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FACULTY OF VETERINARY MEDICINE**

**PREVALENCE OF CAMEL TRYPANOSOMOSIS AND ASSOCIATED RISK FACTORS
IN SELECTED DISTRICTS OF BALE ZONE, ETHIOPIA**

**MSC THESIS
BY
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LIST OF ABBREVIATIONS

AGID	Agar Gel Immuno Diffusion
BCS	Buffy coat smear
BoTat	Bordeaux Trypanosoma Antigenic Type
CATT	Card Agglutination Test for Trypanosomosis
CFT	Complement Fixation Test
DIM	Diminazene Aceturate

ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
IFAT	Indirect Fluorescent Antibody Test
IgG	Immunoglobulin G
ISMM	Isometamidium Chloride
Kg	Kilogram
mAECT	Mini Anion Exchange Centrifugation Technique
mHCT	Mini Haematocrit Centrifugation Technique
MEGA	Multiplex - Endonuclease Genotyping Approach
OIE	Office International des Epizooties
OVI	Onderstepoort Veterinary Institute
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
RoTat	Rhode Trypanosoma Antigenic Type
VAT	Variable Antigenic Type
VSG	Variable Surface Glycoproteins

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ABSTRACT

A cross sectional study was conducted to determine the prevalence of camel trypanosomoses and to identify the risk factors associated with the disease occurrence in Dello-mena and Sawena districts of Bale zone. Blood samples were collected from 619 camels and examined for the presence of *T. evansi* using card agglutination test for trypanosomiasis (CATT / *T. evansi*), and a parasite detection test (buffy coat smear – BCS). Clinical examinations as well as questionnaire survey, regarding information about the host and management practices were conducted. Questionnaire survey results indicated that high milk production, bush encroachment as well as the ability to survive drought are the major reasons for camel keeping. Camel trypanosomoses locally known as “Dhukan” is the most important complaint of camel breeders. Respondents clearly described the diseases and ranked it as the most important disease of camel in the study area. The prevalence of the disease was found to be 12.12% using buffy coat smear and 24.88% with CATT. Camels in Dello-mena had a significantly higher prevalence than camels in Sawena district in both BCS and CATT / *T. evansi* examination results. Thus it was found that camels in riverine areas of Dello-Mena are more at risk of infection than herds in non-riverine areas of Sawena. Age appeared to be a risk factor for individual trypanosomoses serological status and that the highest seroprevalence was observed in camels greater than five years old. Poor body condition was significantly correlated with positive status in both CATT and BCS results. The average PCV of parasitological positive camels was found to be significantly different from the average PCV of parasitological negative camels. However, the average PCV of seropositive but parasitological negative camels did not differ from the average PCV of seronegative camels. From the result of this study it can be inferred that camel trypanosomoses is endemic in dello-mena and Sawena districts with a higher degree of prevalence in Dello-mena than Sawena district. Absence of proper animal health extension and the indiscriminate use of camel trypanocidal drugs often underdosed are major constraints in camel health delivery in the study area. Thus, attention has to be given to provide adequate veterinary service and sensible animal health extension systems. Furthermore, considering the widespread existence of surra and its impact on camel productivity further study on identification of the principal vector species responsible for transmission and investigating the potential for vector control is recommended.

Key words: Camel, trypanosomosis, BCS, CATT/*T.evansi*, Dello-mena, Sawena, Bale Zone.

1. INTRODUCTION

The camels (*Camillus dromedarius*) are the important livestock species uniquely adapted to hot and arid environments. Camels provide milk, meat, wool, hair, hides and serves for riding and use as a draft animal for transport. Camels have been known to have a particular or peculiar physiological feature, by which they regulate body temperature to changes in ambient temperatures, which enable them to survive and adapt to such hard conditions (Wilson, 1984; Yagil, 1985; Higgins *et al.*, 1992). The liveliest ecological zones for camels are the tropical, sub tropical arid areas of Africa and Asia. In Africa, camel production is dominantly exercised in the east and northern parts of Africa . The eastern Africa has been reported to consist population of camels, amounting, 16.5 million (Schwartz and Dioli, 1992; Wilson, 1998).

Ethiopia has one of the largest camel populations in the world. Over one million camels were estimated to exist in the country. The proportion of camels in the total domestic herbivores biomass (DHB) is 4% at the national level and 35% in the semi arid and arid parts of the country. Camels are kept in the arid low lands, which cover 50% of the country and the home range of over 2 million pastoralists. The major product is milk with an official estimate of 174000 tones per year, followed by 20000 tones of meat. Hairs, hides and drought power are recognized as potential products but are not exploited to any significant extent (Schwatz and Dioli, 1992).

Most camels suffer from trypanosomosis (surra) caused by *Trypanosoma evansi* that is transmitted mechanically, independent of tsetse flies. Natural and experimental camel trypanosomosis have been described in different parts of the world (Dia *et al.*, 1997; Njiru *et al.*, 2002). Severe outbreaks, which occurred in different parts of the world where several thousand animals died in the 1970s and, of late, in 1994 and 1995, for instance, in Pantamal, Brazil, have also been well documented (Luckins, 1998). These epidemics pose a major constraint to camel productivity given their importance as a source of meat, milk production, transportation and draught power, as well as by-products (wool, hair, skin and hides). In addition, they also provide foreign currency to their owners from their export (Elamin *et al.*, 1999).

Camels are produced by pastoral societies of the developing countries who dwell in dry marginal areas. 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 research. This affects the depth of our knowledge on the general aspects of and systematic interest (Baumann and Zessin, 1992). In Ethiopia until recently there has been very little systematic research and no development projects that feature the camel in any way (Schwartz and Dioli, 1992). Generally, there is negligence towards the promotion of camel health and production. Only recently that camel became the subject of more intensive and systematic interest (Schwartz and Dioli, 1992). The existing published documents about camels so far either based on small numbers of animals, short observation periods, one-time surveys, interviews or estimates and simulations. Recently, from their continuing importance as an essential element of the life support system, their complementarity with other domestic species, sustainable system of production in harsh and marginal environments increases the interests of scientists, development workers and extension agents (Schwartz and Dioli, 1992; Wilson, 1998).

Although *T. evansi* occurs in a variety of ecological regions in the Old and the New World and affects many different domesticated livestock species, there is a lack of epidemiological data from which it is concluded that surra is of little economic importance - a judgment based on ignorance, rather than fact. Moreover, where there are data on the prevalence of *T. evansi* they almost invariably derive from studies carried out in response to serious outbreaks of disease and diagnosis is often restricted to parasitological techniques or clinical signs. The impact of *T. evansi* is recognized only when epidemic outbreaks of disease cause widespread loss. That economically significant production losses might occur also when less acute forms of disease exist is not often considered. Deficiencies in our knowledge of the epidemiology of *T. evansi* that are required to remedy this situation include, our knowledge and understanding of the natural history of the disease, the absence of any strategies for effective monitoring and surveillance, and the inability to design and implement cost-effective, appropriate strategies for control of the disease. Hence, considering the growing reliance on camels and the importance of trypanosomosis in the pastoral production systems the present study was undertaken to determine the prevalence of camel trypanosomosis in Dello- mena and Sawena districts of Bale lowlands.

The objectives of this study are therefore to determine the prevalence of camel trypanosomosis and to identify risk factors associated with the disease occurrence and to gain some insight into the local perceptions of camel health problems in general and camel trypanosomosis in particular through questionnaire survey, clinical observation and parasitological and serological examinations.

2. LITERATURE REVIEW

2.1. Distribution of camels (*Camillus dromedarius*)

Camelid was probably among the last of major domestic species to be put to regular use by man. The most likely time of domestication is about 4000 years before present or slightly earlier. The presumed area of domestication is the southern Arabian Peninsula, probably the area of Yemen and Oman. From presumed center of domestication, the dromedary has subsequently been distributed to almost the rest of the world (Schwartz and Dioli, 1992; Wilson, 1998).

Environmental, social and cultural factors have great influence on the distribution and production of camels. Arid and semi arid zones of tropical and subtropical countries of Africa and Asia are found to be of convenient ecology. The greatest cultural influences in recent distribution of camels was the advent of Islam, when Arabs spread their gospel, consolidating its ranges north and east wards in Asia, and along the Mediterranean littoral. There have been many attempts to introduce camels outside the “normal” range, in Brazil, Colombia, USA, Cuba, Spain, Italy and France (Wilson, 1998). Generally, there has been steady increase in camel population since about 1980s. However, decrease in numbers has been observed in some countries for instance, where oil is the principal commodity and the nomadic way of life is no longer the major one (Wilson, 1998). Eastern Africa is known to be the heartland for camel production as 80% and 63% of the Africa and world population, respectively, is produced in the region. Subsistence camel production is practiced in dry areas of Ethiopia that cover 61% to 65% of the total land area. The eastern part of the country is considered as the heartland for camel production, which is the home of two – third of the nations camel population (Getahun and Bruckner, 2000).

2.2. Potential importance of camels

Camels are primarily the domestic animals of pastoral communities that ensure their food security (Wilson, 1998). They produce milk, meat, hair and hides, and also serve as a draught animal for agriculture and transport people as well as goods (Schwartz and Dioli, 1992). Milk and meat are the important products that camels produce elsewhere. A study in eastern Ethiopia indicated 3 - 6 liters of daily milk yield over 13 - 15 months of lactation length. Long lactation and ability to maintain milk production over long dry spells are important facets of camel productivity. Apart from home consumption, the majority of the households sell at least one-third of the produced milk to generate cash income (Getahun and Bruckner, 2000). Daily milk yield can be as high as 20 liters with improved management conditions (Schwartz and Dioli, 1992; Wilson, 1998). Until the arrival of motorized transport in the arid and semi arid zones, camels have been the sole means of transport in the areas where they are adapted. They are also used for wheel transport, water lifting and for deriving oil mill. Camel racing and other leisure activities such as camel safaris and trekking have recently become a tourist attraction and luxurious in some part of the world (Schwartz and Dioli, 1992; Higgins *et al.*, 1992; Wilson, 1998).

From global perspective, the economic production of camels seems minimal. In Ethiopia they are also the subset of huge livestock resource when considered from national economic point of view (Getahun and Bruckner, 2000). However, what makes the difference is its adaptation to harsh environment to produce milk from scanty and highly variable feed resources. The most significant merits to perform in areas where other livestock species do not thrive and perhaps do not survive are attributed to the economic use of water in almost all metabolic functions and wide range of feed resource utilization (Yagil, 1985; Wilson, 1998). In mixed species the camel feeds on plants or part of plants that are not eaten by other conventional livestock due to its size to browse the highest strata, thus reducing competitions and enhancing complementarities (Wilson, 1998).

