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REVIEW ARTICLE

TRYPANOSOMOSIS IN ETHIOPIA

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ABSTRACT: Tsetse-transmitted trypanosomosis is widely distributed in western and southern lowlands and the river valleys cutting into the central highlands of Ethiopia. Prior to the 1960's, trypanosomosis had relatively little impact on the economy of Ethiopia. There were pockets of human sleeping sickness in the west and domestic livestock, particularly cattle, could not be kept over extensive areas of the lands. However, much of the country is tsetse-free, either because it is north of the main African tsetse belts or because it is too high, hence too cold to support the fly. As of early 1970's the significance of the disease has increased enormously and is still increasing. The loss of fertility of land in the marginal, high temperature, low rainfall northern regions led to the resettlement of the affected rural population and their livestock in more potentially productive areas, many of which are tsetse-infested. Furthermore, the expansion of tsetse population into higher altitude areas brings them into contact with previously unaffected livestock. Considering the agricultural economy of Ethiopia, livestock, cattle in particular, provide meat, milk and manure; also draught oxen are more extensively used in tsetse-free highlands of Ethiopia than anywhere else in sub-Saharan Africa. The introduction of draught oxen into the resettlement areas in the lowlands was severely constrained by the widespread presence of trypanosomosis. Five species of *Glossina* (*G. morsitans submorsitans, G. pallidipes, G. tachinoides, G. fuscipes fuscipes* and *G. longipennis*) have been recorded in Ethiopia but only four are widespread and of significant economic importance. The most important trypanosomes, in terms of economic loss in domestic livestock are tsetse-transmitted species: *Trypanosoma congolense*, *T. vivax* and *T. brucei*. The closely related *T. brucei* subspecies, *T. b. rhodesiense* cause human sleeping sickness. The other trypanosoma species of economic importance are *T. evansi* of camels and *T. equiperdum* of equines. Tsetse control activities against, mainly, *G. m. submorsitans* were undertaken over 4,500 km^2 of Didessa Valley as part of the Eastern Africa Regional Programme. Apart from this, operation is underway to eradicate tsetse flies from an area of 25,000 km^2 in the southern Rift Valley of Ethiopia using the sterile insect technique. If trypanosomosis could be controlled in Ethiopia, much of the best-watered and most fertile land of the southwest could be utilised. Land suitability studies carried out in areas of low population density in tsetse-infested areas of the country revealed that these areas have the best potential of expanded agriculture, provided that trypanosomosis constraint can be overcome.

Key words/phrases: Ethiopia; Livestock; Trypanosome; Trypanosomosis; Tsetse fly.

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INTRODUCTION

Trypanosomosis is a protozoan disease of both humans and animals caused by different species of the genus *Trypanosoma*. The disease is characterised by intermittent fever, anaemia, lymphadenopathy, splenomegaly and cachexia often followed by death in untreated cases (Mulligan, 1970). Hoare (1964) noted that the mammalian species of trypanosomes fell fairly into two sections depending on their development in the vector and in the mammal. He suggested the rather descriptive names; Stercoraria and Salivaria for these sections.

The section Stercoraria contains genera in which the parasite completes its development in the "posterior station" that is, metacyclic metatrypanosome, appears in the faeces of the vector, and its transmission is by contamination. There is always a free flagellum in these trypanosomes with the kinetoplast being large and situated some distance from the posterior end and is generally pointed. Reproduction in the mammalian host is usually discontinuous and takes place in the amastigote or epimastigote stages.

In the section Salivaria, the development in the vector is completed in the "anterior station" and transmission is by inoculation of the metacyclic stage. A free flagellum may be present or absent and the kinetoplast is relatively small and terminal or sub-terminal. The posterior extremity of the body is often blunt. Reproduction in the host is continuous in the trypomastigote stage. Trypanosomes in this group are usually pathogenic.

The main pathogens in the section Salivaria fall into four subgenera: *Duttonella* (species: *Trypanosoma (Duttonella) vivax; T. (D.) uniforme); Nannomonas* (species: *Trypanosoma (Nannomonas) congolense; T. (N.) simiae); Pycnomonas* (species: *T. suis)* and *Trypanozoon* (species: *Trypanosoma (Trypanozoon) brucei; T. (T.) rhodesiense; T. (T.) gambiense; T. (T.) evansi; T. (T.) equiperdum* (Mulligan, 1970). Trypanosomes are elongated spindle-shaped protozoa ranging from 8.0-39 μm in length. All the species possess a flagellum, which arises at the posterior (usually blunt or narrow) end of the trypanosome from a basal body at the foot of the flagellar pocket. The flagellum runs to the anterior (usually tapered) end of the body and is attached along its length to the pellicle to form an undulating membrane. Thereafter the flagellum may continue as a free flagellum or not. There is a single centrally placed nucleus. Adjacent to the flagellar pocket, there is a kinetoplast, which contains the DNA of the single mitochondria (Hoare, 1970).

Trypanosomosis is a serious parasitic disease, which occurs in large areas of Africa, Latin America, the Middle East and Asia. It affects most species of domestic livestock, many types of wild animals and humans. The most important trypanosomes in terms of economic loss in domestic livestock are the tsetse-transmitted species such as *T. congolense*, *T. vivax* and *T. brucei* (Mulligan, 1970). Closely related *T. brucei* subspecies *T. b. rhodesiense* and *T. b. gambiense* cause human sleeping sickness. Mechanically transmitted trypanosomes such as *T. evansi, T. vivax* and *T. equiperdum* cause major production losses in the respective hosts. The distribution of African trypanosomosis in domestic animals and humans coincides with the known distribution of the tsetse fly vector (Mulligan, 1970). Tsetse flies (*Glossina*) inhabit wide range of habitats covering over 10 million km^2, representing 37% of the African continent and affecting 38 countries (Finelle, 1980; FAO/WHO/OIE, 1982) including Ethiopia. Approximately 30% of the total cattle population in the African continent and about 50 million people are exposed to animal trypanosomosis and human sleeping sickness, respectively (FAO/WHO/OIE, 1963). In the regions infested with tsetse flies, chronic trypanosomosis causes a severe reduction in animal productivity reflected in poor growth, low milk yields, reduced capacity as work animals and infertility. The annual loss in meat production alone due to trypanosomosis is valued at US$5 billion (FAO/WHO/OIE, 1963). The reduced capacity for work animals is also a very important factor where 80% of the traction power in African agriculture is provided by animals (McDowell, 1977). This threat and that of human trypanosomosis are major obstacles to the economic development of the continent and also responsible for the incalculable toll of human health. In addition to the effect of current status, trypanosomosis limits the expansion of natural herds particularly in Africa where the presence of tsetse fly denies access to woodland and savannah areas with good grazing potential. Ethiopia is situated at the extreme east end of the African tsetse belt and its south west part bordering the Sudan suffers from tsetse-transmitted trypanosomosis.

CURRENT SITUATION

Ethiopia has the largest domestic animal population in Africa, with 31 million cattle, 23.2 million sheep, 18.1 million goats, 2.7 million horses, 0.63 million mules, 5.2 million donkeys and 1.07 million camels (FAO, 1993). Eighty eight percent of the national farming population lives on the highland areas which occupy 40% of the country's total area. Overstocking associated with consequent land degradation, is widespread. In 1975, the

government introduced a policy which included resettlement in low-lying areas (PMAC, 1975). Unfortunately, most of these areas, especially in the southwest, are infested with tsetse/trypanososmosis and malaria, which deter animal production and pose a great threat to human life. Trypanosomosis is prevalent in two main regions of Ethiopia, that is, the northwest and the southwest regions (Getachew Abebe *et al.*, 2004). In these regions tsetse transmitted animal trypanosomosis is a major constraint to utilization of the large land resources. The potential area of tsetse infestation has been variously estimated at 66,000 km^2 based on the 1,500 m a.s.l. breeding limit (Ford *et al.*, 1976); 97,855 km^2 based on a 1,600 m a.s.l. breeding limit (Langridge, 1976) and between 135,000-220,000 km^2, based on the maximum dispersal up to 1,700-200,000 m a.s.l. (Slingenberg 1992). Inter-African Bureau for Animal Resources (IBAR, 1989) and Slingenbergh (1992) reported that at least 6 million of the 45 million heads of cattle that are raised under trypanosomosis risk in Africa, are now found in west and southwest Ethiopia. The northwest region of Ethiopia is also affected by tsetse and non-tsetse transmitted trypanosmosis (Getachew Abebe and Yilma Jobre, 1996; Yohannes Afewerk *et al.*, 2000; Alekaw Shinishaw *et al.*, 2005).

Tsetse Flies (*Glossina*)

According to Langridge (1976) the tsetse flies in Ethiopia are confined to the southern and western regions between longitude 33° and 38° E and latitude 5° and 12° N. They infest areas which amount to 97, 855 km^2. Tsetse infested areas lie in the lowlands and also in the river valleys of Abay (Blue Nile), Baro, Akobo, Didessa, Ghibe and Omo (Fig. 1). The infested area extends from the southern part of the Rift Valley, around the south-western corner of the country and along the western lowlands and escarpments to the Blue Nile. Restricting a further eastward spread is the cold limit imposed by highlands that rise to the height above which tsetse cannot survive, or the semi-desert condition along the southern border east of the Rift Valley. Elsewhere there have been advances of tsetse, including extension of the upper altitude limit of the fly from about 1,600 to 2,000 m.a.s.l. in certain areas, although whether flies caught at the highest altitudes are representative of self-sustaining population is uncertain. Tsetse fronts in many places are unstable and tsetse-animal interface is constantly moving. Consequently new areas are being invaded and settled communities are being continually evicted by the advancing tsetse. Such hot spots include the areas in Upper Didessa Valley, the northern and north eastern edges of Lake Abaya in the Rift Valley, the upper reaches of the Omo-Ghibe and its

tributaries.

To date five species of *Glossina* (*G. m. submorsitans, G. pallidipes, G. tachinoides, G. f fuscipes and G. longipennis)* have been recorded from Ethiopia but only four are widespread and of significant economic importance. These are *Glossina m. submorsitans* and *G. tachinoides*, which have a west to east distribution across Africa south of the Sahara desert, and *G. pallidipes* and *G. f. fuscipes* which often occur together in East Africa, although the former extends far to the south whereas the latter has essentially central African distribution. Out of the nine regions of Ethiopia, five (Amhara, Beneshangul-Gumus, Gambella, Oromiya and SNNPR) are infested with more than one species of tsetse flies (Table 1).

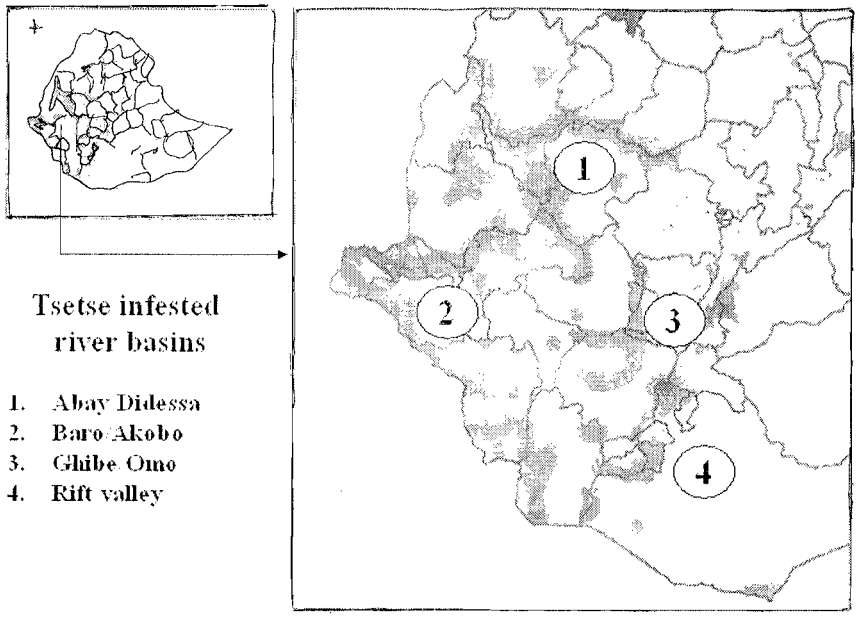

Fig. 1 Tsetse infested river basins of Ethiopia.

