Review on Tsetse Transmitted Bovine Trypanosomosis in Ethiopia

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Abstract: Trypanosomosis is a haemoprotozoan disease, mostly transmitted by the tsetse fly (Glossina spp.), which cause severe disease in humans and livestock in Sub-Saharan Africa (SAA). The disease results in loss of livestock and agricultural productivity with serious socio-economic consequences. In Ethiopia bovine trypanosomosis is widely distributed in western and southwestern part of the country. It is estimated that some 10 to 14 million heads of cattle in Ethiopia and an equivalent number of small ruminants together with a significant number of equines and camels are exposed to the risk of trypanosomosis. Six species of trypanosomes are recorded in Ethiopia and the most important trypanosomes in terms of economic loss are the tsetse transmitted species: *Trypanosome congolense, T. vivax and T. b. brucei*. Annual estimated losses for Ethiopia as a result of trypanosomosis is roughly $200 million, in terms of mortality and morbidity losses in livestock (excluding utilization of fertile land for crop and livestock production) and the costs included in controlling the disease. The pathogenesis of trypanosomosis depends on the pathogenicity of the strain; the host breed, genotype, age, sex, skin type.. Besides clinical diagnosis, parasitological, serological and molecular methods with varying degrees of sensitivity and specificity are available for the diagnosis trypanosomosis. Bovine trypanosomosis could be treated by diminazenaceturate, homidium bromide, homidium chloride, isometamidium and quinaypraminesulphate. However, trypanocidal drug resistance is increasingly reported all over Africa and is now present in 21 sub-Saharan countries including Ethiopia. Bovine trypanosomosis can be controlled by early treatment of infected animal and vector control. Thus, it is recommended that an appropriate use of antiprotozoal drugs, integrated prevention and control program should be implemented to reduce the impact of trypanosomosis.

Key words: Cattle · Epidemiology · Ethiopia · Trypanosome · Trypanosomosis · Tsetse fly

INTRODUCTION

Livestock is backbone of the socio economic system of most of the rural communities of Africa [1]. Ethiopia is known for its large and diverse livestock resource endowments. Livestock is primarily kept on small holdings where it provide drought power for crop production, manure for soil fertility and fuels, serves as a sources of family diet and sources of cash income (from livestock and livestock products). Despite large livestock population, Ethiopia fails to optimally utilize this resource due to different constrains facing the livestock subsector. Shortage of nutrition, reproductive insufficiency, management constraints and animal disease are the major constraints [2]. One of the diseases hampering the livestock subsector is trypanosomosis [3].

Trypanosomosis is a complex disease of protozoa that is caused by different species of unicellular parasites (trypanosome) found in the blood and other tissues of vertebrates include livestock, wild life and people [4]. Trypanosomosis limited to the extension of natural herds particularly in Africa were the presence of the tsetse fly density access to woodland and savanna areas with good grazing potential [3]. It is a serious constraint to agricultural production in extensive areas of the tsetse infested regions which accounts over 10 million square kilometers of the tropical Africa [5].

Ethiopia is one of the countries suffering from the impact of trypanosomosis. In Ethiopia, it is estimated that some 10 to 14 million heads of cattle and an equivalent number of small ruminants together with a significant number of equines and camels, are exposed to the risk of

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trypanosomosis [6]. 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.b. brucei* [3].

Tsetse flies in Ethiopia are confined to western and south-western parts of the country between 33° and 38° E longitude and 5° and 12° N latitude. It is estimated to cover an area of 140, 000 - 220, 000 km² [7]. Tsetse infested areas follow the major river systems namely Abay (Blue Nile), Baro, Akobo, Didessa, Ghibe and Omo river systems [8]. Five species of Glossina (*Glossina morsitans submorsitans, G. pallidipes, G. tachinoides, G.f. Fuscipes and G. longipennis)* have been recorded in Ethiopia [3]. According to National tsetse and Trypanomosis Investigation and Control Center[7], tsetse transmitted animal trypanosomosis still remain as one of the largest causes of livestock production losses in Ethiopia. The effects of trypanosomosis is not only the direct losses resulting from mortality, morbidity, infertility of the infested animals and costs of controlling the disease, but also due to indirect losses, which include exclusion of livestock and animal power based crop production from the huge fertile tsetse infected areas. Annual estimated losses for Ethiopia as a result of trypanosomosis is roughly $200 million, in terms of mortality and morbidity losses in livestock (excluding utilization of fertile land for crop and livestock production) and the costs included in controlling the disease [9]. Thus, the objectives of this review are:

- To review the epidemiological information and economic significance of bovine trypanosomosis with a particular emphasis to Ethiopia livestock sub-sector.
- To highlight the diagnostic techniques in African animal trypanosomosis.
- To discuss the most commonly used and effective control and preventive strategies against trypanosomosis.