2.3. The disease (Camel trypanosomosis)

Most camels suffer from trypanosomoses (surra) caused by *Trypanosoma evansi* that is transmitted mechanically, independent of tsetse flies. Natural and experimental camel trypanosomoses have been described in different parts of the world (Dia *et al.*, 1997; Njiru *et al.*, 2002). Severe outbreaks, which occurred in different parts of the world where several thousand animals died in the 1970s and, of late, in 1994 and 1995, for instance, in Pantamal, Brazil, have also been well documented (Luckins, 1998).

Surra is an animal disease occurring in Africa, Asia and Latin America, caused by *Trypanosoma evansi*. The parasite can infect different host species and is mechanically transmitted by different biting flies such as *Tabanidae* and *Stomoxys* as well as by vampire bats such as *Desmodus rotundus*. Camels and horses are very susceptible to the infection and death can occur within weeks or months. Moreover, *T. evansi* infections of cattle and buffaloes usually lead to a pronounced immunosuppression resulting in an increased susceptibility to other opportunistic diseases such as Pasteurellosis and anthrax.

Diagnosis of a *T. evansi* infection usually starts with clinical signs or the detection of antibodies. Conclusive evidence of *T. evansi* infection, however, relies on detection of the parasite in the blood or tissue fluids of infected animals. Unfortunately, parasitological techniques cannot always detect ongoing infections as the level of parasitaemia is often low and fluctuating, particularly during the chronic stage of the disease.

In Africa trypanosomosis is a major constraint to the expansion and production of livestock and their products on approximately 10 million km² of land, covering 37 countries (FAO, 2000). Most camels suffer from trypanosomosis (surra) caused by *Trypanosoma evansi* that is transmitted mechanically, independent of tsetse flies. Camels are also affected to a lesser extent by the tsetse transmitted trypanosome species *T. brucei* (Evans *et al.*, 1995). *T. evansi* is widely distributed wherever camels are found (Losos, 1980; Luckins, 1988).

2.3.1. Etiology of surra

Camel trypanosomosis, also known as surra, is caused by *Trypanosoma evansi*. *T. evansi* belongs to the subgenus *Trypanozoon*, together with *T. equiperdum* and *T. brucei* (Table. 1). The parasite can infect different host species and is mechanically transmitted by different biting flies such as *Tabanus* and *Stomoxys*. It is hypothesized that *Trypanosoma evansi* is originated from *Trypanosoma brucei* by adaptation to a non cyclical mode of transmission and loss of ability to undergo growth and differentiation in the fly vector (Luckins, 1998). Other species of trypanosomes, e.g. *Trypanosoma congolense*, *Trypanosoma brucei* and *Trypanosoma vivax* have also been isolated from camels in the Sudan, but their role in camel trypanosomoses is insignificant (Mahmoud and Gray, 1980; Elamin *et al.*, 1999).

Table 1: Taxonomy of *Trypanosoma evansi*.

Phylum	Protozoa
Class	Zoomastigophora
Order	Kinetoplastidae
Family	Trypanosomatidae
Genus	<i>Trypanosoma</i>
Subgenus	Trypanozoon
Species	<i>T. brucei</i> , <i>T. evansi</i> , <i>T. Equiperdum</i>
Subspecies	<i>T. b. brucei</i> , <i>T. b. gambiense</i> , <i>T. b. rhodesiense</i>

Source: Hoare, 1972

Morphology

Morphologically, *T. evansi* and *T. equiperdum* cannot be distinguished from the slender form of *T. brucei brucei*. Although pleomorphism may appear to some extent generating stumpy and intermediate forms in some strains, *T. evansi* is generally monomorphic, assuming a long slender

form. Strains from different geographical areas and various host sources are morphologically indistinguishable. The trypanosomes measure 14-33 μm in length with a width of 1.5 – 2.2 μm . They possess a free flagellum and a small sub terminal kinetoplast. Dyskinetoplastic forms, in which the circular kinetoplast DNA (KDNA) is absent, are found in wild strains as a result of mutation, or after treatment with trypanocides such as diminazene or prothidium (Hoare, 1972). Although *T. evansi* and *T. equiperdum* are indistinguishable by light or electron microscopy, the clinical presentation of the disease indicates which organism is most suspect (Brun *et al.*, 1998). Additionally, *T. evansi* is covered by a thick layer of a single glycoprotein (or variable surface antigen), that is the primary immunogen eliciting antibody formation. Periodically, the organism alters the glycoprotein coating and evades the host's defensive responses. Randomly amplified polymorphic DNA analysis showed limited heterogeneity in *T. evansi* stocks from different hosts and geographical regions of the world, or in other species of the subgenus Trypanozoon (Ventura *et al.*, 2001)

2.3.2. Epidemiology

Distribution and host ranges of surra

Trypanosoma evansi is the most widely distributed of the pathogenic animal trypanosomes, affecting domesticated livestock in Asia, Africa and Central and South America (Luckins, 1998). It causes epidemics of a disease called surra, which is of great economic importance in Africa, Asia and South America, where thousands of animals die from *T. evansi* infection each year (Brun *et al.*, 1998).

Trypanosoma evansi has been spread widely by biting insects outside tsetse-infested regions of sub-Saharan Africa and is at present known to occur throughout the Sahel region of Africa, in North Africa, most Near and Far East Asian countries (including southern Siberia, China and Indonesia) and many countries in Latin America, from Argentina in the south to at least Venezuela and Colombia in the north. The situation in Central America is not well defined, but in the past the parasite has been reported from at least one country, Panama (Uilenberg, 1998). Its spread into

South East Asia has occurred only relatively recently, and the serious epidemics of surra that were recorded in the early years of the present century in Indonesia and the Philippines, suggest that it could have spread into these regions within the last 100 years (Luckins, 1988; Lun *et al.* 1993). Similar outbreaks of disease occurred also in South America in the 19th century. In Africa, *T. evansi* occurs in areas beyond the northernmost limits of the tsetse fly and affects livestock in Kenya, Somalia, Ethiopia, Eritrea and the Sudan. Countries in North Africa are also affected. More recently, it has gained additional importance in certain semi-arid areas with increasing use of camelidae for meat production (Horchner, 1983).

Surra is essentially a disease of camels and horses (also mules and donkeys) and is also highly pathogenic for dogs. It is also of considerable economic importance in Asian (domestic) buffalo, and to a less extent in some countries in cattle as well. Many other host species, wild as well as domestic have been found naturally infected (Uilenberg, 1998). Although many species of domesticated livestock can be infected with *T. evansi*, the principal host varies geographically. In Africa, beyond the northernmost limits of the tsetse fly belt, and in parts of East Africa, camels are the most important host (Dia *et al.*, 1997), whilst in Central and South America the horse is principally affected (Monzon *et al.*, 1990; Silva *et al.*, 1995). In Asia, a much wider range of hosts is involved, including the Bactrian camel and dromedaries, cattle, buffalo, horses and pigs (Pathak *et al.*, 1993; Partoutomo *et al.*, 1994; Tuntasuvan *et al.*, 1996). 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 *Trypanosoma evansi*. Nevertheless, there are reports of serological evidence of infection in goats and sheep from the Sudan and in cattle from Brazil (Boid *et al.*, 1981; Franke *et al.*, 1994; Luckins, 1998) and both goats and cattle have been considered as potential reservoirs of infection (Denning, 1989; Luckins, 1998).

It is not clear to what extent wild-animal foci of infection exist. Capybara (*Hydrochoerus hydrochoerus*), wild dogs, (*Canis azarae*) and deer (*Axis axis*, *Rusa timorensis*) have been shown to be infected with *Trypanosoma evansi* but there have been few attempts to investigate their relationships with livestock. *Trypanosoma evansi* is primarily a parasite of domesticated livestock,

and infections in wild animals are acquired secondarily and the disease is often fatal (Clark and Dunn, 1933). Chronic infections in wild animals do sometimes occur, as in the capybara. Some reports indicate that capybara are highly susceptible to *Trypanosoma evansi*, and capybara were implicated as reservoirs of infection in a ranch in the Pantanal region of Brazil. Although not usually considered of zoonotic concern, one case of human infection with *Trypanosoma evansi* recently has been documented in India (WHO, 2005).

Surra is found to be one of the most important disease problems of camels in almost all camel rearing areas of Ethiopia and results from different surveys indicated a prevalence range of 0.3 up to 31.9 percent (Table 2).

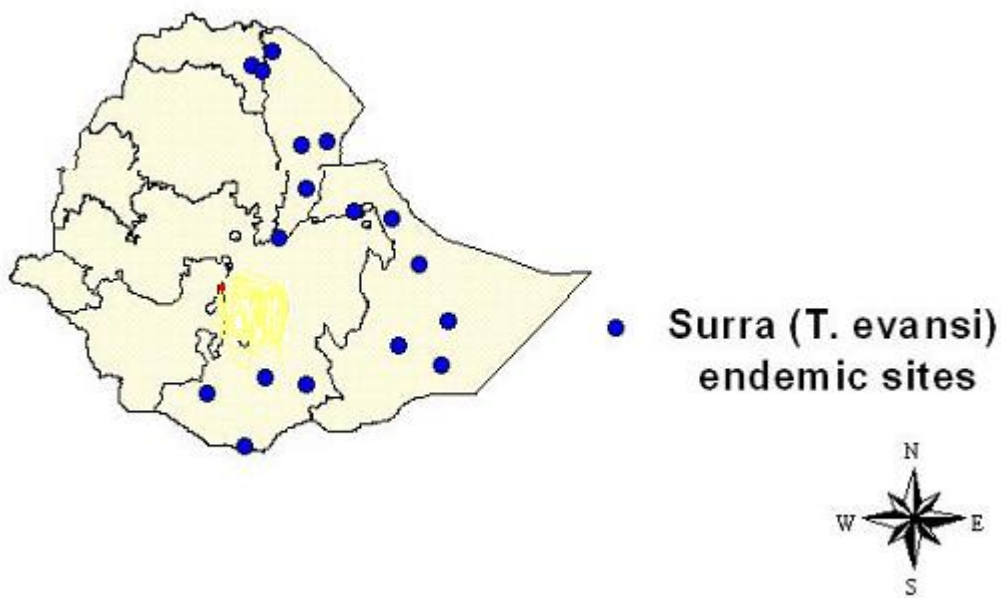


Figure 1: Distribution of surra in Ethiopia

Table 2: Retrospective data on the prevalence of camel trypanosomosis in Ethiopia.