Trypanosomes

Six species of trypanosomes are recorded in Ethiopia and the most important trypanosomes, in terms of economic loss in domestic livestock, are the tsetse transmitted species: *T. congolense, T. vivax* and *T. brucei.* The closely related *T. brucei* subspecies, *T. b. rhodesiense* causes human sleeping sickness. The other trypanosoma species of economic importance are *T. evansi* of camels and *T. equiperdum* of horses. Fig. 2 shows the major

distribution of animal trypanosomosis and human sleeping sickness in Ethiopia.

Table 1 Tsetse infested regions and river basins of Ethiopia.

Region	Major River Basin	Tsetse fly
Amhara	Abay (Blue Nile)	*G. m. submorsitans*
		G. tachinoides
Beneshangul-Gumuz	Abay (Blue Nile)	*G. m. submorsitans*
		G. tachinoides
Gambella	Baro/Akobo	*G. m. submorsitans*
		G. tachinoides
		G. pallidipes
		G. f. fuscipes
Oromiya	Abay/Didessa	*G. m. submorsitans*
	Upper Ghibe/Omo	*G. tachinoides*
	Baro/Akobo	*G. pallidipes*
		G. f. fuscipes
SNNPR	Ghibe/Omo	*G. pallidipes*
		G. f. fuscipes
		G. longipennis
	Rift Valley	*G. pallidipes*

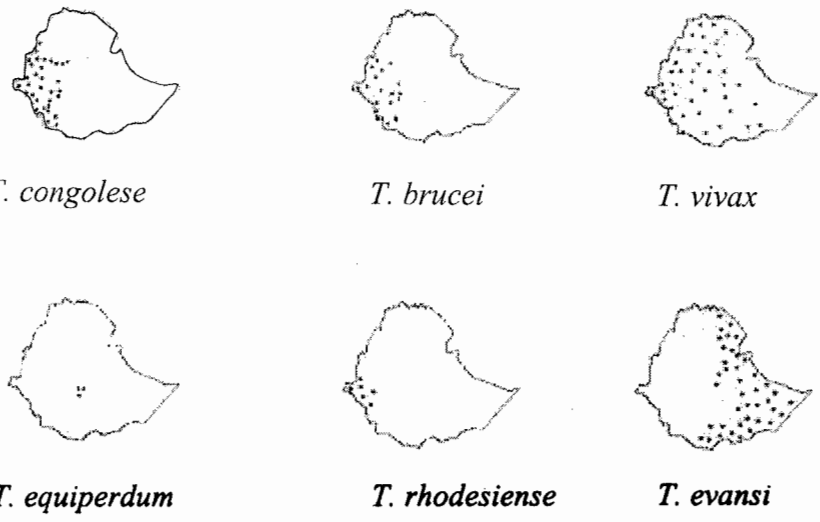

Fig. 2. Distribution of pathogenic trypanosomes in Ethiopia.

Transmission

Trypanosomosis is a disease, which is cyclically transmitted by different species of tsetse flies (Mulligan, 1970). The tsetse fly becomes infected with trypanosomes when feeding on an infected animal. Once the trypanosomes are ingested they lose the surface coat, develop a mitochondrion and

undergo a number of developmental stages before they become infective, once more, to the mammalian host (ILRAD, 1989). These developmental stages are known as trypomastigote, epimastigote and metacyclic forms.

Although the developmental stages are similar for the three species of trypanosomes, the sites within the tsetse in which they occur are different. The initial stage of development of *T. congolense* and *T. brucei* takes place in the midgut. From there the organisms migrate to complete their development in the salivary glands in the case of *T. brucei* and the proboscis in *T. congolense*. By contrast, all stages of development of *T. vivax* take place in the proboscis (Table 2).

Table 2 Morphological characteristics of trypanosomes and site of development in tsetse fly.

Species	Site of development in tsetse fly	Free flagellum	Kinetoplast	Undulating membrane	Size in micrometer	Size & motility in wet film
T. vivax	Proboscis	Present	Large, terminal	Not prominent	20-26	Large, extremely active, traverses the whole field very quickly, pausing occasionally
T. brucei	Midgut Salivary gland	Present in al but not in stumpy form	Small, subtermina l central	Prominent	12-35*	Large, rapid movement in confined areas
T. congolense	Midgut Proboscis	Absent	Medium, subtermina l, marginal	Not prominent	9-18	Small, sluggish active, adheres to red blood cells by anterior end

*Polymorphic- slender, intermediate and stumpy forms

The three species of trypanosomes also differ from one another in the time taken to complete their lifecycle. For *T. vivax* this can be as short as five days, while *T. congolense* usually takes two weeks and *T. brucei* three weeks or longer. The final stage of development to the metacyclic forms is associated with involution of the mitochondrion and reacquisition of a surface coat. At this stage the organism is again capable of establishing an infection when the tsetse fly feeds on a susceptible host.

Other than cyclical transmission, mechanical transmissions of trypanosomal infections can also occur. Mechanical transmission is particularly important in relation to *T. evansi* and *T. vivax* particularly on the fringe of tsetse areas (Hoare, 1970). In the mechanical transmission there are no developmental stages of trypanosomes in the vector. Biting flies of the genus *Tabanus, Haematopta, Chrysops* and *Stomoxys* transmit trypanosomes mechanically

between vertebrate hosts. The trypanosomes are passed from host to host on contaminated insect mouthparts in association with interrupted feeding patterns.

Host Range

Economically the tsetse-transmitted trypanosomoses are of highest importance in cattle, with 14 million heads at risk in Ethiopia (NTTICC, 1996). However, in addition to infection of domesticated livestock, trypanosomes are found in many species of wild mammals. All species of domestic animals are susceptible to infection (Fig. 3) with one or more species of trypanosomes, but trypanosome infections are economically important in cattle, considering its major role in the agricultural economy of Ethiopia (Table 3).

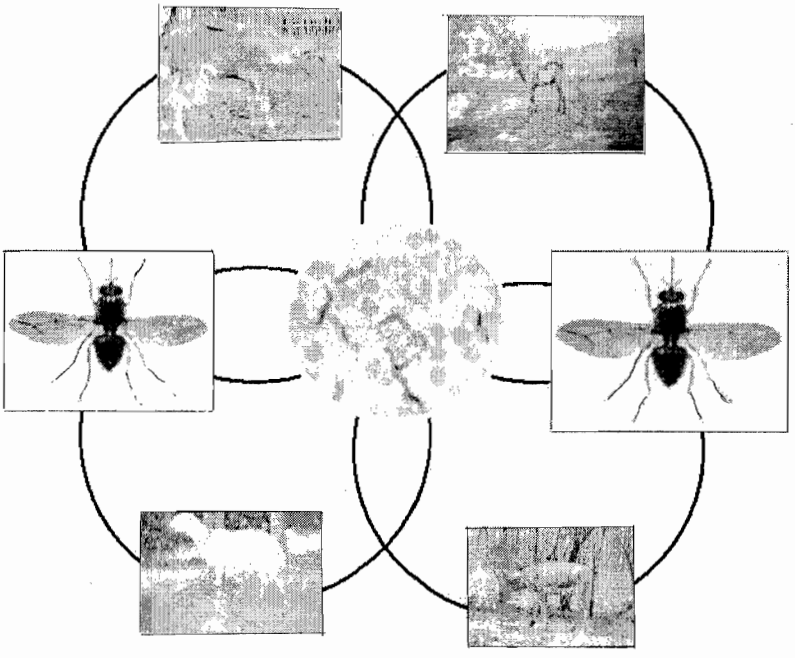

Fig. 3. Tsetse transmitted trypanosomes can circulate in a number of different species of livestock.

Pathogenesis

The pathogenesis of tsetse-transmitted trypanosomosis can be categorized into groups according to the site of host-parasite interaction.

Chancre: The first interaction between trypanosomes and host occur in the skin following a successful feed by an infected tsetse fly. Within a few days

of bite, cattle develop a raised cutaneous swelling called a chancre, which is caused by the reaction to multiplying trypansosmes (Luckins and Gray, 1978, Emery and Moloo, 1980, Akol and Murray, 1982).

Table 3 Trypanosome species reported in Ethiopia.

Trypanosome	Vector	Mainly affected host	Regional distribution
T. congolense *T. vivax and T. brucei*	Tsetse	Cattle	Amhara Benshangul-Gumuz Gambella Oromiya SNNPR
T. vivax	Biting flies	Cattle	All over Ethiopia
T. evansi	Biting flies	Camel	Afar Amhara Oromiya Somali Tigray
T. equiperdum	Via coitus	Horses and donkeys	Oromiya
T. rhodesiense	Tsetse	Human	Gambella Oromiya SNNPR

Lymphadenopathy: Following enlargement of the lymph node draining the chancre, generalized enlargement of lymph nodes and splenomegaly develop (Loses and Ikede, 1972). This is associated with marked proliferation of lymphoid cells in the organs. In the medullary cords of lymph nodes and splenic red pulp there are increases in plasma cells and numerous large active germinal centers are also present (Loses and Ikede, 1972). In addition, in the red pulp of the spleen, there is an increase in the number of activated macrophages, some of which are engaged in erythrophagocytosis.

Anaemia: Plays the major role of pathogenesis of bovine trypanosomosis. The development of anaemia is a well recognized sign of trypanosome infection in cattle. The anaemia in bovine trypanosomosis can be divided into two phases based on the presence or absence of trypanosomes, response to trypanocidal drug treatment and pathological findings (Murray and Dexter, 1988). These are referred to as acute and chronic phases of anaemia. The acute phase anaemia is characterized by progressive anaemia accompanied by parasitaemia (Coetzer *et al.*, 1994). The initial fall in packed cell volume (PCV) values is associated with the first wave of parasiatemia in the blood. During this period the anaemia is extravascular and is possibly the result of increased red cell destruction by phagocytosis in the spleen, lungs, haemal nodes and bone marrow (Murray and Dexter, 1988). Progressive decrease in PCV takes place over a period of 4 to 12 weeks after infection and may result in death. In general trypanosomosis

causes a 40-50% drop in PCV. Cattle that survive the acute process progress into phase two, which is chronic anaemia. This disease syndrome may still result in death, or in either spontaneous recovery or survival with persisting low grade anaemia. This chronic phase is characterized by low and transient parasitaemia or complete absence of detectable parasites in the blood.

Tissue damage: Organs are damaged during the course of infection, some consistently more severely than others. Even though necrosis is not a major feature of bovine trypanosomosis, tissue cell damage and degeneration may be marked (Morrison, *et al.*, 1981a). The heart is constantly damaged by all three species of trypanosomes. Other vital organs or systems, which are commonly affected, include the skeletal muscle, central nervous system (Whitelaw, *et al.*, 1985; Morrison, *et al.*, 1983b), endocrine organs (Ikede and Loses, 1975; Anosa, *et al.*, 1980; Morrison *et al.*, 1981; Getachew Abebe, 1991) and reproductive systems (Loses and Ikede, 1972; Ikede, 1979; Anosa and Itoun, 1980; Mutayoba, 1986).

