 4.777). Hence, a higher risk of seropositivity for camel with poor body condition was seven times at risk than camel with moderate body condition (Table 10). However seropositivity for trypanosomosis was not significantly related with history of abortion even if it had positive association (OR= 2.023, CI: 0.855- 4.787) (Table 10).

Table 10: Summary of multivariable analysis of potential risk factors for CATT/*T.evansi* test seropositive camels.

Risk factors	OR	95% CI	P-value
Poor body condition	7.263	3.680- 14.332	0.000
Previous abortion	2.023	0.855- 4.787	0.109
Pregnancy	2.364	1.170- 4.777	0.017

In the multivariate analysis of potential risk factors for ITS-1PCR test positive camels with poor body condition (OR= 2.96, CI 1.546- 5.668) and history of abortion (OR= 2.458, CI:1.104-5.471) and were associated with increased odds of a camel being exposed to trypanosome infection compared to camels with relatively moderate body condition and non-aborted camels, respectively (Table 11). The OR of the same factors for the thin blood smear positivity were 18.142 (2.062- 159.623) and 6.192 (1.212- 31.635), respectively. The ITS1 PCR test also indicates that factors for infection with camel trypanosomosis were lactation (OR= 2.608, CI: 1.343-5.061) and pregnancy (OR= 2.744, CI: 1.369- 5.500). Hence camels with poor body condition, history of abortion, pregnancy and lactation could be more likely to

acquire infection with trypanosomosis, nearly three times at risk compared to camels with moderate body condition, non-aborted, non-pregnant and non-lactating camels (Table 11).

Table 11: Summary of multivariable analysis of potential risk factors for camel trypanosomes based on thin blood smear examination and ITS-1 PCR test positives.

Risk factors		TBS examination			ITS1 PCR test		
		OR	95% CI	P-value	OR	95%CI	P value
Poor body condition	body	21.9	2.3– 199.96	0.009	2.96	1.546- 5.668	0.001
Previous abortion	6		1.087 – 32.56	0.028	2.458	1.104 – 5.471	0.028
Pregnancy					2.744	1.369 – 5.500	0.004
Lactation					2.608	1.343- 5.061	0.005

4.4. PCV and camel trypanosomosis

There was statistically significant difference in mean PCV between camels ITS-1 PCR test positive (22.15 ± 2.03) and negative camels (26.44 ± 2.4). Similarly, there was significant difference ($P < 0.05$) in mean PCV of camels infected by different species of trypanosomes as indicated in table (Table 12). There was lowest mean PCV was recorded for camels positive for mixed infections of *T. evansi* and *T. vivax* followed by mono-infection of *T. evansi*, likewise a statistically significant difference ($P < 0.05$) in mean PCV was observed between CATT/*T.evansi* positive (21.94 ± 2.061) and sero-negative (26.14 ± 2.54) camels. However there was no statistically significant difference ($P > 0.05$) in mean PCV between parasitologically positive and negative camels by thin blood smear examination (Table 12).

Table 12: Mean PCV of camels on the basis of diagnostic test results

	Mean	Std. Deviation	P-value
Thin blood smear			0.058
Positive	23.15	1.852	
Negative	25.21	2.995	
CATT/ <i>T.evansi</i>			0.025
Positive	21.94	2.061	
Negative	26.14	2.535	
ITS1 -PCR			0.023
Positive	22.15	2.03	
Negative	26.44	2.385	
<i>T. evansi</i>	22.09	1.930	
<i>T. vivax</i>	23.33	1.49	
<i>T. evansi</i> + <i>T. vivax</i>	19.58	1.31	

4.5. Degree of concordance between diagnostic tests used

The study also indicated that out of the 92 camels positive for *T. evansi* by ITS-1 PCR 58 (63%) camels were positive for *T. evansi* by CATT/*T. evansi*. Out of 308 camels negative for *T. evansi* by ITS-1 PCR 270 (87.7%) were also negative for CATT test (Table 13). Likewise out of the 8 camels positive for *T. evansi* by thin smear examination 7(87.5%) camels were produced positive reaction by card agglutination trypanosoma test (CATT). Out of 392 camels negative for *T. evansi* by thin smear examination 303 (77.3%) were also negative for CATT test. The data presented in (Table 13) also showed that 34 (11.2%) negative samples by CATT were developed positive results by PCR and only 1(12.5%) negative sample by CATT developed positive by thin smear examination. It was also noted that there was moderate agreement between the CATT/*T. evansi* and ITS-1 PCR ($k = 0.5$, $P = 0.000$) and slight agreement between the CATT/*T. evansi* and thin smear examination ($k = 0.1$) (Table 13).