Authors and year	Study areas	Prevalence (%)	Trypanosoma species
Richard (1979)	Borena		<i>T. evansi</i>
Zelege (1982)			
Ketema (1990)	Borena	237/1100 (21.54%)	<i>T. evansi</i>
Abebe (1991)	Ogaden	21/321 (6.54%)	<i>T. evansi</i>
Tenaye (1993)	Borena	94/294 (31.9%)	
Tekle and Abebe (2003)	Borena	43/391 (10.9%)	<i>T. evansi</i>
Getahun (1998)	Leben, Borena	33/324 (10.2%) Wet season	<i>T. evansi</i>
		(2.8%) Dry season	Mixed) <i>T. evansi</i> and <i>T. vivax</i>
Ahmed (1998)	Dire Dawa, Jijiga East Hararghe	26/336 (7.7%)	<i>T. evansi</i>
Reshad (1999)	Dire Dawa	12/248 (4.8%)	<i>T. evansi</i>
Demeke (2000)	Afar and Tigray	14/280 (5%)	<i>T. evansi</i>
Elias (2003)	Arsi, Bale and East Shoa	19/347 (5.5%)	<i>T. evansi</i>

Transmission

Surra is transmitted mechanically by the bites of haematophagous flies. *T. evansi* lacks the genes necessary for mitochondrial development (Gibson *et al.*, 1983; Borst *et al.*, 1987; Songa *et al.*, 1990) and is therefore unable to undergo growth and differentiation in the insect vector. Nevertheless, this has not precluded transmission by insects. It is speculated that the widespread occurrence of *Trypanosoma evansi* is largely due to its being spread mechanically by the bites of haematophagous flies, e.g. Tabanus. Stable flies (Stomoxys) have also been incriminated, but based on experimental transmission between horses, guinea pigs and dogs; they do not appear to be important vectors (Losos, 1980). Large biting insects such as tabanids carry more blood and are more likely to act as mechanical vectors than for example mosquitoes. Tsetse flies themselves can of course also act as mechanical vectors. This mode of transmission has proved to be sufficiently effective to maintain *Trypanosome evansi* in South and Central America, North Africa and Asia (Uilenberg, 1998).

More than 20 different species of Tabanus have been shown experimentally to transmit *Trypanosoma evansi* (Luckins, 1998). Furthermore, surveys of Tabanus in the various tropical areas have shown a definite correlation between the seasonal outbreaks of *Trypanosoma evansi* infections and the increase in number of Tabanus during the rains (Mahmoud and Gray, 1980; 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 species 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). However, the efficiency of the different flies to transmit *Trypanosoma evansi* appears to vary in different geographic conditions and is also dependent on the interval between two successive feeds and intensity of the fly challenge (Luckins, 1998).

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).

However, no definitive study has ever been conducted to confirm their role in the epidemiology of trypanosomosis, and it is, therefore, not really clear how important they are.

Risk factors

The occurrence of surra is influenced by climatic and ecological conditions, based on the distribution and abundance of the insect vectors. It has a marked seasonal incidence related to wet and humid condition and increased activity of biting flies during the wet season. Local epidemics of surra occur where conditions exist for the spread of infection with *T. evansi*, such as when many animals are stabled together or close herded and particularly when the biting fly population is abundant during the wet season (Luckins, 1994). Surveys of *Tabanus* in the various tropical areas have shown a definite correlation between the seasonal outbreaks of *Trypanosoma evansi* infections and the increase in number of *Tabanus* during the rains (Mahmoud and Gray, 1980; 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 may explain the sporadic occurrence of the disease during the dry season and outbreaks during the rainy season (Njiru *et al.*, 2002). However, the efficiency of the different flies to transmit *Trypanosoma evansi* appears to vary in different geographic conditions and is also dependent on the interval between two successive feeds and intensity of the fly challenge (Luckins, 1998).

Age of the camel, season and ecological conditions are apparent risk factors for the disease. Important is that in dry season herds in riverine areas are more at risk of infection than herds in non-riverine areas and that more cases of trypanosomoses were encountered in the rainy seasons than in the dry seasons (Getahun, 1998).

2.3.3. Clinical manifestations

Trypanosoma evansi can infect a variety of hosts and causes a species-specific pathology (Enwazor et al., 2005). In camels the disease is manifested by elevation of body temperature which is directly associated with parasitaemia. Infected animals show progressive anaemia, marked depression, dullness, loss of condition, and often rapid death (Mahmoud and Gray, 1980; Luckins, 1998). Anaemia was observed to be a major clinical finding in camel trypanosomosis in Morocco (Rami et al., 2003). Milder 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 death (Tuntasuvan et al., 1997); abortion (Lohr et al., 1986); weight loss, reduced draught power (Luckins, 1998) and nervous signs like circling movement and trembling, unusual aggressiveness, running aimlessly and sudden collapse in severely stressed and over worked animals (Manuel, 1998). At post mortem, necrotic foci in the liver and spleen as well as generalised lymphoid tissue hyperplasia are common in camels suffering from surra (Rottcher et al., 1987).

Experimental studies have shown that marked immunosuppression to vaccine antigens occurs in goats and sheep that have only sub-clinical infections, hence, even in situations where *T. evansi* does not cause overt signs of illness, it could compromise vaccine efficacy or lead to severe disease with intercurrent infections. In the field, reports of disease in camels, buffalo, cattle, horses and pigs are well documented and among the effects seen are death, abortion weight loss and reduced draught power (Mulligan, 1970).

2.3.4. Pathology and pathogenesis

Anaemia is a major component of the pathology of surra and of African trypanosomosis generally. Anaemia in *Trypanosoma evansi* infections of camels is reportedly macrocytic and hypochromic (Jatkar and purohit, 1971). In the early phases of infection the anaemia is haemolytic and haemophagocytic. The mechanism(s) responsible for this increased erythrophagocytic activity are

not fully understood. Several have been proposed, viz, immune complexes, expanded mononuclear phagocytic system per se, haemolytic factor produced by the trypanosome, fever and disseminated intravascular coagulation (Food Agricultural Organization, 1979). 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. 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. The marked depression observed in camel trypanosomosis is a mental state and is a manifestation of depression of cerebral cortical function in various degrees. Other nervous signs reported, such as circling movement, incoordination and dullness, appear to be the results of brain tissue disturbance or damage by the parasites. Evidence of *Trypanosoma evansi* being found in the cerebrospinal fluid has been presented (Rottcher *et al.*, 1987).

The atypical lesions of multiple necrotic foci found in the liver and spleen, as well as generalized lymphoid tissue hyperplasia in camels suffering from surra on post mortem, could be attributed to pathological events that occur in the tissues of animals infected with *Trypanosoma evansi*. The degenerative changes thus observed could be due to tissue anoxia, possibly caused by anaemia, which results in a fall in tissue pH and vascular damage. Other mechanisms may also be involved. It is known that *Trypanosoma evansi* is a member of the Brucei group of trypanosomes, which have a known preference for connective tissues of a host, where they disrupt the collagen bundles and destroy the fibroblasts which produce and maintain the collagen (Boid, 1980). This disruption of host connective tissues, along with the vascular damage attributable to brucei group trypanosomes (Boid, 1980), would be expected to release large quantities of cytoplasmic and mitochondrial enzymes into the serum, thereby causing further tissue damage.

Indeed, a two-step process in the pathology of infection with *Trypanosoma evansi* in camels based on studies of changes in serum enzymes has been proposed (Boid, 1980). The first step coincides with the appearance of trypanosomes in the host bloodstream and is characterized by a sharp and as yet unexplained rise in sorbitol dehydrogenase (SDH) activity. The second step occurs later in the

infection and is characterized by a large increase in serum levels of glutamic oxaloacetic transaminase (GOT) now known as aspartate alanine transferase (AST) and a smaller rise in glutamic pyruvic transaminase (GPT), now known as alanine amine transferase (ALT). The rise in AST level can be attributed partly to cellular damage caused by the trypanosomes lysis, while the increase in ALT probably results from host destruction of trypanosomes. AST is found mostly in cell organelles and rises when there is a great damage to the heart, kidney, skeletal muscles and liver. ALT is a specific liver enzyme found in the cell cytoplasm and its rise is associated with cell membrane damage. The reported increases in these enzymes, especially AST, is not surprising as it is indicative of organ damage and supports the post mortem reports of necrotic foci in the liver and spleen of camels suffering from surra. The fever characterized by high temperature might be due to the effects of toxic metabolites produced by dying trypanosomes (Tizard *et al.*, 1978). In addition, the oedema reported in the dependant parts of the body during the chronic stage could be due to a significant decrease in the albumin levels, resulting in alterations in osmotic pressure of the blood. This leads to excessive accumulation of fluid in tissue spaces caused by a disturbance in the mechanism of fluid interchange between capillaries, the tissue spaces and the lymphatic vessels. All this possibly indicates great liver damage. The haemorrhage and serous exudates that occurred could be caused by haemolysis involving the expanded mononuclear phagocytic system. This has also been observed in *Trypanosoma brucei*-infected donkeys, while the frequent abortions reported may be attributed to endocrine dysfunction (Losos, 1980).

2.3.5. Immune response

Pronounced immune changes occur in African trypanosomosis. An increase in gamma-globulin (IgM) during both acute and chronic *Trypanosoma evansi* infections in camels has been reported (Boyd *et al.*, 1981) but this is not protective, as the majority of the antibodies are auto antibodies. Leucocytosis, neutrophilia and eosinophilia have been reported in *Trypanosoma evansi* infections of camels (Anosa, 1988). These changes occur as a result of an increase in the activity of the mononuclear phagocytic system. The eosinophilia observed is a feature of parasitic infections and is associated with immediate-type hypersensitivity reactions. The cells are expected to accumulate in tissue in response to tissue injury. In the acute phase of the disease, lymph nodes and spleen are remarkably reactive, with plasma cells predominating. This may account for the generalized

lymphoid tissue hyperplasia characteristic of *Trypanosoma evansi* infections, while in the late stages the immune system becomes depleted of lymphoid cells (Losos, 1980). Circulating and tissue-mediated immune complexes have been demonstrated in laboratory animals infected with *Trypanosoma brucei* species, and much of the antibody found in them is directed against the trypanosome (FAO, 1979). Complement has also been found in association with them. These immune complexes are likely to have wide varying pathological effects including anaemia, complement activation, tissue damage and interference with both induction and effector mechanisms of the immune response (FAO, 1979).

Hypocomplementaemia (decreased alteration of the complement system) has been reported, including elevated levels of immunoconglutinin and deposition of complement in the tissues. Further possible evidence of complement reactivity is found in the demonstration that the kinin system becomes activated with the release of pharmacologically active substances, thus causing microvascular dilatation and increased permeability (FAO, 1979). A state of immunosuppression later develops associated with these changes. The host immune response to a variety of antigens has been found depressed in animals under experimental conditions (Baltz *et al.*, 1981; Anene *et al.*, 1989).

2.3.6. Diagnosis

Diagnosis of a *T. evansi* infection usually starts with clinical signs or the detection of antibodies to *T. evansi*. Conclusive evidence of *T. evansi* infection, however, relies on detection of the parasite in the blood or tissue fluids of infected animals. Unfortunately, parasitological techniques cannot always detect ongoing infections as the level of parasitaemia is often low and fluctuating, particularly during the chronic stage of the disease (Claes *et al.*, 2003).