When an animal is infected with trypanosomes, antibodies against the surface coat are produced. However, trypanosomes have multiple genes, which code for different surface proteins, allowing organisms with new surface coat glycoproteins to elude the immune response. This process is called antigenic variation and results in the persistence of these organisms. Antigenic variation has thus far prevented development of a vaccine and permits reinfection when animals are exposed to tsetse-carrying trypanosomes with surface coat glycoproteins of a new antigenic type (Blood *et al.*, 1989).

The pathogenesis of trypanosomosis is, however, rather complex and depends on the trypanosome species and the species of the transmitting vector as well as on the resistance of the host. The real cause that leads to the death of the animal is not fully understood. On the one hand it is believed that the parasite releases toxic substances when it is destroyed within the circulatory system, which damage the lining of the blood vessels. In some cases the sudden release of large amounts of such toxins triggers a chain of reactions, which produce a shock-like syndrome (Seifert, 1996). Therefore, the damage to the host does not depend on nutrients being taken away by the parasite but rather on the production of toxic substances. With this theory, the typical symptoms of trypanosomosis, such as cachexia, oedema, anaemia and nervous symptoms can be explained.

Metabolic disorders are observed in the host due to a trypanosome-induced hypothyroid status (Getachew Abebe and Eley, 1992) and pituitary

dysfunction during trypanosomosis (Getachew Abebe *et al.*, 1993a; Getachew Abebe *et al.*, 1993b). The ability of trypanosomes to change their surface-coat-antigen continuously leads to the exhaustion of the antibody production of the host leading to immunosuppression (Brown *et al.*, 1990). In addition, there is lymphoid enlargement and splenomegally associated with plasma cell hyperplasia and hypergammaglobulinaemia (Urquhart *et al.*, 1992). Acute infections associated with high parasitaemia may lead to the death of an animal still in good body condition. On the other hand, chronic trypanosomosis is associated with progressive emaciation and eventual cachexia. This is usually accompanied by low levels of parasitaemia and death in untreated cases (Coetzer *et al.*, 1994).

Genetic resistance to animal trypanosomosis has been attributed to certain breeds of livestock, most notably to the indigenous West African N'dama (Murray *et al.*, 1979). This resistance is manifested by the N'dama's ability to withstand the adverse effects of trypanosomosis by regulating parasite growth and their ability to prevent or reduce the rate and degree of development of anaemia (Murray, 1988). There is evidence that trypanotolerance has a genetic basis and may be inherited as dominant trait (Trail *et al.*, 1991). A better understanding of the mechanisms involved in trypanotolerance could aid in the research for genetic markers that could be used for the selective breeding of resistant cattle.

TRYPANOSOMOSES

Cattle

The most important trypanosome species affecting cattle in Ethiopia are *T. congolense, T. vivax* and *T. brucei*. Trypanosomosis in cattle locally referred, as "Gendi" is a serious constraint to livestock production in areas of the north and southwest Ethiopia at an altitude of below 2000 m.a.s.l. (Figure 4a and 4b). Cattle bitten by tsetse flies develop fever, anaemia, lose weight, and progressively become weak and unproductive. Breeding animals frequently abort or may become infertile. Several affected cattle die of anaemia, congestive heart failure or intercurrent bacterial infections that frequently take advantage of the weakened immune system.

In the tsetse infested areas of Ethiopia, 20-30% of cattle are affected by trypanosomosis and in some high tsetse-challenge areas the prevalence of the diseases reaches up to 50%. *T. congolens* and *T.* vivax (Getachew Abebe and Yilma Jobre, 1996) are the most prevalent trypanosomes that infect cattle in the tsetse-infested and tsetse-free areas of Ethiopia, respectively. In

the tsetse-infested areas of the country, though the prevalence of *T. congolense* was found to be high (58.5%), a considerable number of examined animals were also harbouring *T. vivax* infection (31.28%).

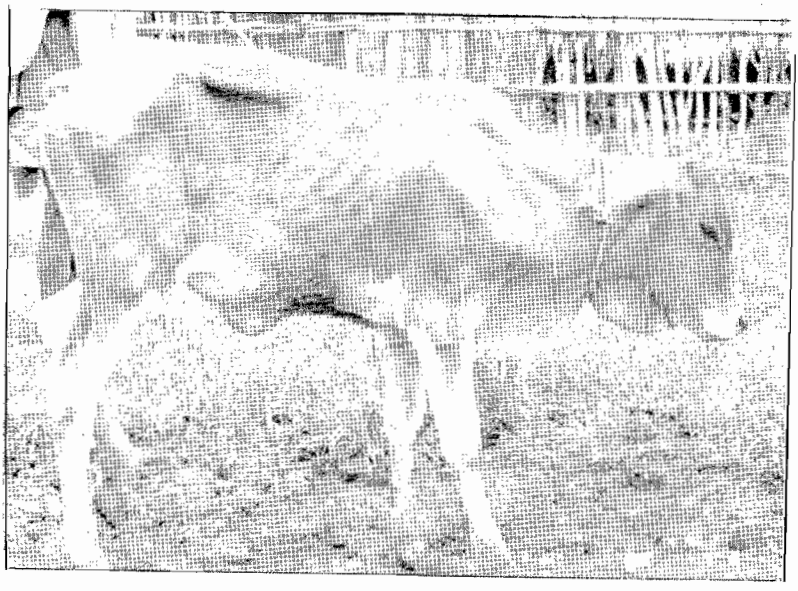

Fig. 4a. Calf infected with trypanosomes in Metekel, Benshangul-Gumuz.

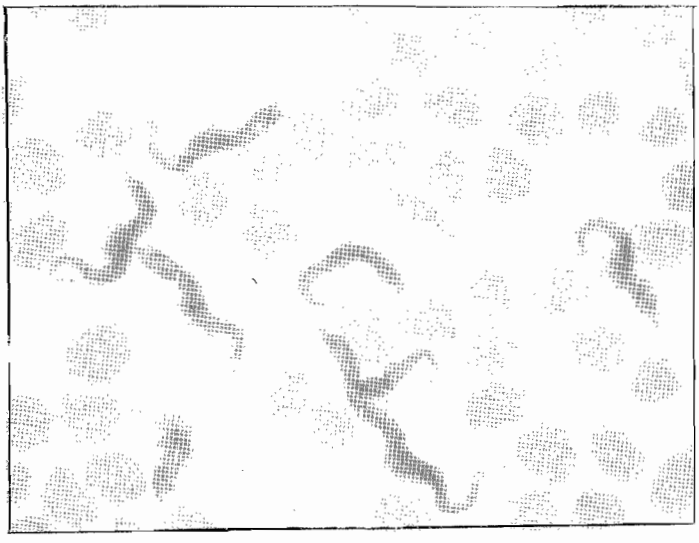

Fig. 4b. Blood stream form of *T. congolense.*

Similar findings were reported by other workers in which they indicated 60% *T . congolonse* and 31% *T. vivax* (Langridge, 1976); and 84% *T. congolense* and 14% *T. vivax* (Rowlands *et al.,* 1993) infections in

southwest part of Ethiopia. Higher proportions of *T. congolense* infection were detected in areas such as Gamo Gofa, Illubabor, Sidamo and Ghibe valley. The predominance of *T. congolense* infection in tsetse-infested areas of Ethiopia indicates the existence of increased contact of cattle with tsetse vectors. The high ratio of *T. congolense* may also suggest that the major cyclical vectors are the savanna tsetse flies *(G. m. submorsitans* and *G. pallidipes)*, which are more efficient transmitter of *T. congolense* than *T. vivax* in East Africa (Langridge, 1976). However, in areas such as Wollega and Keffa, the respective ratios between *T. congolense* and *T. vivax* infections were found to be more or less similar. Bourn and Scott (1978) reported a different situation at Angar Gutin, Oromiya where the chief vector was *G. m. submorsitans*. The study indicated 94% *T. vivax* infections in newly introduced oxen but on keeping these oxen on the settlement site for more than two years, the infections with *T. vivax* had dropped to less than 10%. Some of the factors that could depress the incidence of *T. vivax* could be the use of drugs and the self-cure phenomenon associated with low number of variable surface antigens of *T. vivax* compared to *T. congolense*. In East Africa, except for the haemorragic syndrom, *T. vivax* is generally less virulent than *T. congolense* and consequently cattle develop tolerance to *T. vivax* more readily and easily than to *T. congolense*.

The finding from tsetse-free areas, however, highlights the importance of *T. vivax* infection. Trypanosomosis, in general, is not regarded as an important disease in most parts of the highlands of Ethiopia. However, upon routine blood examination, close to 10 % of the herd was found to harbour *T. vivax* infection. The ability of the parasite to adapt to ways of mechanical transmission has enabled *T. vivax* to establish itself in the vast highland plateaus of Ethiopia (Getachew Terefe and Getachew Abebe, 1999; Alekaw Sinishaw *et al.*, 2005). Both tsetse and non-tsetse transmitted trypanosomes cause marked PCV reduction. According to Getachew Abebe and Yilma Jobre (1996), 60% of *T. vivax* infected cattle in the highland showed anaemia below a PCV value of 20% compared to 50% of *T. congolense* and *T. vivax* infected cattle in the lowland. The high degree of anaemia observed in the highland Ethiopian zebu cattle could possibly be attributed to the compound effects such as fascioliasis, haemonchosis and bunostomiasis, and the high degree of susceptibility of highland zebu cattle to trypanosomosis. In the highland Ethiopia, where 70% of both the human and livestock population exist, there is a chronic shortage of pastureland and high prevalence of bloodsucking gastrointestinal parasites such as fascioliasis and hemonchosis.

Sheep and Goats

Tsetse transmitted trypanosomes of cattle are recognized as a serious constraint to livestock productivity, but the situation with regards to sheep and goats is less clear. A study was undertaken in Didessa and Ghibe valleys, from November 2002 to April 2003, to collect baseline data on small ruminant trypanosomosis, assess the effects of trypanosome infection in sheep and goats and establish the potential role of small ruminants as reservoir of different species of trypanosomes (Hunduma Dinka and Getachew Abebe, 2005). Blood samples from 533 randomly selected small ruminants of different species, sex and age groups were collected and examined with conventional haematological and parasitological techniques. Among the small ruminants examined during the study period, 27 animals (5.1%) were infected with trypanosomes. Most of the infections were due to *T. congolense* (46.7%, 33.3%) followed by *T. vivax* (26.7%, 25.0%) and the rest was due to *T. brucei* (6.7%, 8.3%) and mixed infections of *T. congolense* and *T. vivax* (13.3%, 25.0%), *T. brucei* and *T. vivax* (6.7%, 8.3%) in sheep and goats, respectively. Mean PCV of parasitaemic animals was significantly lower than ($P < 0.01$) that of aparasitaemic animals. Animals infected with trypanosomes were not showing clinical signs and were in good body conditions (Fig. 5).

Goossens *et al.* (1998), Osaer *et al.* (1994) and Snow *et al.* (1996) indicated that small ruminants were not often selected as a source of feeding by tsetse flies. Though these animals do not show signs of trypanosomosis they could however act as carrier of infection and endanger other livestock species. However, when goats are infected artificially with *T. congolense* they develop the disease with typical signs of trypanosomosis (Fig. 6).

In an attempt to identify the vectors involved in the transmission of small ruminant trypanosomosis, both tsetse flies of the morsitans group (*G. pallidipes* and *G. morsitans submorsitans*) and palpalis group (*G. f. fuscipes*) and mechanical vectors of trypanosomosis that belong to the tabanidae family (*Tabanus*) were captured in the lowlands of Didessa (1400-1780 m a.s.l.) and Ghibe (1250-1700 m a.s.l.) valleys. The study revealed that trypanosomosis in sheep and goats is an important disease and small ruminants serve as a potential reservoir of infection to other animals (Hunduma Dinka and Getachew Abebe, 2004).