Table 13: Relationship between CATT/*T. evansi* and ITS-1 PCR in detecting *T. evansi* infections in camels of study area

CATT/ <i>T. evansi</i>	ITS-1 PCR test			Thin smear examination		
	Negative	Positive	Total	Negative	Positive	Total
Positive	38	58	96	89	7	96
Negative	270	34	304	303	1	304
Total	308	92	400	392	8	400
Kappa test			0.5	0.101		
P			0.000	0.000		

The study also indicated that out of the 392 animals negative for thin blood smear examination 278(70.9%) camels were negative for trypanosomes by ITS-1 PCR. Similarly out of 8 animals positive for thin blood smear 8(100%) camels were also positive by ITS-1 PCR (Table 14). It was also noted that there was slight agreement between the thin blood smear examination and ITS-1 PCR ($k = 0.1$).

Table 14: Relationship of TBS examination and ITS-1PCR tests in detecting trypanosome infections in camels of study area

ITS-1 PCR test	Thin blood smear examination		Total
	Negative	Positive	
Positive	114	8	122
Negative	278	0	278
Total	392	8	400
Kappa test			0.089
P			0.000

5. DISCUSSION

In most cases, control of camel trypanosomosis is limited to treating those animals that are considered to be infected on the basis of unreliable clinical signs; using curative and prophylactic drugs. But treatment recommendations and strategies for chemotherapeutic control should depend on prevalence and information of trypanosomosis risk. However the accuracy of the studies depends on the quality of the study design, and on sensitivity and specificity of the diagnostic methods used. In this study, Giemsa stained thin blood smear examination, CATT/*T.evansi* serological test and one of the most sensitive and specific molecular test ITS-1 PCR were used to indicate the prevalence and host related risk factors of camel trypanosomosis in two districts of Afar region, eastern Ethiopia in attempt to generate accurate reliable epidemiological data on camel trypanosomosis.

Questionnaire Survey

Questionnaire survey in this study indicated that the problem of trypanosomosis in camel is expanding from time to time and ranking first among other camel diseases of the study area. The symptoms described by the pastoralists were in consistent with the signs and symptoms of the disease indicated in different literature (Luckins, 1998; FAO, 2000). This indicates that the pastoralists are well aware of the disease in alienation from other diseases. The higher prevalence of this finding using ITS-1 PCR coincides with ranking of camel diseases by pastoralists they placed camel trypanosomosis at first prioritization. However, none of the respondents have know how of the cause, vectors and way of transmission of trypanosomosis in camels. Likewise, none of the respondents have used curative or prophylactic trypanocidal drugs in the study areas. This condition of lack of knowledge of trypanosomosis and its control by the pastoralist in the study site could have contributed to the wide spread of the disease, as the respondents couldn't have any ways of control and prevention of the disease.

Disease prevalence and concordance of detection between different diagnostic techniques

In this study the overall prevalence recorded by Giemsa stained thin blood smear examination indicated that 2 % of the camels examined were infected by *T. evansi*. The present result is in agreement with previous findings where 2.8% in Southern Ethiopia (Getahun, 1998) which

was done at the dry season of the study area and from other African countries, for instance, 2.3% in Kenya (Ngaira *et al.*, 2004) and 2.5% in Nigeria (Egbe- Nwiyi and Chaudry, 1994). However, the prevalence from this study is lower than other studies in different parts of Ethiopia 12.5% (Richard, 1979), 12.1% (Hagos *et al.*, 2009), 10.2% (Getahun, 1998) during wet season of the study area, 6.54% (Abebe, 1991), 5% (Hailu, 2000; Hailemariam *et al.*, 2008) and 3.9% (Tadesse *et al.*, 2012). Likewise, the findings of this study are higher than findings of Melaku (1985) in Ogaden where 0.3% prevalence of *T. evansi* in camel was reported and 1.3% by Dia *et al.* (1997) from Mauritania. The lower parasitological prevalence observed in this study may be due to the differences in the detection methods, course of infection, season of the sample collection, and geographical area of the study as well as management and husbandry regimens of the camels under study.