Bovine Trypanosomosis

Etiology: African animal trypanosomosis (AAT) a disease is caused by trypanosomes, unicellular protozoan parasites of the phylum Sarcomastigophora, order Kinetoplastida, family Trypanosomatidae and genus Trypanosome [10]. *T. congolense, T. vivax and T.b.brucei* are the predominant trypanosome species in Ethiopia. *T. congolense* resides in the subgenus *Nannomonas*, a group of small trypanosomes with medium-sized marginal

kinetoplast, no free flagella and poorly developed undulating membranes. *T. vivax* is a member of the subgenus *Duttonella*, a group of trypanosomes with large terminal kinetoplast, distinct free flagella and inconspicuous undulating membrane, although this organism is considered to be less pathogenic for cattle than *T. congolense*. This trypanosome readily persists in areas free of tsetse, where it is transmitted mechanically by biting flies or contaminated needles, syringes and surgical instruments. *T.b. brucei* resides in the subgenus *Trypanozoon*. *T.b.brucei* is an extremely polymorphic trypanosome occurring as short, stumpy organisms without flagella, long slender organisms with distinct flagella and intermediate forms that are usually flagellated [3].

Life Cycle: The life cycle of trypanosome in tsetse involves cyclical development for varying length of time, depending on species and ambient temperatures. *T.vivax* completes its development cycle in the proboscis and pharynx. The cycle of *T.congolense* involves in the mid gut and proboscis and completed in about 2 week. That of *T.b.brucei* is more complex. It takes 3 or more weeks and involves in the mid gut and saliva glands [11].

Pathogenesis: The pathogenesis of trypanosomosis depends on the pathogenicity of the strain; the host breed, genotype, age, sex, skin type etc.; and most importantly, on the method by which the infection was induced i.e. natural or artificial [12]. The trypanosomes affect firstly the bite site or in other words the inoculation site in the animal skin causing a swelling and a chancre. The fly deposits during the blood sucking process the metacyclic proliferating trypanosomes in a limited number of metacyclic variant antigenic types. This stimulates the immune response causing the chancre. The chancre not only forms a site for the establishment of the infection but also is a focus for multiplication and persistence of trypanosomes before their dissemination into blood stream [1].

The parasites then after spread to the lymph nodes and blood then continue to replicate. *T.congolense* localizes in the endothelial cells of small blood vessels and capillaries. *T.b. brucei* and *T. vivax* localize in tissues like Lymph nodes [4]. Very acute infection with *T. vivax* in cattle causes parasitemias and disseminated intravascular coagulation (DIC) with hemorrhages. Such syndromes resemble a septicemia and anemia may not be severe. *T.b.brucei* and rarely *T. vivax* have the added capability of escaping from capillaries into interstitial tissues and
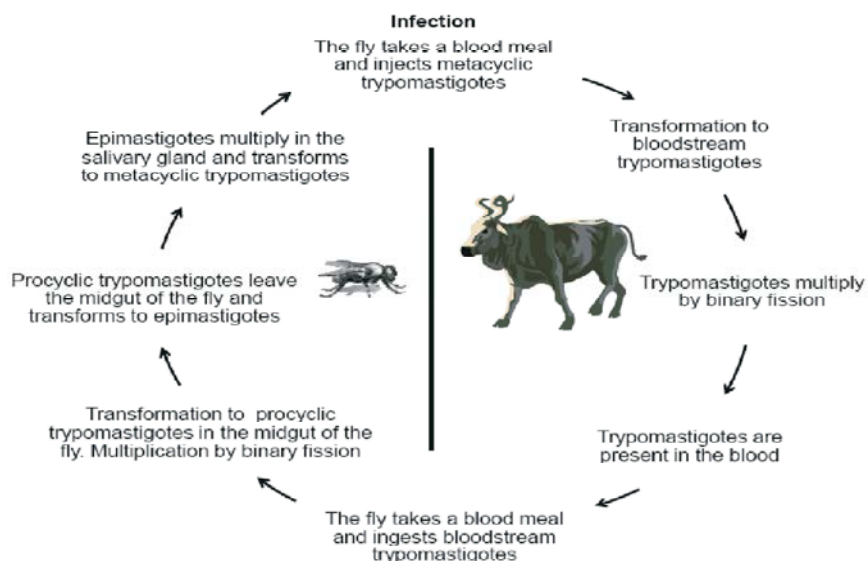
Fig. 1:   The main phases in the life cycle of the trypanosome, both in the intermediate host (Tsetse fly) and in the mammalian host. Source: [6].

serous cavities where they continue to multiply. The cerebrospinal fluid is often invaded by *T.b.brucei* alone or mixed with other species or as a relapse after an apparently successful treatment. Animals chronically infected with any pathogenic trypanosome may develop concurrent and even fatal bacterial, viral and other protozoan infections as a result of immune suppression. Trypanosomes can also pass through the placenta and into the fetus in pregnant animals, as a result some cows abort and still birth could also occur [13]. Generally, the pathogenesis of tsetse-transmitted trypanosomosis can be categorized into groups according to the site of host-parasite interaction as chancre, lymphadenopathy, anemia and tissue damage [4].