Horse and Donkeys

Equines have a prominent position in the agricultural and transport systems

as draught, pack and riding animals. In a country where there is less developed modern transport and communication service, the natural choice rests on the use of human and pack animal mode of transport, as it has been the case in some parts of the world. Thus, in a developing country like Ethiopia, the contribution of equines in the energy scenario is of considerable significance.

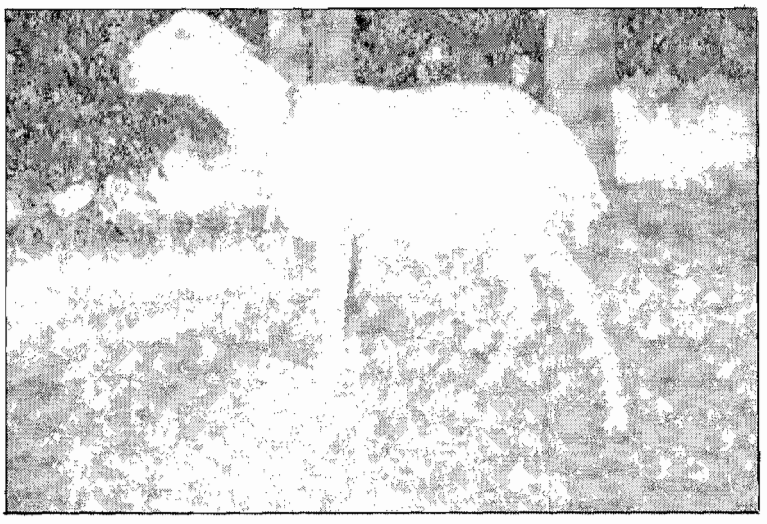

Fig. 5a. Trypanosome infected sheep in Didessa Valley.

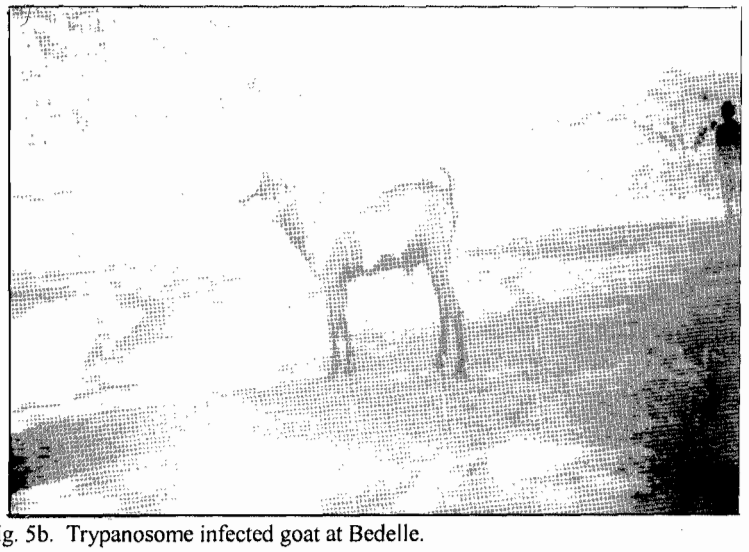

Fig. 5b. Trypanosome infected goat at Bedelle.

Fig. 6. Goat artificially infected with *T. congolense* at FVM-AAU.

Most of the studies conducted in Ethiopia address the problem of bovine trypanosomosis regardless of the fact that equine trypanosomosis exists in tsetse-infested areas of the country. Wondale Yimam (1993) indicated a prevalence of 21% in equines in North Omo, Southern Ethiopia of which 44.1% was due to *T. vivax*, 36.9% due to *T. congolense* and 19% due to *T. brucei.* Kebede Kanchula and Getachew Abebe (1997) reported donkey trypanosomosis with a prevalence of 21% in the same site (Fig. 7).

Recent studies conducted by Ermiyas Assefa and Getachew Abebe (2001) on drug sensitivity of trypanosome infections in donkeys in the four villages of North Omo zone showed 18.2% prevalence of trypanosomosis. Among these animals, 66.2% of the donkeys showed an infection with *T. congolense,* 20.6% showed mixed infection *(T. congolense* + *T. brucei* and/or *T. congolense* + *T. vivax),* 8.8% with *T. brucei* and 4.4% with *T. vivax. T. congolense* with 66.2% of the infection was the most prevalent trypanosome species in the donkey population of North Omo zone. The predominance of *T. congolense* infection in these study sites suggests the existence of increased contact of donkeys with the tsetse vector. This high ratio of *T. congolense* may also suggest that the major cyclical vectors are the savanna tsetse flies *(G. m. submorsitans* and *G. pallidepes),* which are more efficient transmitters of *T. congolense* than of *T. vivax* in East Africa

(Langridge, 1976). *T. congolense* in donkeys causes chronic infection with longer persistence in the blood (Mattioli *et al.*, 1994) and the low incidence in *T. vivax* infection could be due to the mildness of this type of trypanosomosis in donkeys or self cure. In this cross sectional study, an overall mean PCV value of 29.2 % was recorded. About 78% of the parasitaemic donkeys had PCV values of less than 30%. The mean PCV of parasitaemic donkeys (24%) was significantly lower than that of aparasitaemic donkeys (30.7%). In most cases donkeys contract the infection whenever they cross the tsetse-infested areas on their way to markets.

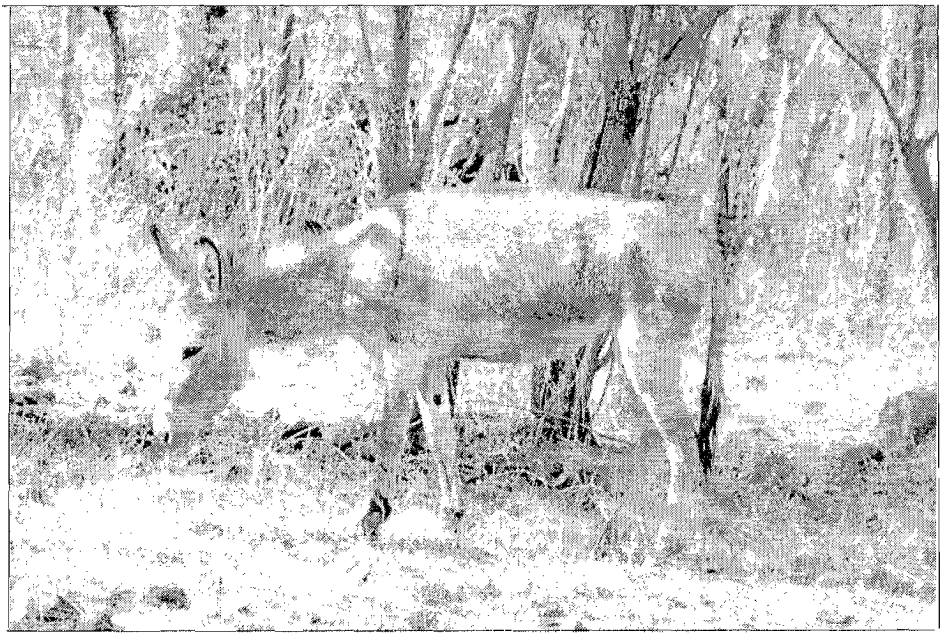

Fig. 7. Trypanosome infected donkey in the Ghibe Valley.

Among the Non-Tsetse Transmitted Trypanosomosis (NTTAT), dourine is included in list B of the OIE notifiable diseases (OIE, 2001). It is the only trypanosomosis that is not transmitted by an invertebrate vector. *T. equiperdum* differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood. It is a chronic, sometimes acute contagious disease of breeding solipeds that is transmitted directly from animal to animal during coitus (Fig. 8).

Local farmers have recognized the problem of dourine in Ethiopia for many years and it has been found to be a threat to the life and productivity of the equine population in the endemic areas.

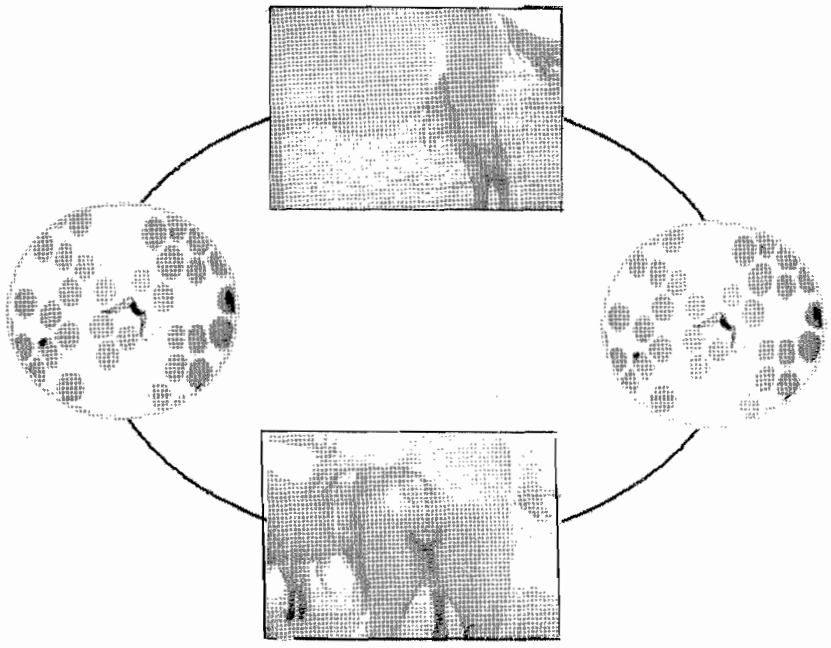

Fig. 8. Transmission of *T. equiperdum* is through coitus.

However, the first official report of the disease was made in 1980 when the Arsi Rural Development Unit (ARDU) requested the Tsetse and Trypanosomosis Survey and Control Department to investigate a persistent disease problem in horses in the administrative regions of Arsi and Bale (Zeleke Dagnachew *et al.*, 1981). Since then dourine was found to be prevalent in the highlands of Ethiopia, particularly in Arsi and Bale zones (Temesgen Alemu *et al.*, 1997). Similarly, multiple positive cases through serological complement fixation test (CFT), enzyme linked immunosorbent assay (ELISA) and trypanozoon polymerase chain reaction (PCR), yet aparasitemic horses were reported in Arsi and Bale zones in Ethiopia (Clausen *et al.*, 1998). However, the presence or absence of dourine in adjacent geographical areas to the Arsi-Bale highlands and other parts of the country where there is high equine population, unrestricted mobility and uncontrolled breeding, remained unknown.

Diagnosis of *T. equiperdum*, the causative organism of dourine in horses, by standard parasitological techniques is difficult, owing to the low numbers of parasites present in the blood or tissue fluids and the frequent absence of clinical signs of the disease. Consequently, the demonstration of

trypanosomal antibodies in the serum has become the most important parameter in determining the disease status of individual animals (Bishop *et al.*, 1995). The principal reason for using serological tests for the diagnosis of trypanosomosis is to overcome the low level of sensitivity of parasitological tests in detecting chronic infections. For the isolation of such parasites, potential loci are Mongolia and Ethiopia (Touratier, 2000). In Ethiopia multiple cases of serological (CFT and ELISA) and Trypanozoon PCR-positive yet aparasitemic horses are reported (Clausen *et al.*, 1998). The difficulty in the diagnosis of *T. equiperdum* lead to difficulties in achieving reliable data on the prevalence and distribution and in the implementation and monitoring of the disease control programmes.