Conventional parasitological techniques, especially the direct smear examination frequently fail to detect the parasite. This is particularly the case in endemic situations where the majority of infections are chronic with low levels of parasitaemia when trypanosomes concentrations in peripheral blood are below the detection limits 500,000/ml. This will be missing considerable percentages of actual infected animals reaching 50-80% of positive cases (Chappuis *et al.*, 2005; OIE, 2010). In addition, the season of sampling can also affect the prevalence of trypanosomosis observed; previous studies have shown that the prevalence is higher during rainy season than the dry season (Lohr *et al.*, 1985). Getahun (1998) also found that prevalence of 2.8% and 10.2% in agiven study area during dry and wet season, respectively. This is directly related to the density of insects transmitting trypanosomosis such as *Tabanid* and *Stomoxys*. Local epidemics of surra occur where conditions exist for the spread of infection with *T. evansi*, such as when many camels are stabled together or close herded and particularly when the biting fly population is abundant during the wet season (Luckins, 1988). The overall sero prevalence of camel trypanosomosis by CATT/*T.evansi* was 24%. This result indicate that camel trypanosomosis is wide spread disease of camels in the study site. The prevalence recorded in this study is in agreement with the findings in Bale zone (Hagos *et al.*, 2009). But it is smaller than the finding of Getahun (1998) in Leben district, south Ethiopia which was 56 % by using ab-ELISA. Ab-ELISA is said to be more sensitive and specific for trypanosomes (Luckins *et al.*, 1988). As lower prevalence was obtained in this study compared to sero prevalence of camel trypanosomosis in other Africa countries like Egypt (Zeyed *et al.*, 2010) and Kenya (Njiru *et al.*, 2004) using the same serological test. Contrary to the

parasitological findings a large proportion of camels were found to be sero positive. This can be explained by the fact that antibodies to *T. evansi* could persist even after the elimination of the parasite from the blood circulation which does not necessarily mean the presence of the parasite in the sera of the animals. Therefore, these methods cannot be used for the diagnosis of patent or sub-patent infections, neither for the differentiation of infected individuals from cured ones, nor for assessing the effectiveness of applied drugs or disease progression because they detect the antibodies that are long lasting after treatment (Zeyed *et al.*, 2010).

In this study an overall prevalence of camel trypanosomosis of 30.5% was detected by ITS-1 PCR. Of which the ITS-1 results clearly revealed that 20% camels were infected with the subgenus *Trypanozoon*, most likely *T. evansi*, 7.5% *T. vivax* and 3% mixed infection (*T. evansi* and *T. vivax*). Even though ITS-1PCR cannot differentiate between the *Trypanozoon* group, the positive results reported in this study are most likely be *T. evansi*, given that there was no record of tsetse fly distribution in that region of the country. In Ethiopia it is widely reported that tsetse fly and tsetse transmitted trypanosome prevail in large magnitude in south west, and north west part of the country following major river basins between longitude 33° and 38° E and latitude 5° and 12° N. The rest of the country, the north, south east and east is considered as tsetse free (NTTICC, 2004, Sinshaw *et al.*, 2006). There was no previous molecular study carried out in Ethiopia, however, the result from the present study is in agreement with reported in Kenya (Thumbi *et al.*, 2010) and in Sudan (Salim *et al.*, 2011). The internal transcribed spacer (ITS-1) region of the ribosomal RNA locus of eukaryotes have a high copy number (100-200 copies), having a small size (300 – 800bp), and vary both in sequence and length between species, but highly conserved in size in a given taxon, making it a preferred diagnostic target for universal test. The ITS1-PCR detection method constitutes a powerful molecular diagnostic tool for *T. evansi* detection and discrimination from other trypanosomes in one PCR. Interestingly, using ITS-1 PCR for the first time in Ethiopia the infection of camel with *T. vivax* was reported with prevalence of 7.5%, and mixed infection of *T. vivax* with *T. evansi* (3.%). Similarly, mixed infections of *T. congolense*, *T. brucei* and *T. vivax* have been reported from camels in Nigeria based on microscopic examination (Mbaya *et al.*, 2010). In contrary to this finding only *T. evansi* was detected using ITS-1 PCR in Sudan (Salim *et al.*, 2011). Though it is stated that *T. evansi* is the most common and pathogenic trypanosome infecting camels, *T. vivax* has also been incriminated to infect this animal (Mihok *et al.*, 2006). Most of the previously conducted studies on camel trypanosomosis in Ethiopia