There are no pathognomonic signs of surra and so laboratory diagnosis has to be carried out to confirm infection. Traditionally, this involves parasitological and serological diagnosis. Parasitological diagnosis is mainly carried out by the direct microscopic examination of blood or buffy coats and/or sub-inoculation of camel blood into rodents such as mice or rats. However, the

test has a poor sensitivity, often less than 50% (Nantulya *et al.*, 1989; Monzon, 1990; Nantulya, 1990; Luckins, 1992; Yadvendra *et al.*, 1998). The implication of this is that in most situations *Trypanosoma evansi* is under-diagnosed and the level of infection is greater than frequently reported. On the other hand, serological techniques, e.g. immunofluorescent antibody test (IFAT), enzyme Linked Immunosorbent Assay (ELISA) and the Card Agglutination Test for Trypanosomosis (CATT), although sensitive, cannot distinguish current from cured infections (Luckins, 1988).

Recent tests, like, latex agglutination test (LATEX) or SURATEX 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). A comparative sensitivity test for *T. evansi* in camels in Kenya revealed 68.8% sensitivity for CATT/*T. evansi* and 58.8% for SURATEX, although the two techniques showed no significant difference (Ngaira *et al.*, 2003). Similarly, at prevalence values between 10 and 100%, CATT/*T. evansi*, as well as SURATEX, had infinitely high positive predictive values, whereas SURATES had a lower NPV than CATT/*T. evansi* and the two techniques were more sensitive than parasitological methods in revealing the true extent of trypanosomosis in camel herds (Ngaira *et al.*, 2003). Atarhouch *et al.* (2003) diagnosed a prevalence of 14.1% (CATT/*T. evansi*) and 18.2% (ab-ELISA) in provinces located in the South and East of the Atlas mountain chain in Morocco. Similarly, Delafasse and Doutouin (2004) using buffy coat technique (BCT) and CATT/*T. evansi* revealed a prevalence rate of *T. evansi* of 5.3 and 30.5%, respectively, in Chad. However, non-validation, standardization, application and deployment are factors militating against their use in the field.

PCR amplification of the RoTat 1.2 VSG gene is a specific marker for *T. evansi* strains, except *T. evansi* type B, and is especially useful in dyskinetoplastic strains where kDNA based markers may fail to amplify (Claes, 2003)

2.3.7. Treatment and control

Control of *T. evansi* is limited to treating animals that are considered infected on the basis of the clinical signs, or treatment of animals showing patent infection. Treatment of proven infected, or clinically affected animals, fails to address the underlying problem of carrier animals. The low sensitivity of parasitological techniques and the unreliability of clinical signs ensure that this approach cannot decrease the number of cases as effectively as treating all infected individuals. If wildlife reservoirs are of no importance, and if small ruminants and dogs present little or no problem, then it should be possible to effect control based solely on the use of chemotherapy (Luckins, 1986). Treatment with trypanocidal drugs is the usual method of control of *T. evansi*, and five compounds, suramin, diminazene, isometamidium, quinapyramine and cymelarsen have been used. Suramin has been the mainstay of treatment for all host species for over 70 years. Quinapyramine has been used in camels and horses whilst diminazene and isometamidium have been used for treatment of cattle and buffalo. Diminazene has been used successfully to treat cattle and buffalo in India and Thailand, but there are some doubts about its efficacy at the recommended dose rate of 3.5 mg/kg; some authorities suggest a higher dose. Although the drugs have been in use for many years the reports of drug resistance from the field are few. Nevertheless, a number of studies have indicated that drug resistant strains of trypanosomes are present in Kenya and Vietnam. Quinapyramine has been used in camels, and only recently melarsomine (cymelarsen) (Rhône Merieux, France) was introduced for the treatment of surra in camels because of the problem of drug resistance (Luckins, 1998; Bourdichon, 1998). Treatment of *T. evansi* infected camels in Morocco with melarsomine (Cymelarsan (R). Rhône-Mérieux) reduced the sero prevalence level from 58 to 19% within a year (Rami *et al.*, 2003). Resistance up to 500 ng/ml has been found in camels against quinapyramine sulphate (Bourdichon, 1998). Cymelarsen is also effective against *T. evansi* infections in cattle and horses (Raynaud *et al.*, 1989) and animals with surra are commonly treated at different stages of the disease. However, relapses in camels after treatment have been reported (Otsyula *et al.*, 1992).

Another drug, Trypan, which is a formulation containing diminazene-di-acetate (diamidinophenyltriazene diacetate tetrahydrate), phenazone and procaine hydrochloride is effective against *T. evansi* infections, as well as infections with *Trypanosoma congolense*,

Trypanosoma vivax, and *Trypanosoma brucei*. The drug is also effective against *Babesia bigemina*, *Babesia canis* or other *Babesia* and *Theileria annulata* (Bourdichon, 1998). It has a synergistic and an additive effect in comparison with other trypanocidal drugs and is reported to have a painless, antipyretic and long-lasting effect (Bourdichon, 1998). With regard to prevention, it has been confirmed that a single injection of 15 ml of Trypan affords an animal protection against a new re-infestation over a period of three months (Bourdichon, 1998). Trypan can be used for curative and preventive treatment. On the whole, control of surra requires treatment of infected animals with effective drugs and reducing blood sucking flies by regular insecticide treatment.

3. MATERIALS AND METHODS

3.1. Study area

The present study was conducted in two selected districts of Bale zone namey Dello-mena and Sawena. Bale zone is found in the Oromiya Regional state south east of the country. Robe the capital of Bale zone is located 430 kms away from Addis Ababa. The two study districts, that had distinct climate and ecology, were selected to represent the camel rearing districts of Bale zone based on their camel population and ecological conditions.

The altitude of the study area ranges from 850 to 2800 m.a.s.l, where a lowland area predominates with a narrow high land area in the Northern part of Dello-mena district. The area experiences a bimodal rainfall occurring from September to November and March to June. An average annual temperature of 20- 25 °C and rainfall of 200 mm are recorded in Dello-mena. Rainfall in Sawena district is short and erratic with mean annual rainfall of below 100mm. Vegetation of the area changes with altitude ranging from scattered trees and bushes in the low land to dense woody forest area in the high land. Dello-Mena district is endowed with several rivers, nine perennial rivers flow in the district namely: Welmel, Yadot, Erba-1, Erba-2, Deyu, Denda, Doya, Gomgoma and Shawae. Besides these deep wells, ephemeral ponds, seasonal streams are sources of water for livestock and people. Contrary to the situation in Dello-mena, surface water is a serious problem in Sawena district, seasonal streams, ephemeral ponds and shallow temporary wells are sources of water in the rainy season and these usually dry out after a few days during the dry season. The deep wells remain the only sources of water, which support a large number of animals in the dry season.

Dello-Mena district has an agricultural vocation and a mixed farming system with crop-livestock production, which is the mainstay of the livelihood of peoples and the leading economic activity of the area. On the contrary, Sawena has a pastoral vocation and livestock rearing is the dominant

economic activity of the district. Livestock plays an integral role for agricultural activity, which also provides meat, milk, cash income and transportation purposes. The livestock species reared are cattle, goat, sheep, donkeys, camels, mules, horses and poultry. Infectious diseases like anthrax, black leg, pasteurellosis, lumpy skin disease, african horse sickness, internal and external parasitic diseases, and protozoan diseases particularly trypanosomoses are the main constraints of livestock production. Furthermore, lack of grazing due to frequent drought conditions in Sawena district also exacerbates the livestock to be affected by the above-mentioned problems.

3.2. Study design and sampling strategies

Dello-Mena and Sawena districts

Cross sectional study

A Cross-sectional study design based on questionnaire, parasitological and serological survey was conducted in two selected camel-rearing districts of Bale lowlands. A combination of purposive multistage and stratified random sampling methods was applied according to Thrusfield (1995). First 19 peasant associations, 10 from Dello-Mena and 9 from Sawena districts (First stage) were randomly selected from the lists of camel rearing peasant associations obtained from the respective Districts Agricultural Development Office, then a total of 38 villages (second stage) two from each selected peasant associations were again randomly selected. In the third stage, 76 herds, two from each village were selected. The selection of a herd was based on the compliance of camel herd owners. Finally, 619 individual camels (Forth stage) were selected at random. From each selected herd, approximately 8 camels were sampled at random, in proportion to sex and age categories in the herd. The sample was composed of 619 randomly selected dromedaries from 76 herds, divided into three age groups: less than two, two to five and greater than five years of age (Table 3).

Taking average prevalence of 28 % based on average results of previous reports in various parts of Ethiopia (Getahun, 1998; Ketema, 1990; Tenaye, 1993), absolute desired precision of 5% and

confidence level of 95% for estimating prevalence in simple random sampling according to Thrusfield (1995) the sample size was determined as follows.

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

Where:

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

The calculated sample size for estimating prevalence in simple random sampling was 309. In order to adjust the sample size required for the present multi stage stratified random sampling method to estimate the disease prevalence at a 5% level of precision, the sample size was inflated two times than in simple random sampling. Therefore, a total of 619 animals were sampled proportionally from Dello-mena district, 319 (51.5 %) and from Sawena district 300(48.5%) based on camel population size. Important study variables including ecological conditions, age, sex, and body conditions were considered and recorded in pre designed data collection format (Annex 3).

Table 3: Profile of the study animals by sex and age group.

District	Sex		Age group			Total
	Male	Female	< 2year	2-5 years	>5years	
Dello-mena	106	213	21	87	211	319
Sawena	110	190	35	94	171	300
Total	216	403	56	181	382	619

3.3. Questionnaire survey

A pre-tested structured questionnaire, designed to include questions about factors either known or thought to influence the spread of surra, was administered in person. The format was filled by directly interviewing randomly selected animal owners (n= 65), professionals and paraprofessionals (Community Animal Health Workers) working in the areas. The questionnaire was pre-tested before the actual survey for time, resource and relevance of type of questions included.

3.4. Parasitological and hematological survey

3.4.1. Collection of samples

Blood samples were collected from the jugular vein where about 10 ml of blood was taken from each animal by EDTA coated vacutainer tubes; another 5 ml for serum separation was collected in plain tubes. The blood in the EDTA coated tube was used for parasitological examination of trypanosomes and for the determination of PCV (Woo, 1970). The blood collected with the plain tube was allowed for overnight to clot, afterwards serum was separated by centrifugation of the tubes at 1500 g for 10 minutes and stored at -20 °C until examined by CATT / *T. evansi*.

3.4.2. Packed cell volume (PCV) determination

Following collection of blood from the jugular vein, a micro haematocrit tube containing 70 micro liter of blood was centrifuged for 5 minutes using the Hawksley micro-haematocrit centrifuge. The PCV was read and registered.