The Faculty of Veterinary Medicine, Addis Ababa University is currently running a sero-epidemiology work on dourine in Arsi and Bale Zones and during the first phase of the study it was possible to appreciate the magnitude of the problem. According to the rapid questionnaire survey conducted, both animal owners and professionals reported that Dourine (*T. equiperdum*) is a major health problem of horses causing high morbidity and economic loss. The disease is locally known by different names as *"Lappessa"* mainly by the Arsi farmers, which refers to the extreme emaciation of affected cases, *" Duda Kuta"* or *"Diressa"* by the Bale people which refers to the hind leg paralysis (literally it means back bone breaker). It was also reported that the disease has a seasonal character which most commonly follows the breeding season (March to May) from June to late September. Sometimes a second peak is observed in the dry seasons of the year, which probably is associated with stressful conditions of lack of feed. Both animal owners and professionals claimed that horses treated against dourine using the available trypanocidal drugs show frequent relapse and said treatment was not effective enough to cure cases.

The most dominant clinical signs observed among the examined clinical cases were: incoordination of hind legs (hind leg paralysis), oedematous swelling of the external genitalia, oedema of the prepuce and glans penis extending up to the ventral abdomen, depigmentation of the genital region in females and poor body condition. The farmers also reported that the first sign of the disease in affected horses to be incoordination, especially of the hind legs (hind leg paralysis) and swelling of the external genitalia (Fig. 9a and b).

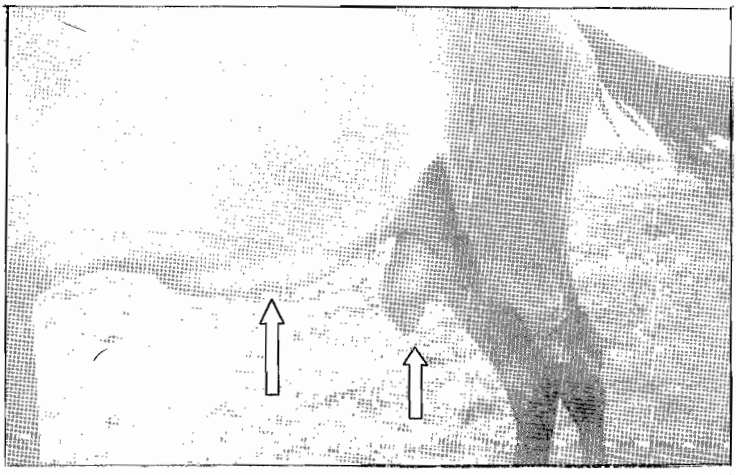

Fig. 9a. Dourine in male horse with oedematous swelling on the ventral side of the abdomen and genital organ.

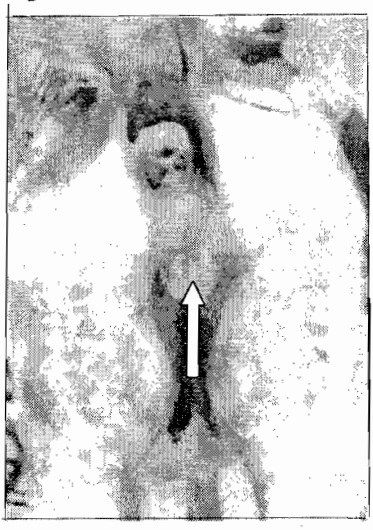

Fig. 9b. Dourine in female horse with depigmented scar around the vulva.

Serum samples from apparently healthy and clinically sick horses were subjected to three different serological tests namely Card Agglutination Test for Trypanosomosis (CATT/ *T. evansi*), LATEX/ *T. evansi* and ELISA obtained from Belgium, Institute of Tropical Medicine, Antwerp. Of 649 horse sera tested for the detection of antibodies against *T. equiperdum*, revealed seropositivity of 184 (28.35%), 161 (24.81%) and 125 (19.26%) for CATT, LATEX and ELISA tests, respectively. Risk factors with significant association to dourine were parity number, previous history of abortion and body condition score (Hagos Ashenafi, 2005).

Camel

Among many diseases that affect camels in Ethiopia, trypanosomosis (Surra) is the most important (Richard, 1979; Getahun Demeke, 1998; Thewodros Tekle and Getachew Abebe, 2001) The disease is widely distributed throughout camel rearing areas of the Ogaden, Borena and Afar regions and causes considerable economic effect due to loss of beasts of burden and food. The causative agent of surra (*T. evansi*) is unicellular flagellated protozoan parasite belonging to the subgenus *Trypanozoon.* Morphologically *T. evansi* is monomorphic and similar to the slender form of *T. brucei.* The similarity between *T. evansi* and *T. brucei* has led to the suggestion that *T. evansi* evolved from *T. brucei* when camels accidentally came in contact with tsetse flies infected with *T. brucei* and gradually disseminated the infection by caravans travelling across the Sahara (Hoare, 1972).

Due to the fact that the disease is characteristically found outside the tsetse belts, it is considered that *T. evansi* is transmitted mechanically by biting flies such as *Tabanus* and *Stomoxys.* The parasite is incapable of cyclical development in tsetse fly because it lacks the genes needed for formation of the mitochondrion, which is a prerequisite for cyclically transmitted members of the brucei groups (Hoare, 1972). The distribution of surra in Ethiopia coincides with the distribution of camels, which is far away from the tsetse belts of the country.

The pathogenesis of *T. evansi* infection in camels varies according to the virulence of the particular stock of the parasite, the susceptibility of the host and the local epidemiological conditions such as the presence of the carrier animal and the vector (Fig. 10).

The acute form of the disease is characterized by intermittent fever, subcutaneous oedematous swelling, progressive anaemia, dullness, lethargy, peticial haemorrhage of the mucosa and discharge from the eye. In the more chronic form, which may last up to three years, there is a continuation of anaemia and progressive emaciation and weakness, often accompanied by development of skin abscesses (Fig. 11a and b). In Ethiopia, surra is observed in more of a chronic form. Recent epidemiological studies on camel trypanosomosis in Borena region indicated a prevalence of 10 and 50% using parasitological and serological tests, respectively (Getahun Demeke, 1998).

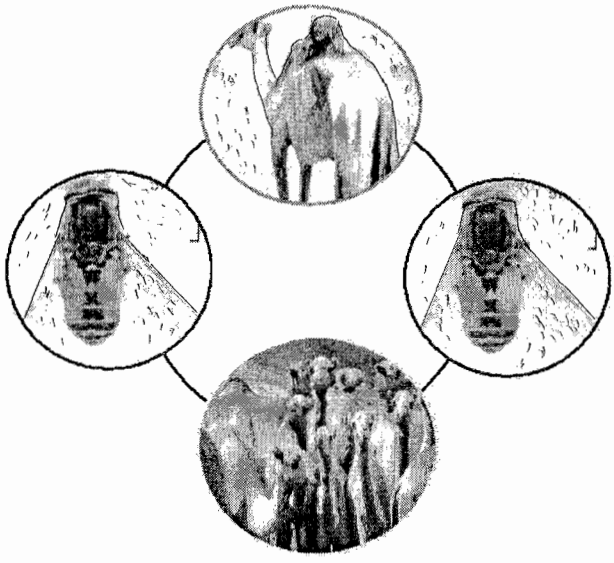

Fig. 10. Transmission of *T. evansi* in the presence of carrier animal and the vector.

The integration of camel husbandry into the traditional pastoral economy of the Southern rangelands is a recent development. The Borena pastoralist who have lost large number of cattle due to droughts have started to rely on camels as a more reliable insurance against climatic calamities (Dessalegn Belete, 1984). Because of limited available grazing lands it is common to see camel herded with cattle and small ruminants. As a consequence, exposure to contagious diseases and various endo- and ecto-parasites leads to high morbidity and mortality and, which in turn, intern restrict herd growth and productivity. In realization of this fact, a study was conducted to determine the prevalence of camel trypanosomosis and helminthiasis in the southern rangelands of Borena, Ethiopia (Thewodros Tekle and Getachew Abebe, 2001). Out of 391 blood samples examined, 43 (10.9%) were found to be positive for *T. evansi.* The disease caused by *T. evansi* infection is well known to the breeders by the local name *"Dhukane"* and is given the first place in its order of importance among camel diseases. Clinical signs observed in the acute cases of the disease were hyperlacrimation, rough hair coat, weakness and depression. The camel owners also reported sharp decline in milk production in lactating and abortions in pregnant animals. Trypanosomes were observed in the blood smear of the animals for the acute cases of the disease. In its chronic form the disease was manifested as emaciation, disappearance of the hump, weakness and general loss of production (decreased milk production and long calving interval as stated by

the camel owners).

Contrary to the parasitological findings, a large proportion of camels were showing the clinical signs of trypanosomosis. The chronic nature of the disease and the possible presence of trypanosomes in extravascular location (Ngernawa *et al.* 1993) might explain the difficulty to demonstrate the trypanosomes in the blood of chronically infected camels by the routine laboratory methods employed in this study. Although blood examinations were showing negative results, the response to treatment resulting in improvement of the health of the camels as stated by the breeders is a good indication for the widespread presence of the disease in its chronic form.

Human Sleeping Sickness

There are three tsetse-borne species of the subgenus Trypanozoon: *T. b. gambiense*, human infective and found in foci of west of the Rift Valley; *T. b. rhodesiense*, also human infective and found in both wild and domestic animals to the east of the Rift, and *T. b. brucei* non-human infective and found in animals across sub-Saharan Africa. All the three species are morphologically indistinguishable. Before the advent of DNA technology, human infectivity of animal isolates of *T. brucei* was determined using either human volunteers or parasite survival in human serum. Recently, a single gene has been identified in *T. b. rhodesiense* that renders human serum sensitivity *T. b. brucei* clones resistant to lysis in human serum (Zong *et al.* 1998). The fact that this gene is not expressed in *T. b. gambiense* highlights the fundamental difference between these parasites.

The distribution of endemic human trypanosomosis and its tsetse vectors appears to be limited to southern and southwestern Ethiopia. The human disease, based on case reports, occurs in Illubabor, Wollega, and Gamo Gofa regions (Makonnen Abebe *et al.*, 1988). *T. b. rhodesiense* is singled out as the species occurring in Ethiopia (Baker *et al.* 1970; Hutchinson, 1971; Baker and McConnell, 1969, 1973). This trypanosome is a flagellated protozoan parasite of humans as well as domestic and wild animals. Morphologically, it is indistinguishable from *T. b. gambiense*, the other human infecting species in West and Central Africa, and *T. b. brucei*, which is a common parasite of cattle and wild game. However, it has been questioned whether sleeping sickness in Ethiopia is caused by *T. b. rhodesiense* or *T. b. gambiense*, as the original diagnosis was based on clinical signs. There is no *T. b. rhodesiense* in the neighbouring Sudan, which is the closest sleeping sickness focus to the Ethiopian outbreak. The closest *T. b. rhodesiense* focus is in the Busoga area of Uganda.

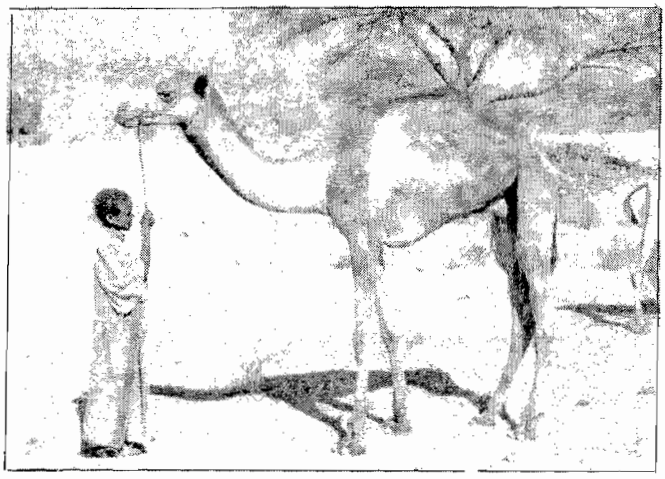

Fig. 11a. Trypanosome-infected camel with poor body condition.