were based on parasitological and serological studies; the low report about *T. vivax* in camels could be due to the inability of the tests to distinguish between the two species of trypanosome or due to less attention paid to *T. vivax* infection in camels. The previous immunological studies which were carried out in Ethiopia and elsewhere (Richard, 1979; Egbe-Nwiyi and Chaudry, 1994; Pays *et al.*, 2004; Hagos *et al.*, 2009) would not be able to survey camels for other trypanosome species, and this an advantage of the ITS1-PCR method used here.

The assessment of the agreement between the tests; parasitological, serological and molecular tests, indicated that there was slight agreement between parasitology with serology and molecular test. The relative sensitivity and specificity of CATT/*T. evansi* in this study were 87.5% and 77.3% respectively; these findings are similar with those of Dially *et al.* (1994) and Gutierrez *et al.* (2000) who reported sensitivity of CATT test to parasitological methods varied from 86 to 100 %. The slight agreement ($k=0.1$) could also be associated with the degree of detection of the tests. In case of parasitological, thin smear examination, the detection limit is parasitaemia level of 500,000 trypanosomes/ml of blood (OIE, 2010). As the CATT principally detects IgM, it for detects early infections or late infections with recent circulation of parasites in the blood, and can detect active infections with a high positive predictive value. The CATT/*T. evansi* test being an antibody-detecting assay of *T. evansi* in camels it has inherent shortcomings to distinguish current from cured infection (as detectable level of antibodies can still be found in self cured animals or after treatment with trypanocidal drugs might explain this enormous difference past and present infections (Luckins, 1988; Ngaira *et al.*, 2003). Furthermore, there was moderate agreement between CATT/*T. evansi* and ITS-1 PCR ($k=0.5$) in the present study. The CATT/*T. evansi* test failed to detect 34/92 (37%) of PCR positive samples, and 1/8 (12.5%) of Thin blood smear examination positive samples. The PCR and thin blood smear positive cases missed by CATT/*T. evansi* may indicate early infections where camels have not yet formed detectable antibody levels or isolates that lack Ro Tat 1.2 gene and/or do not express Ro Tat 1.2 VSG, likewise the case in Sudan (Salim *et al.*, 2011). The phenomenon of parasitological positive samples but CATT/*T. evansi* test based on the predominant variable antigen-type RoTat 1.2 VSG, negative were also observed in Kenya, by Elsaid *et al.* (1998); Davison *et al.* (1999) ; Ngaira *et al.* (2003).

Apart from identifying and confirming infections detected by parasitological and serological methods, PCR detected infections in 114 (29%) camels out of 392 camels which, based on

parasitological methods, were deemed negative this is due to higher sensitivity of the molecular technique, which is able to detect less than 5 trypanosome/ml of blood (OIE, 2010). The present result showed that the highest diagnostic sensitivity of PCR in camels was 100% (8/8) relative to the thin blood smear examination on the other hand PCR detects 58 (60.4%) out of 96 CATT positive camels. This is in agreement with (Ahmed, 2008), and explained by the fact that antibodies to *T. evansi* could persist even after the elimination of the parasite from the blood circulation which does not necessarily mean the presence of the parasite antigens in the sera of the animal (Kashiwasaki *et al.*, 2000; Zeyed *et al.*, 2010). Such difference of prevalence would have a significant impact on the strategy selected for the control of the disease in the affected areas.

Risk analysis

No statistically significant ($P > 0.05$) difference in prevalence of trypanosomosis was recorded among the study districts by all diagnostic tests; this was in contrary with Hagos *et al.* (2009). This may be due to similar agroecology between study districts of the present study. The occurrence of surra is influenced by climatic and ecological conditions, based on the distribution and abundance of the insect vectors.