Epidemiology: The epidemiology of trypanosomosis is highly dependent on the parasite, vector and host factors. Trypanosome species occur in a variety of genotypes with different strains, virulence, immunogenicity and response to chemotherapeutic agent. Since the parasite infects a wide range of animals including wild animals which constitute the reservoirs of the disease, the epidemiology of trypanosomosis is extremely complex. The degree of risk to which domestic animals are exposed to the trypanosomosis depends on the species and density of tsetse present, infection rate in tsetse, species and strain of trypanosomes, source of infection (wild or domestic animals) and feeding preference of the flies [14].

Mode of Transmission: Trypanosomosis is a disease, which is cyclically and acyclically transmitted by different species of tsetse flies and other flies [15]. In principle, any species may occasionally be transmitted congenitally, contaminated syringe and surgical instrument [4]. Transmission by tsetse fly is a complex mechanism in which the fly remains lifelong carrier. In the vector trypanosome changes through several morphological distinct stages (amastigotes, promastigote and epimastigote) until it reaches trypomastigote (metacyclic stage) which is infective for mammals [16]. 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 for the mammalian host [17].

Distribution of Bovine Trypanosomosis in Ethiopia: Trypanosomosis is an important disease of livestock in Ethiopia. There are six pathogenic species of trypanosomes which are discovered in Ethiopia, namely T.vivax, T. congolense, T.b. brucei, T. evansi, T. equiperdum and T. rhodesiense. But the most important trypanosomes in the country are T. vivax and T.congelense. Both species affect a great number of cattle which are the most important species of the domestic animals in Ethiopia. Due to its extensive distribution, T. vivax is more important than T. congolense. Most of the above listed species of trypanosomes are limited in

distribution to Africa which is the home of the cyclical vector. But the mechanically and venereally transmitted trypanosomes have a cosmopolitan distribution [18].

***T. vivax*:** is found in the entire country except in the highlands, which are 2500 meters above sea level. The highlands include: the North Central and the Arsi-Bale Massifs, the Tigrean Plateau, the Showan Plateau, the South-Western Plateau and the Harar Plateau. The wide spread of *T. vivax* is due to its adaptation to mechanical transmission by biting flies in areas outside tsetse fly belt. The distributions of *T. congolense* and *T.b. brucei*, have been limited nearly to the area of the cyclical vector, the Ethiopian tsetse fly belt. This is due to the fact that both species of trypanosomes are not adapted to acyclical transmission. Therefore, the diseases which are caused by *T. congolense* and *T.b. brucei* are limited to southern and western administrative regions, that is Sidamo, Gamo Goffa, Keffa, Illubabor, Wollage,parts of Gojjam and Shoa [18].

Estimates made decades ago indicates that a total area of 220, 000 km^2 is infested with different species of tsetse flies in which case livestock reared in this area are exposed to various levels of trypanosomes risk. Very recent estimate indicate that, more than 140, 000 km^2 otherwise agriculturally suitable land in the western and southwestern parts of the country is found to be potentially suitable for tsetse. More than twelve million livestock population which are reared in this area are at risk of contracting tsetse-transmitted trypanosomosis in Ethiopia [19].

Risk Factor: *Host factor*-The effect of infection varies with the host in that most wild animal and some domestic ones, establish a balance with the parasite and remain as clinically normal carriers for long periods. Specifically, some breeds of cattle indigenous to Africa can tolerate light to moderate challenge with tsetse flies by limiting the multiplication of trypanosomes in their blood and by apparently warding off the infection, especially *T. vivax* [20]. This phenomenon is called trypanotolerance. It is both genetic and environmental in origin and the level of tolerance varies. Crossbreeds of indigenous Taurine and Zebu animals are also more tolerant than pure breed zebu. However, due to the uncertain genetic makeup of animals within these so-called breeds and crossbreeds, the level of trypanotolerance may also vary with individual animals within a given category and it can be overcome by heavy tsetse challenge, malnutrition, or other stress factors [21]. The Sheko breed classified as a humpless Short horn is

the only known breed of Taurine type in eastern Africa which exhibit trypanotolerance [22]. The breed is found in the Bench-Maji zone of southern Region in the south-western parts of Ethiopia [6].