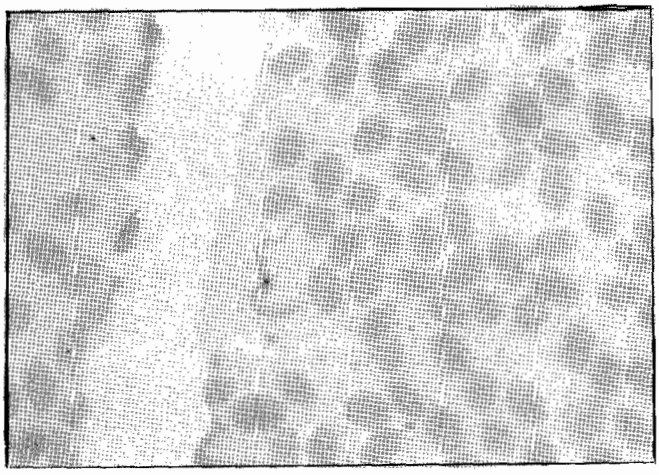

Fig. 11b. *T. evansi* in camel blood smear.

The first confirmed case of human trypanosomosis in Ethiopia was reported in 1967 (Baker and McConnell, 1969) but a major outbreak of the disease was not recorded until the 1969-1970 epidemics (Hutchinson, 1971) and since that time, only sporadic cases have been reported (Makonnen Abebe *et al.*, 1988). The usual mode of transmission is through the bite of several species of tsetse flies belonging to the genus *Glossina*. Common symptoms begin with an inflammation response at the site of the fly bite, which soon forms a chancre. As the disease progresses and parasites enter the lymphatic channel, the lymph nodes, spleen, and liver become enlarged. The parasites also invade the central nervous system, thereby giving rise to nervous symptoms such as somnolence and seizure. Hutchinson (1971) noted that

invasion of the central nervous system does not necessarily occur after invasion of the visceral organs. Dysfunction of the endocrine system causes facial puffiness (moon face) and reproductive disorders, such as irregularities in menstrual and oestrus cycles, infertility, abortion, and impotence. Occasional cases of neonatal deaths and stillbirths have also been associated with trypanosomal infections. Most reproductive disorders are reversible following treatment.

In contrast to *T. b. gambiense*, which causes a milder illness, the parasite *T. b. rhodesiense* reportedly gives rise to an acute disease that results in death within a few months, if not treated. With the development of primary parasitaemia, an accompanying fever, anorexia, headache, and limb pains frequently occur. Some of the commonly reported human symptoms during the 1969-1970 epidemics in Ethiopia included chancres, noted in only a small percentage of the cases; headache, reported in approximately half of the cases; and enlargement of the lymph nodes, noted in less than a quarter of the cases (Hutchinson, 1971).

The endocrine dysfunction observed during sleeping sickness is also observed in cattle experimentally infected with trypanosomes. Cattle infected with *T. congolense* show signs of endocrine dysfunction expressed in terms of low levels of pituitary and target endocrine gland hormones. Getachew Abebe and Eley (1992) have reported the presence of asymptomatic hypothyroidism manifested by low level of T_4 in *T. congolense* infected Boran cattle. Furthermore, they have observed low responsiveness of the pituitary gland towards exogenous Corticotropin-Releasing Factor (CRF) and insulin administration in *Trypanosome* infected compared to control animals (Getachew Abebe *et al.*, 1993a). Low levels of T_4, in particular, could result in defective energy metabolism of the host. It is known that plasma concentrations of thyroid hormone correlate positively with energy intake. Reduced feed and impaired efficiency of feed conversion have been implicated to be the main cause for growth failure in pigs infected with *T. simiae* (Ilemobade and Balogun, 1981). Tumour Necrosis Factor (TNF, Cachectin) is produced during trypanosomosis and inhibit secretion of growth hormones (Walton and Cronin, 1989) and thyroid-stimulating hormone (Pang *et al.*, 1989).

DIAGNOSIS

Accurate diagnosis of trypanosome infections in livestock is required for a proper appreciation of the epidemiology of the disease. However, high parasitaemia are usually evident only in early infections, and in the chronic

phase of the disease, parasites may apparently be absent from the blood for long intervals. This is due to the ability of trypanosomes to establish prolonged infections attributed to the phenomenon of antigenic variation. As parasitaemia rises, a swift antibody response is elicited against the antigen type exposed on the surface of the bloodstream trypanosomes. However, before antibodies reach trypanolytic levels, some trypanosomes switch off the gene that controls the production of the initial surface glycoprotein and activate a gene that codes for a different protein. Trypanosomes, which bear the new surface glycoprotein, are of a different antigenic type and are not destroyed by antibodies against the first antigen type. Consequently, they produce another parasitaemic wave, which is, in turn, removed by antibodies specific to that antigen type. The cycles repeat themselves leading to parasitaemic waves of different peaks (Coetzer *et al.*, 1994). Therefore, diagnostic methods with high degree of sensitivity and specificity are required. Besides clinical diagnosis, direct (parasitological) and indirect (serological) diagnostic methods with varying degree of sensitivity and specificity are available.

Clinical Diagnosis

The disease shows a variety of clinical manifestations, which are also common to other diseases. The fact that the disease may run an acute, chronic or sub-clinical course further complicates the diagnosis of trypanosome infections on the basis of clinical signs. In general, fever can be observed which may be intermittent due to the variation in parasitaemia, and if the animal survives, the disease becomes chronic and there is development of anaemia and emaciation (Blood *et al.,* 1989; Coetzer *et al.*, 1994). This, therefore, makes fever, anaemia and body condition important parameters that are routinely used for the tentative diagnosis of trypanosomosis in areas where this disease is endemic and laboratory services are not available. Definitive diagnosis of the disease is ultimately dependent on the detection of the trypanosome in blood samples from infected animals.

Parasitological Methods

Wet preparations (Baker, 1970; Stephen, 1986)

A drop of fresh blood is placed on a slide and covered with a glass slip. The preparation is examined microscopically with subdued light or by phase contrast. Trypanosome movement is looked for as the actively motile organisms are readily detected by the agitation they produce among the

erythrocytes. The detection limit of this technique is about 8.3 X 10^3 trypanosomes/ml of blood (Paris *et al.*, 1982).

Thin stained blood films (Shute and Maryon, 1966)

A drop of fresh blood is collected on the narrow edge of a microscope slide. This edge is then placed on another slide lying on a flat surface, so that the blood drop lies in the acute angle between the two slides. The inclined slide is then pushed along the recumbent one so that the blood is pulled behind it and spread in a thin layer over the stationary slide. The latter is then air-dried. Ideally, films should be made so that the erythrocytes are fairly close together but not overlapping. The thin films should, for general purpose be stained with Giemsa's, although Leishman's and Wright's stains may also be used. This technique can detect between 2.5-5 X 10^3 trypanosomes/ml of blood (Paris *et al.*, 1982).

Giemsa staining technique (Shute and Maryon, 1966)

A blood smear is air dried and fixed in methanol for 10 minutes. It is then dried and immersed in Giemsa stain (10 % Giemsa in Weise buffer and pH 7.2) for 35-40 minutes. It is then washed under tap water to remove the extra stain and then air dried. The stained smears are then observed under a microscope at X 100 objective and X 10 eyepiece and oil imulsion.

Thick stained blood films (Maclennan, 1957)

Two to three drops of blood are collected onto a clean slide and spread with a needle, or the corner of another slide, into an area about 1 cm in diameter. Gentle heat during drying helps to prevent the film from floating off the slide during staining. About 2-3 hours at 37°C (not higher) should be adequate. After drying thoroughly, the film is stained for 1 second in 0.5 percent aqueous methylene blue before lysing the erythrocytes. After acid lysis, the film may be fixed and stained in the usual manner with Giemsa stain. Because thick films contain much more blood per unit area the chances of finding trypanosomes are greater than in thin films. The detection limit of thick blood films is up to 5.0 X 10^3 trypanosomes/ml of blood (Paris *et al.*, 1982).

Haematocrit centrifugation technique-HCT (Woo, 1970)

A heparinised microhaematocrit capillary tube containing 70 µl of blood is centrifuged for 5 minutes at 15,000 rpm. After the centrifugation, trypanosomes are usually found in or just above the buffy coat layer. Two rectangular pieces of glass from a standard microscope slide (1.2 mm thick)

are fixed 1.5 mm apart an a microscope slide. The capillary tube is placed in the slot and a drop of immersion oil (refractive index 1.524 at 20°C) is placed on top the capillary tube. Oil fills the space between the capillary tube and the two pieces of glass. This arrangement reduces the effect of light defraction caused by the curvature of the capillary tube and facilitates the observation of the trypanosomes. The buffy-coat plasma junction is examined by rotation using an objective with a X 20 magnification and X 10 eyepiece. This technique can detect up to 5.2 X 10^2 trypanosomes/ml of blood (Paris *et al.*, 1982).

Buffy coat darkground-phase contrast technique-BCT (Murray *et al.*, 1977)

Following blood centrifugation at 10,000 rpm for 5 minutes in heparinised microhaematocrit capillary tubes sealed on one end, the trypanosomes are concentrated mainly in the buffy coat zone. The capillary tube is then cut using a diamond pen 1 mm below the buffy coat to include the uppermost layer of the red blood cells and 3 cm above to include the plasma. Using a microhaematocrit tube holder, the contents of the capillary tube are expressed onto a slide, mixed and covered with a coverslip (22 X 22 mm). The preparation is then examined using a condenser with X 25 objective and X 10 eyepiece. The advantages of the condenser is that it allows the use of brightfield, phase and darkground illumination. The dark illumination makes it easy to recognise the trypanosomes by their fluorescing appearance and by their movement. *T. congolense* is recognised by its small size in relation to the red blood cell diameter, its sluggish activity and its invariable attachment to the red blood cells. *T. vivax* on the other hand is seen large and strikingly apparent by the speed with which it traverses the microscopic field. *T. brucei* is distinguished from its large size and going round in circles in a particular location. The buffy coat tecnique is able to detect up to 2.5 X 10^2 trypanosomes/ml of blood (Paris *et al.*, 1982). Accordingly estimation of parasitaemia can be done using the buffy coat darkground-phase contrast technique (Table 4).

Table 4 Buffy coat darkground-phase contrast technique (Paris *et al.*, 1982).

Score	Trypanosome/field	Estimated parasitaemia Trypanosomes/ml
6+	Swarming > 100 per field	$>5 \times 10^6$
5+	>10 per field	$>5 \times 10^5$
4+	1 – 10 per field	$10^4 - 5 \times 10^5$
3+	1per 2 fields – 1 per 10 fields	10^4
2+	1 – 10 per preparation	$10^3 - 10^4$
1+	1 per preparation	$10^2 - 10^3$

The miniature-anion exchange centrifugation technique (m- AECT, Lumsden *et al.*, 1977)

The salivarian trypanosomes can be separated from infected blood by adsorbing the particulate blood components on to DEAE-cellulose columns and eluting the trypanosomes (Lanham and Godfrey, 1970). The elute is collected and centrifuged to concentrate the trypanosomes. A stock solution of phosphate-buffered saline-glucose (PSG) is prepared. The DEAE-cellulose is washed three to four times with PSG. The pH of the supernatant is checked and must be within 0.05 units of the equilibrating buffer. The lower end of the barrel of a 2 ml syringe is plugged with a disc of Whatman No. 41 filter paper and a cylindrical piece of cellulose sponge wet with PSG. The DEAE-cellulose is packed to a height of 2 cm. The column is then rinsed with PSG (three times the column volume). A needle is attached to the syringe and a discard tube provided to collect the elute. A sample of 200 µl blood is prediluted half with PSG on top of the adsorbent. After all the blood has entered the adsorbent, buffer is added and a sealed Pasteur pipette that is filled to constriction replaces the discard tube. The Pasteur pipette is centrifuged at about 525 g for 10 minutes. The tip of the sealed pipette is examined by means of a microscope at 250 magnification with oil.