The result of the logistic regression models suggested that there was association between camel trypanosomosis and the host factors: age, sex, body condition and reproductive status. In the present study a female camel was two times more likely to be infected with camel trypanosomosis compared to a male camel (OR = 2.16, CI: 1.351-3.470) as detected by ITS-1 PCR this is in agreement with Singh *et al.* (2004). This may possibly be related to the stress of lactations and successive pregnancies in females, while an old camel was four times more likely to be infected compared to young (OR = 4.27, CI: 1.742-10.448). Zero prevalence of camel trypanosomosis in young camels (< 5years) detected by thin blood smear examination in the present study was exactly in line with the finding Tadesse *et al.* (2012) in Jijiga Zone where similar prevalence was reported. The observation that seropositivity increases with age is also in agreement with previous studies (Dia *et al.*, 1997; Gutierrez *et al.*, 2000; Atarhouch *et al.*, 2003) and is probably related to, life time exposure to biting flies and possibility to get infected in their long life time, when infection is chronic and self-curing is unusual and the possibility that antibodies persist and detected in seropositivity. However the results of the

present study with different age group don't agree with the findings of Singh *et al.* (2004) who had found the highest incidence of *T. evansi* infection in camels of 5 years of age, 21.75%. In this study all the diagnostic tests employed by thin blood smear, CATT/*T. evansi* and ITS-1 PCR results were affected by the explanatory variables specifically poor body condition, pregnancy, history of abortion and lactation. In this finding pregnant (OR= 2.74, CI:1.37- 5.50) and lactating (OR= 2.6, CI:1.34-5.06) camels were 2.7 and 2.6 times more likely to be infected by trypanosomes compared to those non pregnant and non lactating camels respectively as detected by ITS-1PCR, this may possibly be related to the stress of those with the heaviest metabolic load due to successive pregnancy and then lactation, this result is in line with findings by Singh *et al.* (2004) in Kenya and Berline *et al.* (2010) in Israel. History of previous abortion was significantly associated with thin blood smear examination (OR= 6, CI: 1.08- 32.56) and ITS1 PCR positivity (OR= 2.46, CI: 1.10- 5.47) however this factor was not significantly associated, even though it had positive association with CATT/*T.evansi* sero positivity (OR= 2.02, CI: 0.855- 4.787) is suggesting that aborted camels were parasitemic and the acute parasitemia could cause abortion this is in agreement with (Gutierrez *et al.*, 2005). The Infection rate according to body condition score was recorded and camels with poor body conditions (OR= 2.96, CI: 1.55- 5.67) were three times at risk compared to those moderate body condition. Higher infection recorded in poor body conditioned camel might be due to lowered body resistance due to nutritional stress or other concurrent infections and therefore rendering them more susceptible to the immunosuppressive trypanosome infection.

Assessment of the association of PCV values with parasitological, serological and molecular tests indicated that significantly lower PCV was detected in seropositive and camels positive by ITS-1 PCR. In contrary to this, even though lower PCV values were recorded in parasitologically positive camels there was no statistically significant difference in mean PCV between parasitologically positive and negative camels by thin blood smear. It's stated in other part of this manuscript that parasitological tests are very less sensitive than the other tests. Implying those animals which are actually positives are missed by parasitological test. On the other hand, this is due to chronically infected camel, mostly detected by serological tests, show anaemia compared to acute infection (Singh *et al.*, 2006). Seropositive camels showed a significant decline in PCV values. These values were in agreement with the values of 21.4 and 25.3% for affected and healthy camels, respectively, recorded by Atarhouch *et al.* (2003) in

Morocco. In another study conducted in Mali, Diall *et al.* (1994) also found an association between low PCV levels, CATT/*T.evansi* positivity and presence of trypanosomes in the blood and the researcher also concluded that low PCV levels (18–22%), associated with CATT positivity are suggestive for *T. evansi* infection. Camels infected with *T. evansi* showed relatively lower PCV value compared to *T. vivax* positive camels, which is an indicative *T. evansi* is pathogenic than *T. vivax* in camel. Camels positive for mixed infection showed lowest PCV value than single infection of *T. evansi* or *T. vivax*. However, the PCV record values have to be considered with caution because of the involvement of several factors Diall *et al.* (1993) reported the importance of food and water availability, sex, age and even season on this factor.