Environmental Factor: The density of tsetse population in the area and the level of their contact with the host, will determine the level of infection. This is further influenced by the vectorial capacity of the fly and the availability of its preferred host, which may not be livestock. Trekking of cattle through tsetse-infested vegetation is a risk nomadic farmer's face from time to time and the risk is even greater where cattle routes converge, for example, at major bridges or watering holes [7]. Agricultural and industrial developments generally lead to a lowering of tsetse density by destroying its habitat, whereas the establishment of game or forest reserves provides large numbers of preferred hosts or a suitable habitat for tsetse, respectively. Herds located near such reserves are therefore at a higher risk [23].

The vector for trypanosomosis, the tsetse fly (Glossina spp), requires a habitat that strongly influenced by ecological and climatic features particularly rainfall, soil type, temperature and vegetation type. Fly larvae can die as a result of drying soils. Temperature extremes, particularly above 36 °C and below 10°C also lead to adult fly mortality through starvation and water loss via respiration. Moisture levels directly related to precipitation is also involved in fly mortality, though the exact mechanism is not clear [24].

Cumulative effects of long rainy season or dry season are thought to have been important in influencing advances and recession in tsetse population [25]. The effect of altitude on tsetse distribution is through its effect on climate, mainly temperature. As temperature fall with increasing altitude the geographic limitations of different species may be due to their inactivity in lower temperature [26]. Different species of tsetse flies require particular vegetation type that would provide an optimal condition for growth and survival and vegetation is also important that provides shelter for their hosts, all environmental factors that affects the tsetse fly indirectly affects the occurrence of trypanosomosis [25].

Pathogen Factor: Living and dead trypanosomes produce a number of biologically active substances which are involved in the causation of trypanosomosis. These include variant surface glycoproteins (VSG), enzymes, B-cell mitogen and T lymphocyte triggering factor (TLTF) [27]. Variant surface glycoproteins: In the mammalian

host, the whole parasite is covered with a glycoprotein coat of a single molecular species, called the variant surface glycoprotein (VSG). The surface coat of one trypanosome consists of about 10^7 VSG molecules [28]. It is the predominant surface antigen of African trypanosomes [29]. African trypanosomes undergo antigenic variation of their VSG coat to avoid immune system-mediated killing by their mammalian host. The VSG of trypanosomes is attached to the cell surface by means of a phosphatidylinositol containing glycolipid membrane anchor. The membrane form of the variant surface glycoprotein (mfVSG) of live trypanosomes can be transferred from the parasite plasma membrane to that of erythrocytes. This transfer of mfVSG may sensitize the erythrocyte cells to immune destruction (anti-VSG antibody-mediated complement lysis) and contribute to the development of anemia [30].

Trypanosome Enzymes: Trypanosome has many enzymes which used as pathogen factor, some of them are, sialidases, protease and phospholipases [31].

Sialidases: A membrane-bound sialidase have been reported in *T. congolense, T.b. brucei and T. vivax* [32]. Blood stream trypanosome sialidases cause red blood cell (RBC) surface alterations thus, leading to their subsequent phagocytosis [31].

Proteases: African trypanosomes contain proteases such as cysteine proteases and a serine oligopeptidase that may be released into the bloodstream of their infected hosts [33]. Peptidases are widely implicated as virulence factors and chemotherapeutic targets in trypanosome infections [34].

Phospholipases: Phospholipases and lysophospholipases are generated by autolysed trypanosomes. Phospholipases possess a variety of significant biological activities that are of relevance to the lesions observed in trypanosome infections [35].

B-Cell Mitogen: Trypanosomes produce a B-cell mitogen which, by allowing generalized differentiation and expansion of clones of B-cells, preempts the subsequent specific responses to new antigens. Serum-immunoglobulins, especially IgM, increase considerably in trypanosomosis. This immunoglobulin contains not only specific anti-parasite antibodies but also antibodies reacting with other non-parasite antigenic determinants, including those of host tissue. It is suggested that the immunoglobulin response seen in trypanosomosis is due in part to the production by trypanosomes of a non-specific B-cell mitogen. Stimulation of a non-specific and disordered immunoglobulin response could help the parasite to survive [36].

T lymphocyte Triggering Factor: Trypanosomes express a protein called T lymphocyte triggering factor (TLTF), which triggers CD8 (+), but not CD4 (+) T lymphocytes to Proliferate and to secrete IFN-gamma. TLTF is localized to small vesicles that are found primarily at or near the flagella pocket, the site of secretion in trypanosomes. TLTF is likely to be only the first example of a class of proteins that are designated as trypanokines, i.e., factors secreted by trypanosomes that modulate the cytokine network of the host immune system for the benefit of the parasite [37].