Serological Diagnosis

Diagnosis of bovine trypanososmosis by conventional parasitological techniques is often difficult since trypanosomes cannot always be detected in the peripheral blood and some species do not readily infect laboratory rodents (Killick-Kendrick, 1968; Robson and Ashakar, 1972). These parasitological detection methods have a limited analytical sensitivity (i.e. lower detection limit) and may lead to under-reporting of the prevalence of disease (Paris *et al.*, 1982). As a consequence, more sensitive diagnostic methods, including the detection of *Trypanosoma* specific antibodies and antigens have been developed (Luckins, 1977; Luckins and Mehlitz, 1976; Nantulya *et al.*, 1992). However, immunocompetence, maternal antibodies and physical exhaustion are important biological factors and it is good practice to consider age, sex and breed as potential biological factors a priori when analysing serological data (Greiner and Böhning, 1994; Jacobson, 1998).

Antibody-ELISA

Ab-ELISA was developed by Engvall and Perlmann (1971) and was adopted by Voller *et al.* (1977) for the diagnosis of protozoal diseases. This

technique is used throughout the world for the detection of antibodies for the diagnosis of infectious diseases in veterinry medicine (Wright *et al.*, 1993). In their study of bovine trypanosomosis, Luckins and Mehlitz (1976) used microplate-ELISA system and found that cattle developed positive ELISA values after infection, but it was not possible to differentiate between *T. vivax, T. congolense, T. brucei* or *T. rhodesiense.* Nevertheless, the serological tests in current use suffer from lack of well defined antigens necessary for designing simple and accurate tests that are easily adaptable for field use. In addition, the detection of anti-trypanosomal antibodies in serum cannot distinguish between an active infection and a cured one. This is due to the fact that, in cattle, the length of time taken for antibodies to disappear from circulation after a successful therapy is not yet clear. Antibodies are in circulation even up to 9 months after cure (Voller *et al.*, 1977).

The test is carried out using multiple-well, polystyrene microtitre plates. The wells are coated with antigen prepared from trypanosomes; the serum under test is added to the wells in suitable dilutions and if it is homologous, a reaction occurs and the immunoglobulins bind to the antigen and cannot be removed by washing. An anti-animal antiserum (e.g., Rabbit anti-bovine IgG) which has been previoulsy conjugated with an enzyme (e.g., horse radish peroxidase) is then added to attach to the test serum. Enzyme-substrate is then added which produces a colour change. Completed reactions may be read visually, or more precisely, photometrically (Stephen, 1986).

Antigen-ELISA

An antigen-detection enzyme-linked immunosorbent assay (antigen-ELISA) for trypanosomosis has been described (Nantulya and Lindquist, 1989) and is now available for the diagnosis of *T. vivax, T. congolence and T. brucei* infections in cattle. However, field evaluations of the test have given inconsistent results. Works done in various countries have shown unexpected results such as high prevalence of *T. brucei* infections which is not usual. Therefore, additional work is needed to discover and overcome the cause of those inconsistencies before the test can be used in the routine diagnosis of trypanosomosis (OIE, 2002). Specific circulating antigens can be detected in cattle from 8-14 days after infection, but within 14 days of treatment they are not longer detectable. Therefore, this test seems to be an important tool for controlling the efficiency of trypanocidal treatment, or whether or not a treatment has eliminated premunity (Nantulya, 1990;

Nantulya *et al.*, 1989).

Molecular Tests

New tools developed by molecular biologists now make it possible to characterize trypanosomes both in the vectors and the hosts. The use of molecular biological tools, and in particular the Polymerase Chain Reaction (PCR), introduced an exceptional sensitivity and especially the possibility of characterization at the specific or infra-specific level, which had been impossible previously (Solano *et al.*, 2000).

The principle of molecular tests (DNA probes, PCR) is the demonstration of the occurrence of sequences of nucleotides, which are specific for a trypanosome subgenus, species or even type or strain. Nucleotides are the constituents of DNA, the molecules which constitute the genes on the chromosomes in the cell nucleus. 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.

Animal Sub-inoculation

The inoculation of blood and other fluids into susceptible animals has been employed from earlier times (Stephen, 1986). *T. b. brucei* and *T. b. rhodesiense* multiply readily in many mammals, including those commonly used in the laboratory such as mice, rats, rabbits, guinea-pigs, hamsters and monkeys (Baker, 1970). Because of the expenses and logistic problems incurred when using large animals, mice and rats have been used by many workers for diagnosis and characterisation of drug-resistant trypanosome populations (Peregrine, 1994). Such systems reduce experimental costs and the time taken to characterise a population to approximately two months (Peregrine, 1994; Geerts and Holmes, 1998). The disadvantage is that most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice (Geerts and Holmes, 1998). Because of their ready availability, these animals have been used most frequently and as a result, some *T. vivax, T. congolense, T. simiae* and *T. suis* infections have gone undetected (Montgomery and Kinghorn, 1908; 1909).

The sub-inoculation of blood into rodents is particularly useful in revealing sub-patent infections with trypanosme parasites in animals, including cattle (Godfrey and Killick-Kendrick, 1961). This method is especially useful in detecting camel infection with *T. evansi*, in which case the trypanosome is a

serious pathogen. Godfrey and Killick-Kendrick (1962) examined 145 camels in Northern Nigeria by inoculation of blood into rats. Later, 27.6% of the rats developed *T. evansi* infections indicating that 40 of the camels were habouring the parasite. Examination of thick smears revealed only 18 infected animals (12.4%). Work carried out by Paris *et al.*, (1982) indicated that mouse inoculation has a detection limit of 1.25 X 10^2 trypanosomes/ml for *T. brucei.*

CONTROL

Control strategies in trypanosomsis concentrate on vector control, parasite control with chemotherapy and chemoprophylaxis and use of the inherent trypanotolerant trait in some breeds of animals (Holmes, 1997).

Vector Control

Previous control techniques included vegetation clearing, ground and aerial insecticide spraying and selective game destruction. These methods have been discouraged due to the high costs involved in addition to being environmentally un-friendly (FAO, 1992). The development of insecticide impregnated, odour-baited traps (Dransfield *et al.,* 1990) and targets (Vale *et al.,* 1988) and insecticide-treated cattle as pour-on (Shereni, 1990) which attract and kill tsetse offer the prospect of cheaper alternatives with less damage to the environment (Jordan, 1988). Application of deltamethrin pour-on to cattle proved to be very efficient in controlling tsetse in the pastoral zone of Samorogouan, Burkina Faso (Bauer *et al.,* 1995). On the other hand, baits (traps, targets or animals) are nowadays used widely to replace air and ground broadcasting of the insecticides (Vale, 1993). In addition, various trap designs have been used in various countries in the efforts to control tsetse. To control *G. pallidipes* and *G. longipennis* in Kenya, Brightwell and Dransfield (1992) indicated that NGU traps baited with acetone, cow urine and octenal had been used while biconical traps without odours were used to help control *G. m. submorsitans* in Burkina Faso. Targets are usually more effective as a control method because they do not require the flies to enter (Vale, 1993). In Zimbabwe, Kenya, Ethiopia, Rwanda and Burkina Faso, insecticide-treated targets have been used for control of both *G. pallidipes* and *G. morsitans* (FAO, 1992). Work carried out in Kenya indicated that the populations of *G. pallidipes* and *G. longipennis* were reduced by 99.9% when using insecticide-impregnated targets (Dransfield *et al.,* 1990). In Zimbabwe, Vale *et al,* (1988) indicated that use of targets consisting of a black cloth and netting baited with Octanol, acetone and coated with deltamethrin, reduced *G. pallidipes* and *G.*

m. morsitans populations by 99.9%. In conclusion, he indicated that targets offered a simple and ecologically clean method of controlling tsetse and preventing re-invasion.

In Ethiopia, these techniques have been tried and are still in use in the different tsetse infested areas. In Didessa Valley, Ethiopia, the Technical Co-operation Programme (TCP) from FAO initiated a tsetse control/eradication programme in 1986. A low-cost, non-pollutant, effective and community-based odour-baited, insecticide-impregnated target/trap technology was selected in an initial area of 30 km^2 and later to include some 800 km^2. Other programmes include collaborative work between the Ethiopian government and ILRI in Ghibe Valley with different techniques being tested including target technology and pour-on. In addition, there was the ESTC/SEPAR/ICIPE collaborative programme near Bedessa township north of Lake Abaya using NGU-3 traps through community participation on self-help basis. Another project just completed with EU funds in Didessa Valley as part of the East African Region African Trypanosomosis Control Programme is "Farming in Tsetse Control Areas (FITCA)" as an extension of the previous activities, over a total area of 4,500 km^2 of the upper Didessa valley to control tsetse flies and rehabilitate mixed farming practices. Apart from this, operation is underway to eradicate tsetse flies from an area of 25,000 km^2 in the Southern Rift Valley Ethiopia Tsetse Eradication Project (STEP) area using the Sterile Insect technique (SIT). The use of insecticide impregnated target and application of pour-on on cattle in the area has suppressed the tsetse population from 4.1 to 0.9 fly/trap/day. As a result the prevalence of bovine trypanosomosis has dropped from 27 to 6% in two years time (Getachew Abebe *et al.* 2004). Clausen *et al.* (1992) stressed that efficient tsetse control will lead to a reduction in use of trypanocidal drugs and this will retrict their role to being efficient means of curing the disease in case of an outbreak.

The Sterile Insect Technique (SIT)

This is a biological method of control in which sterilised male tsetse are released and compete with wild male tsetse for mating with females (Dame and Schmidt, 1970). The principles of this method state that it becomes more economical when the natural population is low. The technique has been used successfully in some parts of Africa e.g. eradication of three species of flies in Burkina Faso (Clair *et al.*, 1990) and in Zanzibar, Tanzania. In Ethiopia, a major SIT project coordinated by the Ethiopian Science and Technology Commission and implemented by the Agricultural

Bureau of the Southern Nations and Peoples Administrative Region of Ethiopia in the Southern Rift Valley has been initiated. Southern Rift Valley Ethiopia Tsetse Eradication Project (STEP) is a ten-year tsetse eradication programme of two five-year phases prepared in line with the understanding between the Ethiopian Government and the International Atomic Energy Agency (IAEA) of the United Nations. The SIT is envisaged to supplement the national effort of tsetse and trypanosomosis management, using area wide eradication approach of the resident fly species in the Rift Valley, *G. pallidipes*. The programme has two main components: (1) establishment of sterile insect production plant, which is centrally organised and operated, and (2) the actual field operation of the fly eradication process, which is implemented on regional level (Getachew Abebe *et al.* 2004).

Parasite Control

Chemotherapy/Chemoprophylaxis

Chemotherapy and chemoprophylaxis are presently the major methods of control of trypanosomosis in livestock. Diminazene, homidium and isometamidium are primarily used for treatment and prophylaxis of trypanosomosis in cattle, sheep and goats. Quinapyramine, suramin and melarsomine are primarily used as therapuetic agents for infections with *T. evansi*, although quinapyramine is also used for prophylactic purposes (Table 5). These latter three compounds are, therefore, generally restricted to use in camels, equines and buffaloes (Peregrine, 1994). All the three compounds have been available for at least 45 years. After the introduction of isometamedium in 1961 (Berg *et al.* 1961) the development of new trypanocidal drugs has made little progress. Recently, however, quinapyramin sulphate (Antrycide) has been reintroduced because of the need especially, to control camel trypanosomosis. The incidence of resistance to these drugs is apparently increasing (Peregrine, 1994) and the main means of controlling the disease is therefore under threat.