6. CONCLUSION AND RECOMMENDATIONS

From this study it is concluded that trypanosomosis is an important disease and a potential threat affecting the health and productivity of camel in the study area. The pastoralists of the study area were not aware of the way of transmission as well as use of trypanocidal drugs as a treatment of camel trypanosomosis. Mono-infection of *T. evansi*, followed by *T. vivax*, as detected by ITS-1 PCR test were the species of trypanosomes affecting camels in which *T. evansi* takes the lion share. ITS-1 PCR detected significantly higher prevalence of camel trypanosomosis than the other two tests. It has added value of detecting multiple trypanosome infection in single reaction. Apart from identifying and confirming infections detected by parasitological and serological methods, PCR detected infections in 114 (29%) camels out of 392 camels which, based on parasitological methods, were deemed negative this is due to higher sensitivity of the molecular technique, therefore the molecular test was more sensitive and able to detect more species of trypanosome in higher magnitude of distribution. Pregnant, lactating and poor body conditioned camels are at a greater risk of acquiring trypanosomosis than non-pregnant, non-lactating and camels with moderate body condition. Those populations could therefore be prioritized targeted in the control of camel trypanosomosis in the districts surveyed. Therefore, based on the findings of the study, the following points are forwarded:

- ☛ The pastoralists should be educated on the means of transmission and management of the disease so as to enhance control of the problem through active community participation.
- ☛ Control of trypanosomosis in Ethiopia should not focus only on tsetse transmitted trypanosomes but emphasis should also be given to mechanically transmitted camel trypanosomosis.
- ☛ Further studies are necessary using more sensitive tests like PCR to establishing the real distribution and prevalence of camel trypanosomosis in different parts of the country to obtain a better understanding of their economic importance.
- ☛ Risk factors other than host- factors that cause epidemic outbreaks of the disease should be studied.

- ☛ Host parasite interaction of *T. evansi* and *T. vivax* with camel need to be studied, to understand the pathogenicity of the parasites on came.

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Online source: <http://www.ilri.org/InfoServ/Webpub/fulldocs/ilrad89/image/FIG20.jpg>

Online Source: <http://www.fao.org/docrep/006/x0413e/xo413E02.htm>

8. ANNEXES

Annex 1: Questionnaire set to interview camel owners of pastoralists

I. Animal description

Owner's name: -----Zone-----District: -----Pastoral Association -----
-----Date.....Code.....

II. Livestock Diseases

1. What are the most common diseases affecting your camel ?

.....
.....

2. Does trypanosomosis occur in this area? (Yes, no, other)?

If yes, what is the rank of trypanosomosis with regard to animal losses compared to other diseases?

.....

3. Which livestock does trypanosomosis most affect?

Cattle (yes, no, other)

Sheep (yes, no, other)

Goat (yes, no, other)

Camel (yes, no, other)

Others (specify)

4. What signs do you commonly observe when your camels get sick with trypanosomosis?

.....
.....

5. In which season/month do camels most often get the disease (trypanosomosis)?

.....

6. Do you know how camel trypanosomosis transmitted? (Yes, no, others)

.....

7. Do you think that the problem of trypanosomosis is expanding to new areas?
(Yes, no, we do not know) If yes what are the new areas affected?

.....

II. About drug use and treatment

- 1 .Previously sick with trypanosomes and treated: Yes.....No..... if yes
how long was the treatment.....

drugs used.....

2. Where are the common treatment sources?

Veterinary clinic

Local farmers

Smugglers

Others (specify)

Thank You! Name of interviewer

Signature.....