3.4.3. Parasitological examination

Buffy coat smear technique: The haematocrit tube was cut with a diamond pen 1 mm below the buffy coat/red cell junction, to include the upper layer of red cells and one centimetre above the buffy coat, so that some plasma was included with the buffy coat and the contents of the intermediate section thus obtained was expressed on the slide. The expressed contents were carefully mixed, and then a smear was made on a clean microscope slide. The film was air-dried, fixed in methyl alcohol for 2 minutes and allowed to dry followed by staining using Giemsa stain for 25 minutes. This preparation was poured off the slide was washed in tap water and dried. Finally slides were examined under oil emersion objective lens.

3.5. Serological study

Study on the seroprevalence of camel trypanosomoses was conducted using the card agglutination for trypanosomosis test (CATT/*T. evansi*). The CATT/*T. evansi* is a direct rapid card agglutination test, which uses formaldehyde fixed, freeze-dried trypanosomes expressing a predominant variable antigen type of *T. evansi* (RoTat 1.2) stained with Coomassie blue. The end point titre was defined as the highest dilution of the test serum still showing a single positive result and positive samples were determined at cut-off point dilutions 1:8 and above. The test procedures and details of the diagnostic steps applied were as follow.

Reconstitution of the CATT antigen

- Using the syringe, add 2.5 ml of CATT buffer to a vial of freeze dried CATT antigen
- Immediately shake the vial for few seconds so as to obtain a homogenous suspension
- Put a dropper on the vial. The antigen suspension is ready for use.

Reconstitution of the controls

- Using the syringe, add 0.5 ml of CATT buffer to a vial of the positive and negative control
- After reconstitution of each vial of CATT antigen, test one drop of the positive control and one drop of the negative control to check the quality of the antigen.

Preparation of test samples

- Prepare serial twofold dilutions 1:4, 1:8, 1:16, 1:32 and 1:64 of the test sample in CATT buffer
- Using a micropipette put 25 µl of the serial twofold dilutions on a test area of the card
- Add one drop (about 45 µl) of the well homogenized CATT antigen in each test area
- Using a stirring rod, mix and spread out the reaction mixture to about 1 mm from the edge of test area. Wipe off the stirring rod after each use
- Rotate the test card on a flat bed orbital for 5 minutes at 70 rpm

Reading and interpretation

Evaluate the agglutination reaction as indicated below in Table 4 as follows:

Table 4: Result interpretation of CATT/ *T. evansi* test

Agglutination	Test result
+++	Strongly positive (very strong agglutination)
++	Positive (strong agglutination)
+	Positive (moderate agglutination)
<u>+</u>	Weakly Positive (weak agglutination)
—	Negative (absence of agglutination)

3.6. Data analysis

Data from questionnaire, parasitological and serological survey were inserted into MS Excel Spread Sheets (Microsoft Corp.) to create a database and transferred to the SPSS software programme of the computer before analysis.

Collected data were subjected to SPSS, 2002 software of the computer program for the statistical analysis. Data on the questionnaire was summarised using frequency distribution and

percentages. The impact of explanatory variables district (ecological condition), sex, age, and body condition on the parasitological and serological test results was assessed by logistic regression models. The exponentiated estimates of the coefficients of the models were interpreted as odds ratio (OR). Univariate logistic regression analysis was employed to determine the association of risk factors with the prevalence of surra. Only risk factors of highly significant on univariate analysis were subjected to multivariate analysis to determine major risk factors. The relationship between the parasitological and serological prevalence of trypanosomal infections and average PCV was examined by regression analysis using the estimates of the average PCV as the dependent variable and the prevalence of trypanosomal infections as the independent variable. The prevalence is defined as the proportion of the number of positive animals to the total number of animals examined and expressed in percent.

4. RESULTS

4.1. Questionnaire survey

The results of the questionnaire survey indicated the presence of major diseases of camels such as: surra, anthrax and internal and external parasitism. Both camel herders and professionals interviewed reported that surra is the most important diseases of camels in the study area. The respondents described surra as the major cause of reduced milk production, loss of body condition, poor reproductive performance and some times abortion and death. The disease is locally known by the name “Dhukan” that refers to the marked emaciation of camels affected by the disease.

Among the 65 camel herd owners interviewed 47 (72.3 %) had an average of 0.84 hectares of cultivated land. The rest 18 (27.7%), most of them in Sawena district, are pure livestock keepers. Farming activity in Sawena is unreliable due to short and erratic rainfall. The respondents experience in camel keeping l varying from 2.5 years to as long as lifetime. Among the investigated camel owners those who have recently started camel keeping indicated that high milk production, bush encroachment and the ability to survive drought are the major initiating factors for the beginning of camel rearing. Although camel provides transport service and a source of cash income from sale of live animals and meat, the main purpose of camel keeping in the area is for milk production. Other livestock species kept by the camel owners include cattle, goat, sheep, donkey and mule in that order. In Sawena district the main sources of water for livestock are the deep wells which support large number of animals during the dry season and temporary ponds are used in the rainy season which soon dries out later, where as in Dello-mena the perennial rivers flowing in the district are main sources of water for livestock. In general, camel herding is practiced by girls and boys except in Sawena where the elder members of the family involve in watering activity.

Animal health service in the area is rendered through government clinics found in the capitals of the districts and village health posts. Besides these in Sawena 42 community animal health workers who have been trained and equipped by pastoral development project are also involved in treating animals. Although the aforementioned institutions involve in delivering veterinary services, since

livestock particularly camels are herded far from these centers most of the time the herdsmen themselves treat their camels with trypanocides, antibiotics, anthelmintics, acaricides and herbal medicines. According to the respondents Trypamedium, Berenil and Cymelarsan are drugs commonly used to treat camel trypanosomoses. They also stated that although its availability is limited to government clinics and health posts Cymelarsan is the most preferred drug.

4.2. Clinical observation

During the survey various clinical signs of surra were observed. Loss of body condition, anaemia which was manifested by paleness of the visible mucous membranes and loss of hump were the dominant clinical signs observed (Table 5). Other signs observed include lacrimation, diarrhoea and loss of hair coat. In a few cases there was a ventral abdominal oedema was observed in highly emaciated camels. Camel owners and animal health professionals working in the area reported that the development of oedematous swelling is an indication of chronic infection and in most of the cases terminates by death.

Table 5: Observed clinical signs in camels while sample collection in both study sites.

Symptoms observed	Frequency		
	Female	Male	Total
Loss of condition	79	33	112
Anemia	49	28	77
Loss of hump	38	31	69
Abdominal oedema	8	3	11
Diarrhoea	19	8	27
Lacrimation	15	18	33
Loss of hair coat	24	14	38
Deprssion	18	13	31
No sign	261	148	409

No. of examined camels: Females=403, Males=216, Total=619

4.3. Parasitological and serological study

4.3.1. Overall results

Out of 619 camels examined, using buff coat smear, parasitological prevalence was found to be 2.12 % (Table 6). The parasites had a morphology characteristic of *T. evansi*. Parasitemia varied on the microscope field between a few parasites per slide and over 10 trypanosomes per field. Parasites were detected in blood smears of 75 camels out of which 52 were from Dello-mena and the rest 23 were from Sawena district (Figure 2).

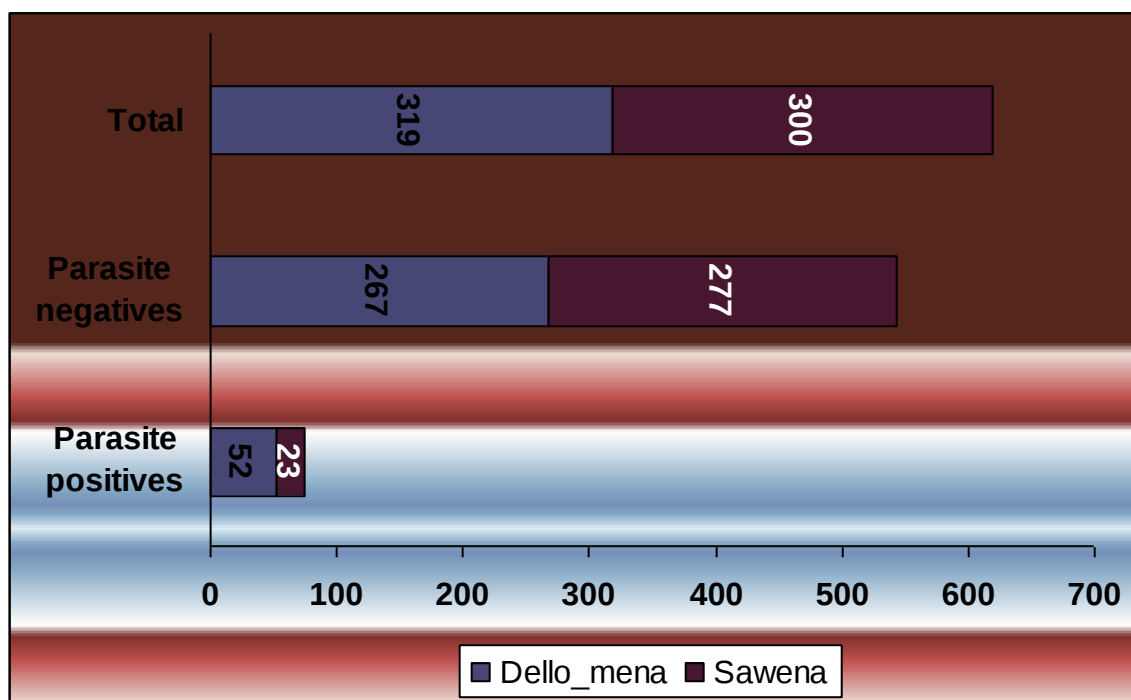


Figure 2: The proportion of parasitological positive and negative camels in Dello-mena and Sawena districts

Out of a total of 619 camels tested by card agglutination test for the detection of agglutinating antibodies against *T. evansi* the overall seroprevalence was found to be 24.88%(Table 6) and uncertain cases amounted to 1.77% (11).

Table 6: Parasitological and serological prevalence of surra in both study district of the Bale lowlands.

District	No. samples	Parasitological		CATT	
		No. of Pos.	Prevalence (%)	No. of Pos.	Prevalence (%)
Dello-mena	319	52	16.3	98	30.72
Sawena	300	23	7.67	56	18.67
Overall	619	75	12.12	154	24.88

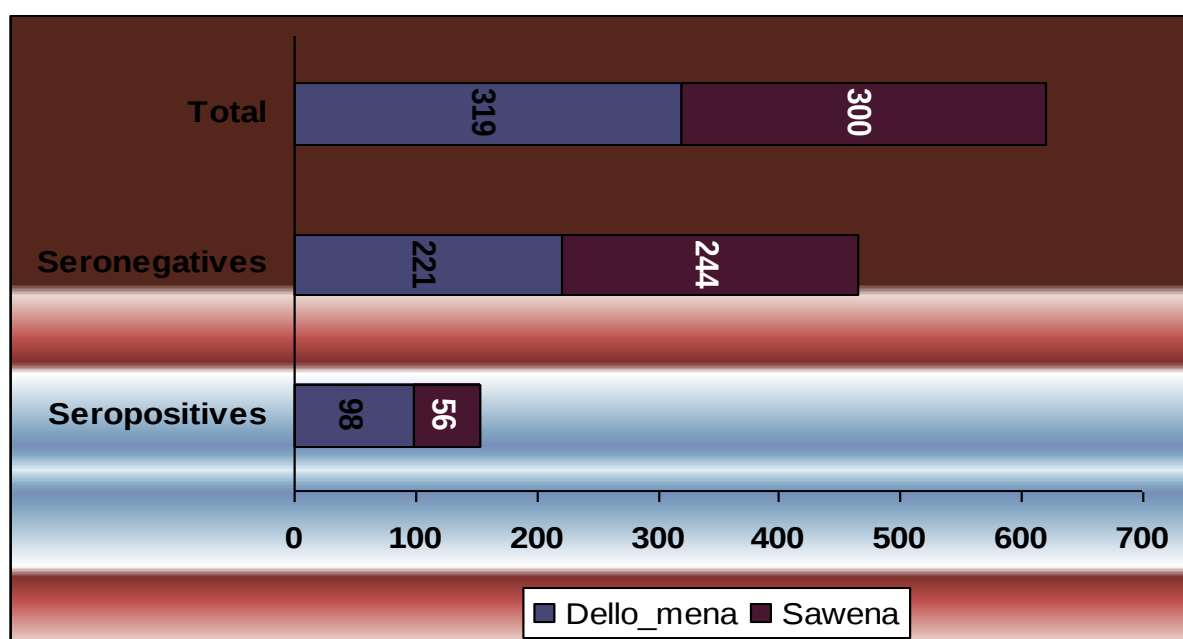


Figure 3: The proportion of seropositive and seronegative camels in Dello-mena and Sawena districts

4.3.2. Ecological condition

The results obtained in both parasitological and serological methods showed a higher prevalence of surra in Dello-mena than in Sawena district (Table. 6).

Table 7: Parasitological and serological prevalence of *T. evansi* and comparison of risk factors in Sawena district.

Factors	No. of observation	Parasitological results				Serological results			
		Prevalence	P-value	OR	95% CI	Prevalence	P-value	OR	95% CI
Sex			0.287	0.500	0.140, 1.792		0.039	2.149	0.10, 4.446
Female	190	10 %				18.9%			
Male	110	3.6 %				18.2%			
Age			0.269	1.70	0.662, 4.382		0.000	3.881	1.946, 7.740
< 2 yr	35	0				2.8%			
2 to 5 yr	94	6.4%				11.7%			
> 5 yr	171	9.9%				8.2%			
Body cond.			0.000	0.45	0.302, 0.676		0.001	.660	0.514, 0.848
V. poor	27	29.6%				40.7%			
Poor	49	14.3%				28.6%			
Medium	80	3.8%				11.2%			
Good	91	4.4%				17.6%			
V. good	53	1.9%				11.3%			

Table 8: Parasitological and serological prevalence of *T. evansi* and comparison of risk factors in Dello-Mena district.

Factors	No. of observ ation	Parasitological results				Serological results			
		Prevale nce	P- valu e	OR	95% CI	Prevale nce	P- value	OR	95% CI
Sex			0.268	0.66	0.326, 1.366		0.502	0.815	0.448, 1.483
Female		18.3%				33.3%			
Male		12.3%				25.5%			
Age			0.19	1.45	0.830, 2.541		0.000	2.928	0.172, 4.986
< 2 yr		9.5%				9.5%			
2 to 5 yr		16.1%				26.4%			
> 5 yr		17%				34.6%			
Body cond.			0.00	0.54	0.408, 0.722		0.000	0.352	0.262, 0.472
V. poor		41.7%				83.3%			
Poor		28.2%				53.8%			
Medium		13.3%				27.5%			
Good		14.4%				22.2%			
V. good		4.3%				8.7%			

Statistical analysis of the serological result indicated a significant difference ($p=0.16$) in the prevalence of surra between the two districts (Table 9). Similarly logistic regression analysis for the effect of district Dello-Mena, vs. Sawena on parasitological results revealed an OR of 2.162 (1.254, 3.727). The chance of positive status on parasitological result for camels in Dello-Mena was about more than twice the chance of camels in Sawena district.

Table 9: Odds ratio comparison of risk factors with CATT result

Factors	P value	OR	95.0 % C.I	
			Lower	Upper
District	.016	1.615	1.092	2.388
Sex	.978	1.006	.651	1.554
Age	.003	1.682	1.19	2.378
Body condition	.000	.615	.501	.755
PCV	.161	.949	.883	1.021

4.3.3. Sex

The seroprevalence rates observed for females and males were 21.76 % and 26.55 %, respectively (Table 10).

Table 10: Parasitological and serological prevalence of surra on basis of sex in both study districts of Bale zones.

Sex	No.of Observations	Parasitological		CATT	
		No. of Pos	Prevalence (%)	No. of Pos	Prevalence (%)
Female	403	58	14.39	107	26.55
Male	216	17	7.87	47	21.76
Total	619	75	12.12	154	24.88

Logistic regression analysis for the independent variable effect of sex, males versus females revealed an OR of 1.002 (0.651, 1.554) for the outcome variable CATT /*T.evansi*. A similar model for the

outcome parasitological result revealed an OR of 0.66 (0.351, 1.239) for effect of sex (Table 11). Hence, neither parasitological nor serological results were affected by the sex of the camel.

Table 11: Odds ratio comparison of risk factors with parasitological result

Factors	P value	Odds ratio	95.0% C.I.	
			Lower boundary	Upper boundary
District	.006	2.162	1.254	3.727
Sex	.196	.660	.351	1.239
Age	.090	1.511	.937	2.436
Body condition	.025	.733	.559	.961
PCV	.000	.789	.715	.871

4.3.4. Age

Infection was observed in animals of all age group with the highest prevalence in camels of greater than five years age group (Table.12). Statistical analysis of serological results using logistic regression revealed a significant difference among all age groups (Figures 4 and 5). The highest significance was observed between camels less than two years of age and those aged greater than five years ($p=0.003$). On the other hand the effect of age on parasitological result was not significant ($p=0.19$).

Table 12: Parasitological and serological (CATT/*T.evansi*) prevalence in different age groups in both study districts in Bale lowlands.

Age group	No. of observations	Parasitological result		CATT result	
		No. of Pos	Prevalence	No. of Pos	Prevalence
< 2 years	56	2	3.57 %	3	5.36 %
2 – 5 years	181	20	11.05 %	34	18.78 %
> 5 years	382	53	13.87 %	117	30.63 %
Total	619	75	12.12 %	154	24.88 %

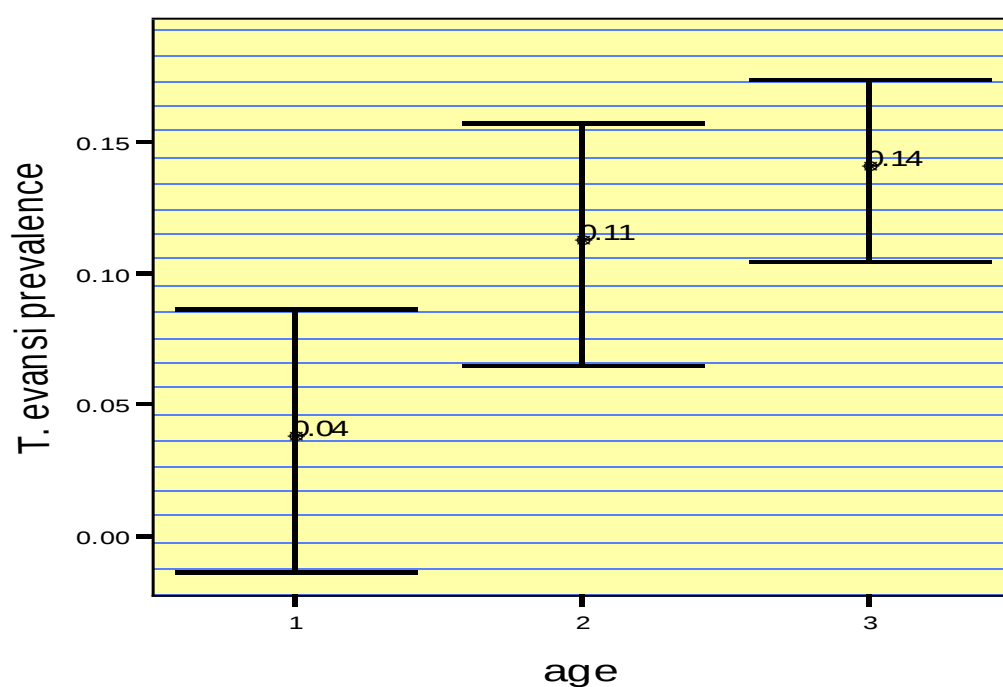


Figure 4: Box plots showing parasitological prevalence in camels of different age groups

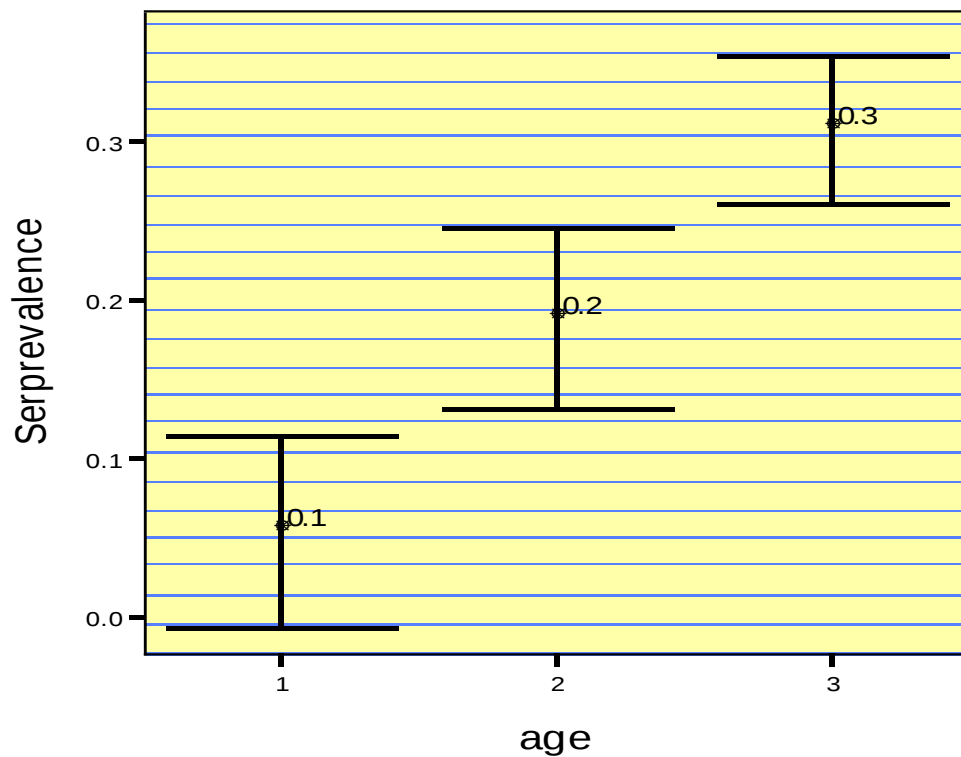


Figure 5: Box plots showing seroprevalence in camels of different age groups

4.3.5. Body condition

Infection was observed in all the five categories of body conditions (1= very poor, 2= poor, 3=medium, 4=good and 5=very good) with the group specific prevalence of positive animals increasing with increasing severity of emaciation. Parasitological and serological results obtained with different body condition groups showed that the highest prevalence was in camels of very poor body condition group (Table. 13).

Table 13: Parasitological and serological prevalence of *T. evansi* in camels of different body conditions in both study districts of the Bale lowlands.

Body condition Score	No. of observations	Parasitological		Serological	
		No. Pos.	Prevalence	No. Pos.	Prevalence
1	51	18	35%	31	60.8%
2	88	18	20.5%	35	39.8%
3	200	19	9.5%	42	21.0%
4	181	17	9.4%	36	19.9%
5	99	3	3%	10	10.1%

A significant effect of the body condition on parasitological result was observed with an OR of 0.733 (0.559, 0.961). The explanatory value of the body condition for the CATT/*T. evansi* result was similar with an OR of 0.615 (0.501, 0.755). Thus, it was shown that very poor and poor body conditions were significantly correlated with positive status of both tests. As shown in Fig. 6 and Fig. 7 the highest significant was observed between camels of less than two years age group and camels of age group five.

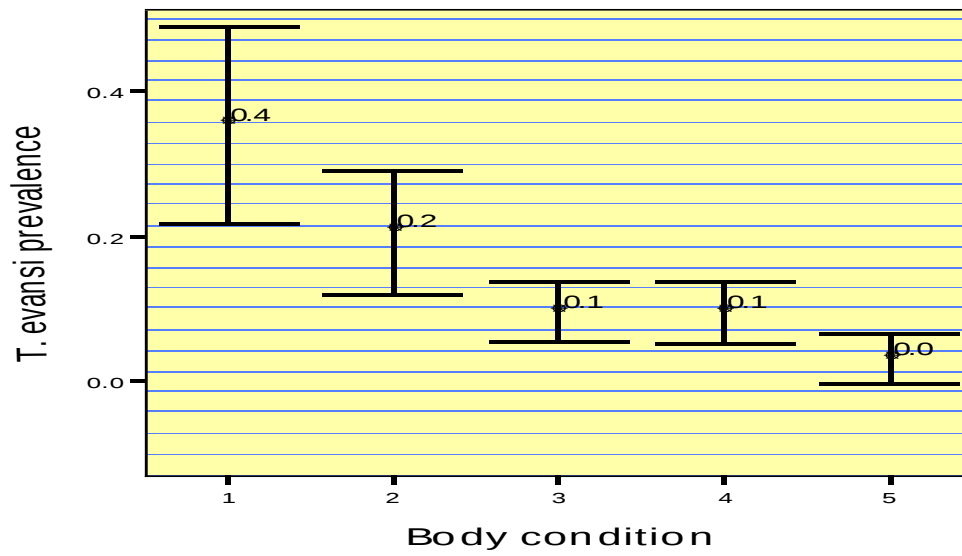


Figure 6: Box plot showing parasitological prevalence in camels of different body condition groups

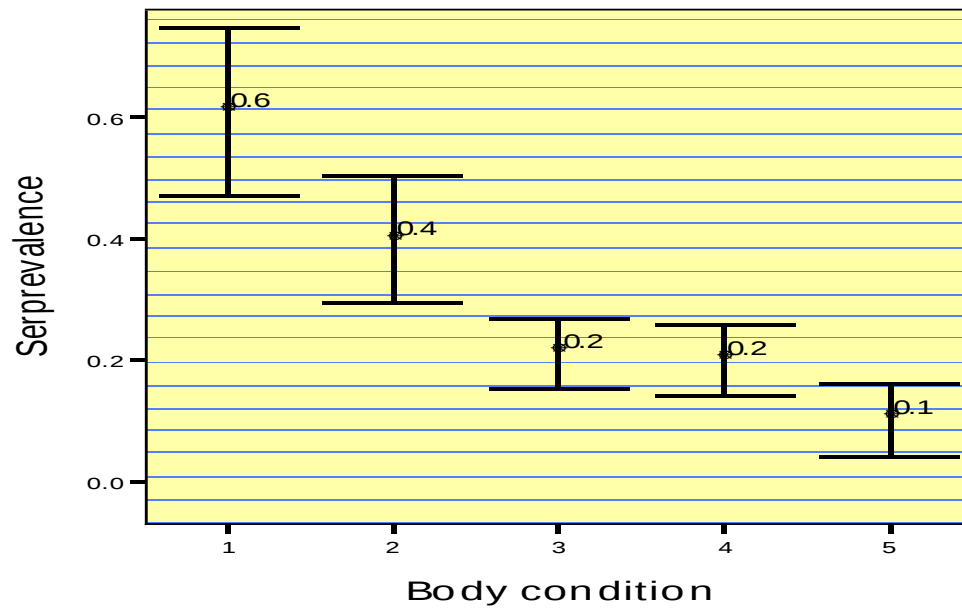


Figure 7: Boxplots of seroprevalences of surra among the 5 body condition groups

4.4. PCV and camel trypanosomosis

A marked depression of packed cell volume was observed in trypanosome infected camels compared to non infected ones. The mean PCV of animals negative for both parasitological and CATT test was found to be 25.11 % and for animals positive for both tests was 21.59 % (Table 13).

Table 14: Mean PCV of different groups of camels based on parasitology and serology results.

Parasitology	Serology	Mean PCV	Std. Deviation	No. of observations
Negatives	Negatives	25.117	2.7989	444
	Positives	24.750	2.8146	100
	Total	25.050	2.8028	544
Positives	Negatives	23.452	3.7746	21
	Positives	21.593	3.7894	54
	Total	22.113	3.8525	75
Total	Negatives	25.042	2.8659	465
	Positives	23.643	3.5192	154
	Total	24.694	3.0982	619

The relationship between the parasitological and serological prevalence of trypanosomal infections and average PCV was examined by regression analysis using the estimates of the average PCV (Table 14) as the dependent variable and the prevalence of trypanosomal infections as the independent variable using data obtained from 619 camels. Out of 75 camels parasitological tested positive 51 were seropositive and 21 were seronegative .

Table: 15. Estimates of mean PCV of different groups of camels based on their trypanosomosis status

Groups of camels on trypanosomosis status	Mean PCV	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1 ¹	25.117	.139	24.844	25.391
2 ²	23.452	.641	22.194	24.710
3 ³	24.750	.294	24.173	25.327
4 ⁴	21.593	.400	20.808	22.377

¹ Serologically and parasitological negatives

² Parasitological positives but seronegatives

³ Seropositives but parasitological negatives

⁴ Serological and parasitological positives

The average PCV of parasitological positive camels was found to be significantly different from the average PCV of parasitological negative camels. However, the average PCV of seropositive but parasitological negative camels did not differ from the average PCV of seronegative camels (Table 17).

Table 16: Pair wise comparisons of mean PCV of different groups of camels based on estimated marginal means.

Pair wise comparison of Mean PCV	Mean Difference	Std. Error	p-value	95% C.I	
				Lower Bound	Upper Bound
1 ¹	2 ²	1.665*	.656	.011	.377
	3 ³	.367	.325	.259	-.271
	4 ⁴	3.525*	.423	.000	2.694
2 ²	1 ¹	-1.665*	.656	.011	-2.952
	3 ³	-1.298	.705	.066	-2.682
	4 ⁴	1.860*	.755	.014	.377
3 ³	1 ¹	-1.665*	.656	.011	-2.952
	2 ²	1.665*	.656	.011	.377
4 ⁴	1 ¹	-1.665*	.656	.011	-2.952
	2 ²	1.665*	.656	.011	.377

* The mean difference is significant at the .05 level

¹ Serologically and parasitological negatives

² Parasitological positives but seronegatives

³ Seropositives but parasitological negatives

⁴ Serological and parasitological positives

4.5. Degree of concordance between buffy coat smear and CATT/T.evansi

Out of 75 animals that had a positive reaction in the parasitological test, 54 were positive and 21 were negative using CATT/T. *evansi* (Table 17). Statistical test using *kappa* indicated a moderate agreement between the two tests ($k=4$)

Table 17: Cross tabulation of parasitological and CATT/*T.evansi* test results

Test		Parasitological result		
		Positive	Negative	Total
CATT/ <i>T.evansi</i>	Positive	54	100	154
	Negative	21	444	465
Total		75	544	619

5. DISCUSSION

All the survey methods used in the present investigation (questionnaire, clinical observation, serological and parasitological examinations) provided strong evidence that camel trypanosomosis caused by *T. evansi* is widespread in Dello-mena and Sawena districts and is a major threat to the well-being and productivity of the camel population.

The problem of surra was well recognized by the farmers in the study area in that they described the disease and ranked it as the second most important disease of camel. The disease rankings revealed which camel disease were thought by farmers to cause economic and production losses. They were aware also that the major features of the disease were loss of body condition and reproductive failure. As explained by individual farmers in the study area and observed during clinical examination most of the surra suspected camels had a poor body condition. The questionnaire result agrees with the serological and parasitological results obtained in this study.

The overall parasitological prevalence of 12.12% obtained in this study is close to those reported by Richard (1979) : 12.5%, Getahun (1988): 10.2% and Tekle and Abebe (2001): 10.9% However, the present result is higher than reports made by other investigators Abebe (1991): 6.54%, Dia *et al.* (1994): 7.34% and Demeke (2000): 5%. The highest parasitological prevalence observed in this study may be linked to ecological conditions of the study area especially in Dello-mena district where there are numerous animal watering points and the existence of wooded area near permanent and temporary waterways might have created favorable environment for the insect vectors of *T. evansi*. The occurrence of surra is influenced by climatic and ecological conditions, based on the distribution and abundance of the insect vectors. It has a marked seasonal incidence related to wet and humid condition and increased activity of biting flies during the wet season. Local epidemics of surra occur where conditions exist for the spread of infection with *T. evansi*, such as when many animals are stabled together or close herded and particularly when the biting fly population is abundant during the wet season (Luckins, 1994). Surveys of *Tabanus* in the various tropical areas have shown a definite correlation between the seasonal outbreaks of *Trypanosoma evansi* infections and the increase in number of *Tabanus* during the rains (Mahmoud and Gray, 1980; 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 (Njiru *et al.*, 2002).