Chemoprophylaxis against bovine trypanosomosis has been in widespread use in tropical Africa for many years. Isometamidium (Berg *et al.*, 1961) has been marketed since 1961 as a prophylactic and therapeutic drug. Prophylaxis can be useful under high challenge situations and enables cattle to remain productive, as demonstrated on a commercial ranch at Mkwaja, in Tanzania. On this ranch, cattle maintained under isometamidium prophylaxis were 80% as productive as high quality Boran cattle on trypanosomosis-free ranch in Kenya (Trail *et al.*, 1985). This form of mass treatment exerts a strong selection pressure on the trypanosome population

(Geerts and Holmes, 1998). As a result, multiple drug resistance later developed in this ranch (Peregrine, 1994; Geerts and Holmes, 1998). Thus, in order to maintain the efficacy of the currently used compounds, it is important that chemotherapuetic and chemoprophylactic regimens are rationalised on the basis of the drug-susceptibility phenotype of trypanosome populations in a given locality (Peregrine, 1994). Reduction of the number of trypanocidal treatments by integrating drug treatment with other control measures may help alleviate the problem (Geerts and Holmes, 1998).

Table 5 Drugs available for the treatment of trypanosomosis in domestic animals.

Drug	Trade name	Host
Diminazene aceturate	Berenil (Intervet)	Cattle, buffalo
Homidium choloride	Ethidium "C" (Boots) Novidium (May & Baker)	Cattle, horses
Homidium bromide	Ethidium bromide (Boots)	Cattle, horses
Isometamidium chloride	Samotin (Merial) Trypamidium (Merial)	Cattle, horses, camel, dogs
Suramin	Naganol (production stopped)	Cattle, horses, buffalo, camel, dogs
Quinapyramine sulphte	Antrycide (ACCI)	Pigs, camels, buffalo, horses
Cymelarsan	Mel Cy (Merial)	Camels

Drug resistance

Treatment and prevention of animal trypanosomosis relies essentially on three drugs, namely; homidium (homidium chloride-novidium® and homidium bromide-ethidium®) diminazene aceturate (berenil®) and isometamidium chloride (samorine®, trypamidium®). However, almost all of these trypanocides are gradually losing their efficacy due to drug resistance (Williamson, 1970). Experimental studies have demonstrated the occurrence of resistance in trypanosomes to both diminazene (Mwabubu and Mayende, 1971; Gitatha, 1979; Ainanshe *et al.*, 1992) and isometamidium (Schönefeld *et al.*1987; Clausen *et al.*, 1992). This has been confirmed by studies carried out in Ghibe valley, Ethiopia (Codjia *et al.*, 1993; Leak *et al.*, 1993; Peregrine *et al.*, 1994). Trypanosomes are usually not resistant to both diminazene and isometamidium at the same time. Thus these compounds have been termed as sanative pair for the control of bovine trypanosomosis (Whiteside, 1960). However, there is recent evidence for the development of multiple drug resistance in Burkina Faso (Clausen *et al.*, 1992) and Ethiopia (Codjia *et al.*, 1993; Wubet Mulugeta *et al.*, 1997; Yohannes Afewerk *et al.*, 2000; Nega Tewelde *et al.*, 2004) that suggest that the concept of sanative pairs might no longer be valid.

Previous studies have shown the prevalence of drug resistant trypanosomes

in cattle herd of Ethiopia. Scott and Pegram (1974) described the occurrence of homidium-resistant population of *T. congolense* in Didessa and Angar valleys in Wollega province. Yohannes Afewerk (1998) reported that *T. congolense* field isolates from Metekel region, expressed resistance to both isometamidium chloride and diminazene aceturate in mice. Meanwhile, Mesfin Ademe and Getachew Abebe (2000) identified population of trypanosomes in North Omo, which express resistance to both isometamidium chloride and diminazene aceturate. Recently Yohannes Afewerk *et al.* (2000) has confirmed multiple drug resistance in cloned *T. congolense.*

A longitudinal drug sensitivity study in naturally infected donkeys with trypanosomes demonstrated 8/37 (21.62%) relapse/breakthrough infections in the field within four weeks of prophylactic treatment with 1.0 mg/kg body weight of isometamidium chloride (Ermiyas Assefa and Getachew Abebe, 2001). Most cases of relapse/breakthrough infections (71.43%) were due to *T. congolense.* The results have shown that the period of prophylaxis conferred by isometamidium against trypanosomes, mainly *T. congolense,* was very short, namely less than one month. This is further supported by the results of drug sensitivity tests in mice. Sutherland *et al,* (1991) reported that the period of prophylaxis conferred by 1.0 mg/kg body weight of isometamidium chloride was less than 28 days in cattle challenged with a clone of *T. congolense* which, in therapeutic trial, had been shown to be highly resistant to isometamidium chloride. A similar finding was reported by Yohannes Afewerk (1998) in Northwest Ethiopia, in which the prophylactic period conferred by 1.0 mg/kg body weight isometamidium was less than one month. In the work done in Ghibe valley, Ethiopia, the presence of multiple-drug resistant strains of *T. congolense* species was described (Codjia *et al.*, 1993). Moreover, recent field observations in Ethiopia, based on cloned populations, showed that the drug-resistant phenotype of *T. congolense* had not altered over a period of four years (Wubet Mulugeta *et al.*, 1997).

A similar study by Hassen Chaka and Getachew Abebe (2003) in mice and cattle confirm the results of the field study. Isometamidium chloride administered intraperitoneally at doses of 0.5 to 4 mg/kg body weight and diminazene aceturate at doses of 3.5 to 28 mg/kg body weight failed completely to cure mice infected with the two different *T. congolense* field isolates. Since both drugs failed to cure the infections in all doses tested, the minimum curative dose for each of the isolates could not be determined. However, from the results obtained the minimum curative dose value for the

two isolates appeared to be greater than 4 mg/kg body weight of isometamidium chloride and greater than 28 mg/kg body weight of diminazene aceturate. It is not known whether the double resistance phenotype of these stocks is because they contained at least two distinct populations or because the stocks contained parasites that expressed resistance to both drugs. If the later were the case, then the use of sanative pairs would be ineffective to treat such infections. Yohannes Afewerk *et al.* (2000) confirmed that three clones that were derived from an isolate, expressed high level of resistance to both isometamidium and diminazene when tested in mice.

The epidemiology of drug resistant populations of trypanosomes is dynamic; once established the incidence progressively spreads within the population. For instance, the incidence of recurrent infection in the Ghibe valley of Ethiopia was 7% in 1986 and it increased to 14% in 1989 (Rowlands *et al.*, 1993). Transmission by tsetse flies does not appear to affect the drug sensitivity of trypanosomes and drug resistant strains remain resistant after passage through tsetse flies (Moloo and Kutuza, 1990). The long-term occurrence of *T. congolense* resistance to diminazene, isometamidium and homidium in the Ghibe valley of Ethiopia (Wubet Mulugeta *et al.* 1997) indicated the magnitude of the problem once drug resistance was established in a herd. The resistance trait is known to be stable for a long time and such stocks can spread to wider areas through animal movement and/or the spread of tsetse populations.

Integrated Control of Trypanosomosis

In view of the facts that trypanosomosis has proved difficult to eradicate and the risk is compounded by emergence of drug-resistant strains of trypanosomes, it is therefore imperative to have a new and integrated approach in the control of tsetse and trypanosomosis so as to reclaim the tsetse infested lands of Africa (Holmes, 1997). There are three levels at which integrated control of tsetse and trypanosomosis can be addressed: integration with rural development, integration with other disease control measures (integrated disease management) and integration of various tsetse and trypanosomosis control measures. In Ethiopia, the emergence of multiple drug resistance has seriously hampered the control of animal trypanosomosis in the Abay-Didessa tsetse belt in Metekel district, North-west Ethiopia (Yohannes Afewerk *et al*, 2000; Nega Tewelde *et al.* 2004); Ghibe valley adjacent to the Didessa valley (Codjia *et al.* 1993; Rowlands *et al.*, 1993; Leak *et al.* 1993; Wubet Mulugeta *et al.*, 1997; Hassen Chaka and

Getachew Abebe, 2003), Omo valley (Mesfin Ademe and Getachew Abebe, 2001) Arbaminch area (Hassen Chaka and Getachew Abebe, 2003) and recently in the Abay (Blue Nile) river basin (Shimelis Dagnachew *et al.*, 2005). In areas of south-western Ethiopia, for example, where high level of trypanosome resistance to several drugs were found, the International Livestock Research Institute (ILRI) introduced experimental tsetse control programme to complement chemotherapy and this has had beneficial effects. The tsetse control programme has reduced the relative density of tsetse flies by over 90%, and reduced trypanosome prevalence in cattle by 70 % (Leak *et al.*, 1995).

The alternative tsetse control measures include method such as aerial and ground spraying with insecticides and deployment of targets, traps and screens. A particular concern with large-scale insecticide application is the pollution it may cause, as most insecticides are harmful to aquatic and terrestrial animals. If live bait animals are used without any other form of tsetse control, difficulties arise with persistence of flies in areas where the treated animals do not go. In case of sterile male technique, the effect on the population only becomes apparent after a period and a substantial fly suppression has to precede the application of SIT. Traps and screens may be stolen for the cloth they are made up of and during rainy season the rapidly growing vegetation may camouflage the trap or screen, which thus loses its visual activities for the flies (Uilenberg, 1998). Trypanotolerant cattle that stand up to challenge in a particular region may suffer from disease when introduced into another area, and so far, all attempts at developing a vaccine against trypanosomosis have failed (Uilenberg, 1998).

In general, all of the available methods have advantages and disadvantages and the various techniques act in a complementary way; an advantage of one may off set a disadvantage of another. The economic and feasibilities of employing various control methods must be compared for any given tsetse infested area. In Ethiopia, reducing the risk of trypanosomosis by employing more effective control methods may well increase both livestock and crop production. The use of vector and parasite control in an integrated package has effectively reduced the burden of tsetse and trypanosomosis in cattle in the Ghibe (Leak *et al.* 1995) and Didessa valleys (Feyesa Regassa, 2004).

One of the major components of sustainability of these methods is the active participation of the majority of the communities contributing to a relevant production system in a given environment or region. Successful strategies for controlling animal trypanosomosis must be based on accurate appraisals

of the impacts of the disease constraints on village farming system and the development of cost-effective sustainable disease control packages which can be adapted by producers.

CONCLUSION

Tsetse and Trypanosomosis have kept farmers and poor livestock keepers out of areas that have very high potential for agricultural development. The problem caused by tsetse and trypanosomosis is not only that of disease but also a significant negative impact on natural resource conservation and sustainable utilization. Increasing gap between population growth and food production creates an increasing pressure for utilization of new land and diversification of food resources. In Ethiopia, the emergence of multiple drug resistance has seriously hampered the control of animal trypanosomosis. The resistance trait is known to be stable for a long time and such stocks can spread to wider areas through animal movement and/or the spread of tsetse populations. Tsetse transmitted animal trypanosomosis is, therefore, one of the most significant and costly diseases in Ethiopia hindering the effort made for food self-sufficiency. It is therefore important to remove the burden of tsetse and trypanosomosis and make the infested areas of Ethiopia accessible for wise and sustainable land resource utilization.

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