In cattle, *T. vivax* generally produces a higher level of parasitemia than other species. And since, its life cycle in the tsetse is also shorter*; T. vivax* is more readily transmitted than the others when animals are newly introduced into a tsetse infested area. Higher parasitemias also facilitate mechanical transmission. On the other hand*, T.b. brucei* is rarely detectable by direct examination of cattle blood, even though infection can be confirmed through other diagnostic methods [38].

Importance

Economic Importance: According to the Food and Agricultural Organization of the United Nations, trypanosomosis is probably the only disease which has profoundly affected the settlement and economic development of a major part of SSA of the approximately 7-10 million km^2 of land that are infested by tsetse fly, only 20 million cattle are raised. Under different circumstances, this land could support more than 140 million cattle and increase meat production by 1.5 million tons [39].

Trypanosomosis threatens 50 million head of cattle in SSA. Every year, trypanosomosis causes about 3 million deaths in cattle while approximately 35 million doses of trypanocidal drugs are administered to enable livestock to survive in tsetse-infested areas. While the economic losses in cattle production alone are in the range of US$1.0-1.2 billion, the indirect impact engendered by the disease on the total agriculture-livestock production is estimated at US$4.5 billion a year. The overall negative impact extends to the access and availability of cultivable areas, changes in land use and exploitation of natural resources, restriction of opportunities for diversification and intensification of agricultural activity. The magnitude

of the problem requires a multidisciplinary approach for effectively promoting sustainable agriculture and rural development strategies [40].

The disease directly affect the milk and meat productivity of animals, reduces birth rates, increases the abortion rates as well as mortality rate; all of these affect the herd size and herd composition [41].

Indirect impact of trypanosomosis mostly lies on crop production through the availability and cost of animals that provide traction power [42]. Animal trypanosomosis reduces work efficiency of oxen for cultivation, reducing access to animal traction or discourages the introduction of drought animals in to crop farming [43]. Evaluation on effect of trypanosomosis incidence on the productivity of oxen used for traction showed that relative inefficiency in the high risk area was 38% less efficient than oxen in the low risk area [44]. Additional traction capacity allows farmers expand the area that they cultivate, increase yield of existing crops; grow different mix of crops or allocated labour land and fertilizer more efficiently. In other study [45] discussed the economic benefits from intervening against bovine trypanosomosis. These authors reported significant benefits especially for Ethiopia, because of its very high livestock densities and the importance of animal traction. The estimated maximum benefit per square kilometer of tsetse infested area is US$ 10,000. Consequently, the total maximum benefits from dealing with bovine trypanosomosis in Ethiopia could be as much as US$ 1 billion.

Zoonotic Importance: The animal pathogens do not infect humans, but animals can serve as reservoirs *of T. brucei rhodesiense and T. brucei gambiense*, the causes of human sleeping sickness, which are morphologically indistinguishable from *T. brucei brucei*. Human infections result from tsetse bites, generally in game parks, forest reserves and along streams or other rural setting [46]. There is no report of sleeping sickness cases from Ethiopia since 2000 [47].

Clinical Finding: There are no pathognomonic signs that would help in pinpointing a diagnosis. The general clinical picture is as follows but there are many variations determined by the level of tsetse challenge, the species and strain of the trypanosome and the breed and management of the host. Acute episodes last for a few days to a few weeks from which the animal dies or lapses in to a sub-acute to chronic stage, or the illness may be chronic from the beginning. Chronic cases may run a steady course, may be interrupted by periodic incidents of severe illness, or undergo spontaneous recovery [48].

The basic clinical syndrome appears after an incubation period of 8-20 days. There is fever, which is likely to be intermittent and to last for a long period. Affected animals are dull, anorexia and apathetic, have a watery ocular discharge and lose condition. Superficial lymph nodes become visibly swollen, mucous membranes are pale, diarrhea occasionally occurs and some animals have edema of the throat and underline. Estrus cycles become irregular, pregnant animals may abort and semen quality progressively deteriorates. The animal becomes very emaciated, cachectic and dies within 2-4 months or longer. Thin, rough-coated, anemic, lethargic cattle with generalized lymph node enlargement are said to have 'fly-struck' appearance. Furthermore, intercurrent bacterial, viral, or other parasitic infections may mask or complicate the basic clinical syndrome. Immune response to bacterial and some viral, vaccines is also depressed but is restored if trypanocidal therapy is given at the time of vaccination [49].

Diagnosis: Diagnosis of Trypanosomosis in tsetse, humans or domestic livestock is a basic requirement for epidemiological studies as well as for planning and implementing chemotherapy and for monitoring vector control operations. Accurate diagnosis of trypanosome infection in livestock is required for a proper appreciation of the epidemiology of the disease in any geographical locality. Besides clinical diagnosis, parasitological, serological and molecular methods with varying degrees of sensitivity and specificity are available for the diagnosis trypanosomosis [50].

Clinical Diagnosis: Severity of disease varies with species and age of the animal infected and the species of trypanosome involved. *T. congolense* and *T. vivax* are highly pathogenic for cattle and *T.b. brucei* infections are generally regarded as being of low pathogenicity. The primary clinical signs are intermittent fever, anaemia and weight loss. Cattle usually have a chronic course with high mortality, especially if there is poor nutrition or other stress factors [51]. Clinical diagnosis was found to have a good sensitivity (78%) but a low specificity (27%) when compared to parasitological tests. Under field conditions, in the absence of simple and portable diagnostic tools or access to laboratory facilities, veterinarians could rely on clinical diagnosis to screen and treat cases of bovine trypanosomosis [52].

Parasitological diagnosis- Wet blood film: These are made by placing a drop of blood on a microscope slide and covering with a cover-slip. The blood is examined microscopically using a 40x objective lens. Approximately 50-100 fields are examined. Trypanosomes can be recognized by their movement among the RBC. The method is simple, inexpensive and gives immediate results. Depending on the trypanosome size and movements a presumptive diagnosis can be made of the trypanosome species [53]. Final confirmation of the species is made by the examination of the stained preparation. The diagnostic sensitivity of the method is generally low, but depends on the examiner's experience and the level of parasitaemia. Sensitivity can be improved significantly by lysing the RBCs before examination using a haemolytic agent such as sodium dodecyl sulfate [54].

Thick blood smear: The method is simple and relatively inexpensive, but results are delayed because of the staining process. Trypanosomes are easily recognized by their general morphology, but may be damaged during the staining process. This may make it difficult to identify the species [55].

Thin blood smear: Usually, both a thin and thick smear is made from the same sample. Thick smears contain more blood than thin smears and, hence, have a higher diagnostic sensitivity. Thin smears on the other hand allow trypanosome species identification. Trypanosome species can be identified by the following morphological characteristics [56].

Criteria of the Office International Des Epizooties
T. vivax: 20-27 ìm long, undulating membrane is not obvious, free flagellum present at the anterior end, posterior end rounded, kinetoplast large and terminal [23].

T.b. brucei is a polymorphic trypanosome species. Two distinctly different forms can be distinguished, i.e. a long slender form and a short stumpy form. Often, intermediate forms, possessing characteristics of both the slender and stumpy forms, are observed. The cytoplasm often contains basophilic granules in stained specimens [23].

T.b. brucei (long slender form): 17-30 ìm long and about 2.8 ìm wide, undulating membrane is conspicuous, free flagellum present at the anterior end, posterior end pointed kinetoplast small and sub terminal. *T.b. brucei* (short stumpy form): 17-22 ìm long and about 3.5 ìm wide, undulating membrane is conspicuous, free flagellum absent, posterior end pointed kinetoplast small and sub terminal [23].

T.congolense: 8-25 ìm (small species), undulating membrane not obvious, free flagellum absent, posterior end rounded, kinetoplast is medium sized and terminal, often laterally positioned. Although *T. congolense* is considered to be monomorphus, a degree of morphological variation is sometimes observed [56].

Haemotocrit Centrifugation: In the mild clinical or sub clinical cases (carriers) with low parasitaemia in which it is difficult to demonstrate the parasites concentration methods become necessary. Blood is collected (70 ìl) into heparinised capillary tubes (75 x 1.5 mm), which are then sealed at the dry end and centrifuged, sealed end down. Then capillary tube is placed under microscope and the buffy coat junction where the trypanosomes will be concentrated is checked for trypanosomes. The buffy coat can also be placed on a slide and checked under dark field microscope [57]

Serological diagnosis- Indirect Enzyme-Linked Immunosorbent Assay (ELISA): The binding of anti-trypanosome antibodies to the antigen is shown by a conjugate of antibovine (if the test serum is bovine) immunoglobulin's labeled with an enzyme, which can be visualized by adding an appropriate chromogenic substrate (i.e. the interaction between enzyme and substrate will create a color). Usually solubilized antigens obtained from disrupted trypanosomes (successive freezing and thawing cycles or ultrasound) are used and the soluble antigens are coated in the wells of microtrays. Each microtray contains usually 96 wells. This makes it possible to process many sera at the same time, using multichannel pipettes. Only small quantities of sera and conjugate are used [58].

An ELISA reading instrument will quickly give the optical density of each well (showing quantitatively the intensity of the interaction between the enzyme and the substrate), thus helping to speed up the processing of large numbers of sera. Various ELISA systems have been constructed exploiting different reagents for detection of antibodies, but still require laboratory and field validation studies to be further assessed for their capacity to improve diagnosis of African trypanosomiasis [38].

Recently, the ability to use mitochondrial heat shock protein 70 (MTP) of *T. congolense* as a diagnostic antigen was examined by [59] and with encouraging results, but the technique still needs to be further validated and evaluated for natural infections in cattle [58].

Indirect Fluorescent Antibody Test: The test is used to detect trypanosomes antibodies. It has proven to be sensitive test but it has the disadvantage of that it can only be carried out in laboratories and the procedure is rather long and complicated as well as some extent subjective (i.e. titration, but different operators may give somewhat different results) [4].

Antigen-Detecting Tests: These tests have been developed for the detection of circulating trypanosome antigens, but are not reliable [60; 61].

Consideration of why the Ag-ELISA fails to detect trypanosome antigen(s) in serum samples is worthwhile and must take into account the following five factors; the reactivity of the reagents (number of available epitopes of the antigenic target), the specificity of the reagents, the nature of the test sample, e.g. the compartmentalization of trypanosomes between plasma, serum and red blood cells, possible interference by immune complexing; and the biology of the host/trypanosome relationship to gain an understanding of the fluctuations in trypanosomes in the systemic circulation [38]

Molecular Diagnosis: Polymerase chain reaction (PCR) provides tools for sensitive and specific diagnosis based on DNA sequence recognition and amplification. PCR permits identification of parasites at levels far below the detection limit of the commonly used parasitological techniques. PCR assays for trypanosome detection have been developed using species specific DNA hybridisation probes. This method requires either prior knowledge of the species to be found or the use of several probes for each sample to be tested [62].

Treatment: If detected early, trypanosomosis can be treated with trypanocidal drug. Therapeutic drugs for treatment includes: diminazenaceturate, quinapyraminesulphate, homidium bromide and homidium chloride. Prophylactic drugs for cattle include homidium bromide, homidium chloride and isometamidium [63].

Diminazeneaceturate: have remarkable curative properties. It is very active, stable and easy to use and have very low toxicity. These advantages make it a practical and risk free trypanocides. Diminazene solution can only be kept for two to three days. It is injected subcutaneously in cattle (slight local reaction is possible) or intramuscularly (very rapid absorption) at a dose of 3.5 mg/kg live weight for treating *T. vivax and T. congolense* infections. Infections due to *T.b. brucei* can be treated in cattle with the dose of 7mg/kg.

Diminazene derivatives bind to DNA and interfere with parasite replications. This class of drugs has tendency to accumulate in tissue, therefore half-life is very long, which may lead to residual problems in food producing animals [51].

Quinapyramin Sulphate: Quinapyramine methylsulphate is sold in the form of white powder that dissolves easily in water. It is prescribed as a curative drug for cattle and small ruminants and is given subcutaneously as a 10% aqueous solution at dose 5mg/kg. It was used in all the African countries, giving excellent result for cattle trypanosomosis (especially *T. congolense*); it was slightly less successful against *T. vivax*. It causes appreciable systematic reactions and intramuscular injection cause painful local reactions leading to discomfort or lameness. Trypanosomes resistant to this compound should be treated with dimenazene [10].

Homidium: Homidium salts are effective against *T. vivax* infections in cattle, but less against *T. congolense* and *T. b. brucei*. Their limited and protective activity in cattle depends on severity challenge and may last three to five weeks. Homidium resistant trypanosome can be controlled by diminazene or isometadium [52].

Isometamidium: Isometamidium is a phenanthridine aromatic amidine with a narrow therapeutic index which has been marketed for both a prophylactic and therapeutic trypanocidal drug. Isometamidium chloride is used as curatively at lower dosage rates and prophylactically at higher dosage rates. It is usually prepared as red powder easily soluble in water. It is used in a one or two percent aqueous solution and administered by deep intramuscular injection at the rate of 0.25-1mg/kg, depend on drugs resistant risk. Strain of trypanosomes resistant to isometamidium and other phenanthridine appear frequently, but they remain susceptible to diminazeneaceturate. It is given to the animal at dose rate of 0.51mg/kg and it will be protected for two to four months depending on the extent infections risk [63]

Trypanocidal drug resistance is increasingly reported all over Africa and is now present in 21 sub-Saharan countries including Ethiopia [64]. Drug resistance is the heritable loss of sensitivity of a micro-organism to a drug to which it was before sensitive. The exposure of trypanosomes to sub therapeutic concentrations of trypanocidal drugs, the treatment frequency and the degree of drug exposure of the parasite population are important factors influencing the development of drug resistance [65].
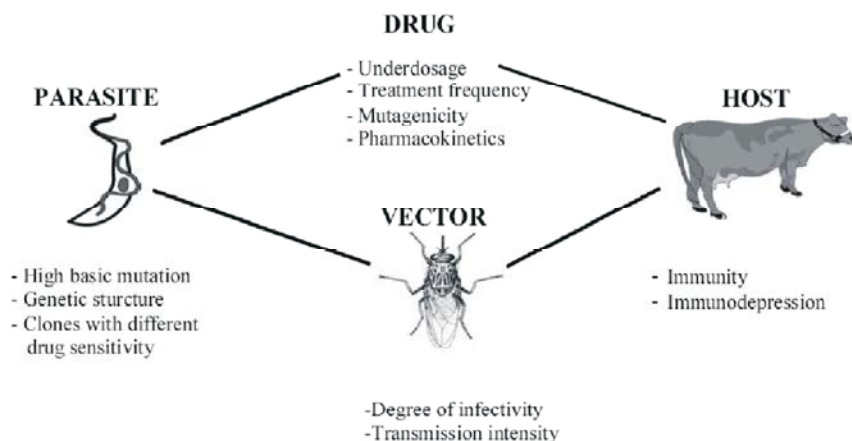
Fig. 1: Factors influencing the development of drug resistance.
Source: [65].

Control and Prevention: The control of trypanosomosis in enzootic countries involves control of tsetse fly population, prophylactic treatment, good husbandry of animals at risk and use of trypanotolerant animals. Control of tsetse has been successfully attempted, but reinvasion is frequent if the land is not properly utilized. The earliest methods involved bush clearing and elimination of game animals on which tsetse feed. More recent methods involved the use of insecticides applied strategically in the form of ground and aerial spraying over large expanses of land [66].

As tsetses are sensitive to insecticides and no resistance has developed, considerable successes were achieved in some countries. However, spraying insecticides is costly and harmful to the environment. These harmful effects are considerably reduced if the insecticides, for example, synthetic pyrethroids, are applied directly on the animal in the form of spray or pour-on formulation. Other effective methods involve targets impregnated with insecticides and traps that attract and catch tsetse. These are simple and cheap and can be constructed and maintained by local communities. Furthermore, they do not pollute the environment and are suitable for both small and large-scale fanning [67].

Another method is the sterile male technique. Since the female tsetse only mates once in a life time, this technique is theoretically able to eradicate a targeted tsetse species in areas where other methods have been used to reduce its density but it is expensive. Finally, it should be stated that development of the land for agriculture, industries, highways, etc. will effectively destroy the habitat for tsetse flies [48].

Attempts at trypanosomosis control have also been directed to prophylactic dosing with chemicals such as homidium bromide, homidium chloride and isometamidium. Prophylaxis is used along with other methods in areas where there is a heavy tsetse challenge. The prophylactic effect is supplemented by the development of antibodies and the total period of protection may be as long as 5 months. However, it is customary to give four or five treatments per year [60]. The productivity response to this pattern of treatment is good if general husbandry is also adequate. The downside of this approach is that it has reportedly led to drug resistance in many countries. In the absence of a vaccine, control methods must combine reduced exposure to the vectors (large scale tsetse trapping and pour-on applications) with strategic treatment of exposed animals (chemotherapy Chemoprophylaxis) along with use of trypanotolerant animals when feasible [48].

CONCLUSIONS AND RECOMMENDATION

In Ethiopia, bovine trypanosomosis is the main constraint of livestock production and rural development. The disease resulted in serious economic losses specially western and southwestern parts of Ethiopia posing a significant impact on the country development. Handfuls of options are available for the diagnosis of bovine trypanosomosis; however, in Ethiopian practical situation the diagnosis of bovine trypanosomosis is mainly relies on the less sensitive parasitological diagnosis techniques. Earlier tsetse and trypanosomosis strategies relied bush clearing and elimination of game animals on which tsetse feed. These methods are environmentally unfriendly and less effective. The current initiatives to control trypanosomosis are mainly based on tsetse fly control (area-wide integrated pest management). The sterile insect

technique have been practiced in Ethiopia for the last 10 or more years and resulted in significant tsetse fly reduction where it have been applied. Bovine trypanosomosis can be treated by both the prophylactic and curative drugs. The extensive and uncontrolled use of trypanocidal drug in tsetse infested areas resulted in trypanocidal drug resistance. Trypanocidal drug resistance is reported from different African countries including Ethiopia. Based on this conclusion, the following recommendations are forwarded.

- ▸ Integrated control strategy, proper management (restriction of pasture grazing in the tsetse belt), vector control (control of tsetse fly) and treatment of the infected animal should be practiced in tsetse infected areas to reduce the economic impact of bovine trypanosomosis.
- ▸ The government and concerned animal health professionals should monitor the use of trypanocidal drugs to avoid further drug resistances.
- ▸ Government and other non-governmental originations should provide financial support for researches on new and alternative drugs.
- ▸ Restriction of cattle movement from an infected area to the disease free area and vice versal to prevent and control of further expansion of bovine trypanosomosis.

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