The result of the logistic regression models suggest that neither parasitological nor serological results were affected by the sex of the camel. When comparing results of both tests the positive status of camels older than two years with camels less than two years of age by logistic regression, it was found that the odds of positivity in older camels was markedly higher than the odds in younger animals. Whereas no age effect was observed between age groups 2 to 5 years and camels older than 5 years of age.

Result obtained with different age groups showed that both parasitological and serological prevalence are at their highest rate in camels aged greater than five years old. The significant increase in seroprevalence with increasing age groups may be explained by the greater probability of encountering *T. evansi* carrying insects in older animals compared to the younger ones. This result is in accord with the findings of Getahun (1988) and Dia *et al.* (1997) who had found the highest seroprevalence in camels older than 5 years. However the results of the present study with different age groups doesn't agree with the findings of Singh *et al.* (2004) who had found the highest incidence of *T. evansi* infection in camels of up to 5 years of age, 21.74%.

The average PCV of parasitological positive camels was found to be significantly different from the average PCV of parasitological negative camels. However, the average PCV of seropositive but parasitological negative camels did not differ from the average PCV of seronegative camels. The significant difference observed in packed cell volume between infected and non infected camels suggest that anemia is one of the pathologies of surra. Enwazor *et al.*, (2005) described that anaemia appears to be a major component of the pathology of surra. Its development and persistence in the course of the disease induce anoxic conditions which manifest signs of dysfunction in various organs as a result of a fall in tissue pH and vascular damage. This is followed by the release of large quantities of cytoplasmic and mitochondrial enzymes, especially aspartate alanine transferase (AST) and alanine transferase (ALT), among others, into serum,

causing further cellular and tissue damage. The net effect associated with the above changes is immunosuppression which later develops and predisposes the animals to other infections and death if untreated.

Anaemia is a major component of the pathology of surra and of African trypanosomoses generally. In the early phases of *Trypanosoma evansi* infections of camels the anaemia is haemolytic and haemophagocytic. Its development and persistence in the course of the disease induce anoxic conditions which manifest signs of dysfunction in various organs as a result of a fall in tissue pH and vascular damage. In the late stages, anaemia continues to be a major factor, with probably additional causes (Enwazor *et al.*, 2005). The prevalence varied between the two study districts. This is strongly linked to district characteristics. A higher prevalence in Dello-mena where there are numerous permanent and temporary rivers and watering points and the vector activities capable of transmitting *T. evansi* being abundant in this district. This suggests a correlation between the prevalence of infection and the existence of wooded areas near permanent or temporary water ways where the animals can go to drink.

Contrary to the parasitological findings a large proportion of camels were found to be seropositive. The poor sensitivity of parasitological methods, often less than 50% (Monzon, 1990; Nantulya *et al.*, 1989; Luckins, 1992), to detect low level Parasitemia in the chronic stage of the disease, the possible extra vascular location of trypanosomes and CATT/*T.evansi* can not distinguish current from cured infection (Luckins, 1988) as detectable level of antibodies can still be found in self cured animals or after treatment with trypanocidal drugs might explain this enormous difference.

The higher parasitological prevalence obtained in this study may be linked to the ecological conditions especially in Dello-mena where there are several permanent rivers and numerous animal watering points, which create conducive environment for the vector activity. This suggests a correlation between the prevalence of infection and the existence of wooded areas near permanent waterways where the animals can go to drink.

6. CONCLUSION AND RECOMMENDATIONS

High milk production, bush encroachment as well as the ability to survive drought are the major reasons for camel keeping in the study area. Among many diseases known to affect camel and camel production camel trypanosomosis locally known as Dukane, is the most important complaint of camel breeders. The investigations carried out in this particular study indicated that camel trypanosomosis caused by *T. evansi* is endemic in Dello-mena and Sawena districts with a significantly higher prevalence in Dello-mena than Sawena district. The degree of disease prevalence is probably linked to the presence of permanent and temporary waterways and the plant canopy where the animals can trek. Thus it was found that camels in riverine areas are more at risk of infection than herds in non-riverine areas. Low PCV value and poor body condition are strongly associated with positive trypanosomosis status of the camel. Age of the camel is apparent risk factor for the disease and that camels older than five years are more at risk of infection than camels below five years of age. Inadequate veterinary service, absence of proper animal health extension and the indiscriminate use of camel trypanocidal drugs often underdosed are major constraints in camel health delivery in the study area. In light with the above mentioned major problems associated with camel trypanosomosis in the Bale lowlands the following recommendations were forwarded:

- Considering the widespread existence of surra and its impact on camel productivity further study on identification of the principal vector species responsible for transmission and investigating the potential for vector control is recommended.
- Absence of proper animal health extension and the indiscriminate use of camel trypanocidal drugs often underdosed are major constraints in camel health delivery in the study area. Thus, attention has to be given to provide adequate veterinary service and sensible animal health extension systems.

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8. ANNEXES

Annex 1: CATT / *T. evansi* test result recording format used in the present study.

No.	Name (Sample code)	CATT/ <i>T. evansi</i> test						End titre
		1:2	1:4	1:8	1:16	1:32	1:64	
1								
2								
3								
4								
5								
6								
7								
8								

Annex 2: Questionnaires for individual camel owners

Date.....

Region Zone District.....

PA. Village.

Name of respondent.....Age.....Sex.....

Herd size: (a. Small, b. Middle, c. Large)

Owner experience (years) Family size.....

1. What is the main activities you are engaged in?

- Mixed farming (Agro pastoralist) _____
- Sedentary pastoralist_____

2. Plot of cultivable land_____

3. Years of experience in farming_____

4. What type of livestock are kept by you their number and importance? (Excluding camel)

Species	Purpose	Relative importance	Count
Cattle			
Goats			
Sheep			
Camels			
Equines			

- Purpose includes milk,meat,draft,sale,security,burden of beast and riding

5. How do you herd your camels?

- Private herding_____
- Communal herding_____

6. Water points in different seasons

Seasons	Water sources				Frequencies days
	River	Ponds	Traditional wells	Flood water	
Dry					
Wet					

7. Activities and labor divisions

Activities	Youngsters		Adult	
	Male	Female	Male	Female
Herding				
Watering				
Milking				
Delivery assistance				
Mating assistance				

8. Where do you herd your camels?

No.	Area	Dry season	Wet season	Remark
1	Wooded plateau			
2	Wooded savanna			
3	River bank			

9. What are the 10 top major diseases affecting your camel herd? (according to the order of importance)

Use local names

- | | |
|----------|-----------|
| 1. _____ | 6. _____ |
| 2. _____ | 7. _____ |
| 3. _____ | 8. _____ |
| 4. _____ | 9. _____ |
| 5. _____ | 10. _____ |

10. Did you treat your herd for trypanosomoses last year?

Yes _____

No _____

11. How many camels from your herd were treated with trypanocidal drugs last year? _____

12. What was the result of treatment?

Success _____

No change _____

Death _____

13. What types of trypanocidal drugs are used in your area, the cost, source and who renders the service?

Drug type	Cost	Sources	Service rendered by	Quality of drug
Cymelarsen				
Naganol				
Berenil				
Trypanidum				

14. How is veterinary service rendered in your area?

Field service (Government) _____ Animal health technicians (village based) _____

Private practitioners _____ Community based animal health workers _____ The owner _____

15. What are the traditional treatment methods for different diseases if there are any?

No.	Diseases	Traditional treatment	Application
1			
2			
3			
4			
5			

16. What types of flies are known to transmit the disease their seasonality, abundance and ecological preference

No.	Local name of fly	Seasonality	Ecological preference	Remark

17. If flies are known to exist in the area what are the strategies of avoidance?

No strategies _____

Keeping away camels from the area at specific season _____

Keeping away camels from the area all the year round _____

18. How many years of experience do you have in camel keeping? _____
19. If camel keeping is a recent exercise what were the major reasons to start camel husbandry?
 Bush encroachment _____ High milk production _____
 Better ability to cope with drought condition _____ Others _____
24. Herd composition or Herd inventory
- Breeding females dry (non pregnant)..... pregnant..... Lactating.....
 - Breeding bulls..... Castrated male.....
 - Non-breeding males (below 5 years).....non-breeding females
 - Un weaned males Un weaned females

Annex 3: Clinical and parasitological survey sheet

Region_____ Zone_____ District _____
 Peasant association _____ Date_____

Code No.	Sex	Age	Body condition	Clinical symptoms	PCV
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Annex 5: Body Condition Scoring:

Score 1: Marked emaciation hump lost lumbar transverse processes project prominently, ribs visible, dorsal spines appear sharply

Score 2: Ribs and hips and lumbar transverse processes clearly visible, individual dorsal spines pointed to touch, hump is barely visible

Score 3: Ribs usually visible, less prominent transverse process, dorsal spines not visible But could be felt, animal is smooth and well covered hump is visible

Score 4: Body is smooth and well covered, hump conspicuous, ribs not visible covered with Muscle, lumbar transverse process covered with muscle

Score 5: Hump, thigh and neck muscles well developed, ribs heavily covered with muscle

9. CURRICULUM VITAE

Personal Information

Name:	Esayas Tesema Belihu
Nationality:	Ethiopian
Sex:	Male
Place of birth:	Asebetefery, W. Harerge
Date of birth:	20 January 1964
Marital status:	Married
Academic qualification:	Doctor of Veterinary Medicine (DVM)
Scientific Membership:	Member of the Ethiopian Veterinary Association (EVA)

Educational Background

1. 1972-1977: Grade 1- 8. Number 1 elementary and junior secondary school (Asbetefery).
2. 1979-1982: Grade 9-12. Chercher comprehensive secondary school (Asebetefery). Award: Ethiopian School Leaving Certificate Examination (ESLCE) GPA - 3.60 (Distinction).
3. 1982-1988: Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit. Award: DVM degree, cGPA- 2.78.
4. 2005 to the present: Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit. Postgraduate MSc Student in Tropical Veterinary Epidemiology

Work Experience

1. 1988- 1996: Veterinary officer in various districts of Bale zone.
2. 1997-2002: Veterinary team leader of bale zone.
3. 2003 to the present: Pan African control of epizootics project coordinator of Dodola branch.

Research and Publications

1. Tesema, E. Short account on major medicinal plants of Ethiopian flora: Seminar on current topics in livestock production and development. In DVM course work. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, 1987.
2. Tesema, E. Study on the prevalence of gastro-intestinal Helminthosis in Ogaden goats of Ethiopia. DVM Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, 1988.
3. Tesema, E. Epidemiology of Dourine and Surra: Comparison of Morphological, Biochemical and Molecular Characteristics of *T. equiperdum* and *T. evansi*. Seminar on current topics on basic sciences in MSc course work, June, 2006

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10. SIGNED DECLARATION SHEET

I, the undersigned, declare that the thesis is my original work and has not been presented for a degree in any university.

Name:

Signature:

Date of submission:

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

Academic Advisors:

1. Dr. Yilkal Asfaw

2. Dr. Hagos Ashenafi