Annex 2: Data collection format for individual camel

ID	Dist rict	Age	Sex	BCS	Aborti on	Pregna ncy	Lactat ion	Diagnostic result			
								GTBS	PCV	CATT	ITS- 1PCR

Annex 3: Body condition scoring for dromedary camel based on Coombs (2002)

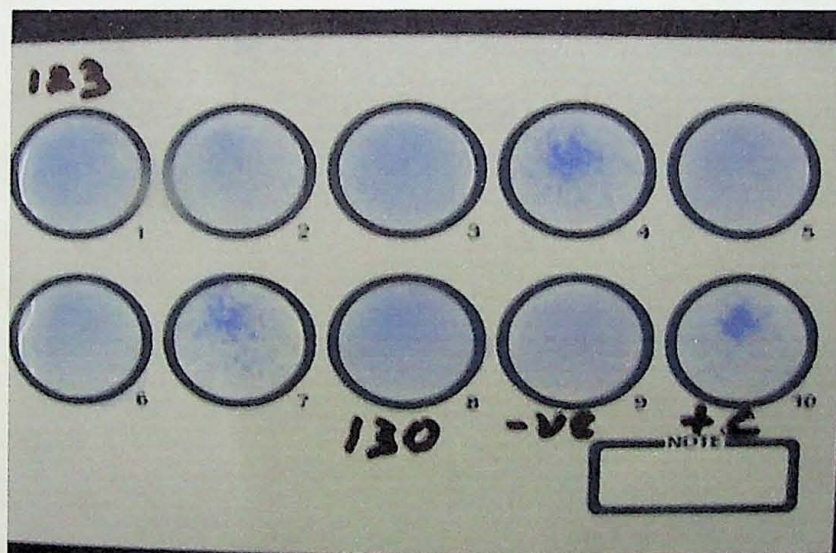
Poor body condition

- a. Transverse processes project prominently; individual dorsal spines are pointed to the touch
- b. Hips, tail-head and ribs are prominent.

Moderate body condition

- a. The animal is smooth, dorsal spines cannot be seen, but are easily felt.
- b. Ribs usually visible, little fat cover, dorsal spines are barely visible. Fatty
- c. Fat cover in critical areas can easily be seen and felt; transverse processes cannot be seen or felt.
- d. Heavy deposits of fat is clearly visible on tail-head, brisket, dorsal spines, ribs and hooks.

Annex 4: CATT/*T.evansi* test results



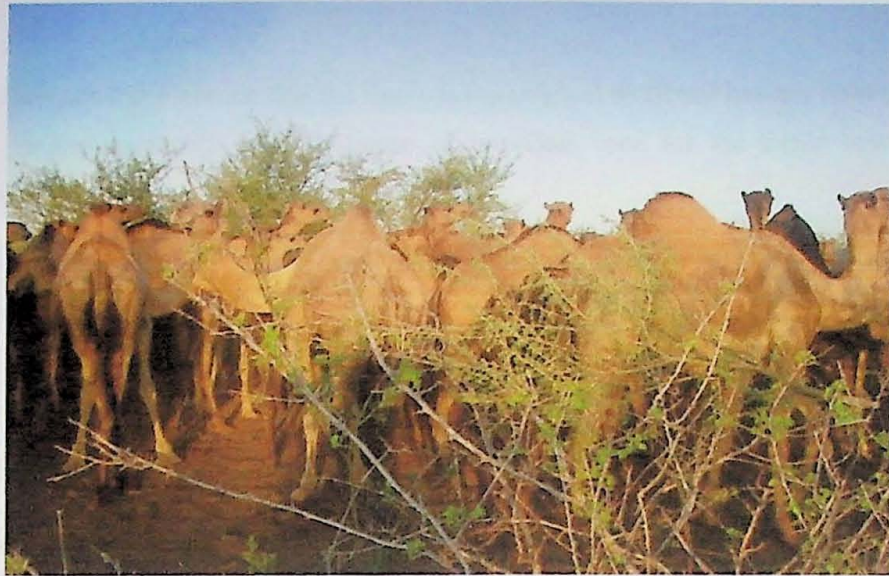
Annex 5: Selection of young camels for sampling from Dewe district



Annex 6: Camel calf, less than 6 month age, congregated at there stable



Annex 7: Adult male congregated at separate stable Chifra district



Annex 8: During extension work, treating sick camels after sampling in Dewe district



9. SIGNED DECLARATION SHEET

The undersigned, declaration that the thesis is my original work and has not been presented for degree in any university and all source of materials used for the thesis have been fully acknowledged.

Name _____

Signature _____

Date of submission _____

This thesis has been submitted for examination with my approval as a University advisor

Name _____

Signature _____

Date _____

Name _____

Signature _____

Date _____

: