Acta Parasitologica Globalis 8 (1): 39-49, 2017
ISSN 2079-2018

DOI: 10.5829/idosi.apg.2017.39.49

# Review on Characterization of *Trypanosoma congolense*; A Major Parasite of Cattle in Africa

*[1]Ayele Abrham, [1]Dagnachew Shemeles, [2]Adugna Takele and [3]Dessie Berhanu*

[1]Department of Paraclinicalstudies, Faculty of Veterinary Medicine, University of Gondar, Ethiopia
[2]Department Veterinary Clinical Medicine, Faculty of Veterinary Medicine, University of Gondar, Ethiopia
[3]Faculty of Veterinary Medicine, University of Gondar, Ethiopia

**Abstract:** Trypanosomosis is a protozoan disease caused by the genus *Trypanosoma* affecting animals and human mainly in sub-Saharan Africa and also in Latin America. *Trypanosoma congolense* (*T. congolense*) is one of the important pathogen of livestock in Africa transmitted cyclically by tsetse flies. The disease results in clinical syndromes such as anemia, emaciation and mortality. *T. congolense* is a complex species comprising three distinct genotypic types: (1) the savannah-type, (2) the West African riverine/forest-type and (3) the kilifi-type. Each genotype of T. congolense contains different strains of *Trypanosoma*. The ability of trypanosomes to change their surface-coat-antigen continuously leads to the exhaustion of the antibody production by the host leading to immune suppression. *T. congolense* infection can be diagnosed by clinical, parasitological, immunological and molecular methods. Control of the disease should combine treatment of infected animals and vector control. Even though, trypanocidal drugs will continue to play an important role in the integrated control of trypanosomosis, the development of trypanosome resistance to trypanocides is a continuous threat to their sustainable use. The available information on the molecular characteristics of different strains and genetic composition of *T. congolense* in relation to its pathology in different species of hosts is not yet elucidated in Ethiopia and hence requires thorough investigations.

**Key words:** Molecular Characteristics · *Trypanosoma congolense* · Pathogen · Africa · Ethiopia

## INTRODUCTION

Trypanosomosis is a worldwide disease caused by the species of the genus *Trypanosoma*, which affects humans, as well as domestic and wild animals. This wasting disease is spread by the bite of the infamous tsetse (*Glossina*). Tsetse flies inhabit 8.7 million km$^2$ of sub-Saharan Africa known as the "tsetse belt" [1]. This area represents approximately a third of Africa, or to put it into perspective, an area greater than that of the entire Australian continent. In the areas where tsetse is prevalent, agricultural output is suboptimal because of the risk of African animal trypanosomosis (AAT). Every year approximately 40 million cattle are threatened and 3 million are killed by trypanosomosis. The economic loss resulting directly from animal death is in the range of US$ 1.0–1.2 billion annually. When secondary losses such as reduced manure and draft power and thus decreased crop yields are included, the total gross domestic product lost can be as much as $4.5 billion per annum [1].

AAT is a collection of symptomatically similar diseases caused by a number of different trypanosome species. In cattle, the most widespread and virulent of these species is *T. congolense* [2]. *T. congolense* is an animal pathogenic trypanosome species which discovered in 1904 by Broden, next to *T. brucei (*in 1895 by Bruce) but before *T. vivax (*in 1905 by Ziemann) [3]. Although this species of trypanosome is the smallest of the trypanosome, it remains the most pathogenic to animals [4]. A wide range of domestic animals such as cattle, horses, camels, donkeys, mules, water buffalo, pigs, goats and dogs are victim to trypanosome infection [5]. The main pathological symptoms of animal trypanosomosis are weight loss, anaemia and immunosuppression; but the mechanisms involved are poorly understood [6].

Parasitemia in all strains of *T. congolense* infection is developed between 7 and 11 days post-infection (dpi). The savannah-type causes consistently higher levels of parasitaemia and lower packed red cell volume percentages and leukocyte counts than the other two

**Corresponding Authore:** Abrham Ayele, P.O. Box; 196; University of Gondar, Gondar, Ethiopia.
Cell: +251946236928, E-mail: abrhamts21@gmail.com.

types. The syndrome was also more severe in the savannah-type and led inexorably to death between 29 and 54 dpi while animals with the forest or the kilifi-types recovered from earlier symptoms and hematological alterations after 3 months of infection [7]. The parasite's life cycle have: bloodstream forms (BSF) proliferate in the blood of the infected mammalian host and are ingested by tsetse during the blood meal, procyclic forms (PCF) differentiate in the insect midgut, migrate to the proboscis (mouth parts) where they attach as epimastigote forms (EMF) and finally differentiate into infective metacyclic forms (MCF) that are transmitted to a new mammalian host during the next blood meal. *T. congolense* is a strictly intravascular parasite and the whole life cycle of it can be reproduced in vitro cultivation of all the developmental stages accomplishing all the differentiation steps [8, 9]. In Ethiopia *T. congolense* is one of the most important trypanosomes species, in terms of economic loss in domestic livestock (which include: *T. congolense*, *T. vivax, T. b. brucei, T. equiperdum* and *T. evansi)* [9]. In tsetse-infested areas of Ethiopia, *T. congolense* and *T. vivax* are the dominant species causing the exhaustive disease, trypanosomosis, problem and the disease is fatal if untreated [10].

This shows that *T. congolense* strongly hinders the agricultural development of sub-Saharan regions including Ethiopia and it has complex characteristics that should be studied intensively to cope up its huge burden and economy crisis. An understanding of its characteristics such as the ways of its complex life cycle, the virulence mechanisms and methods of detection and subsequent protection mode of the parasite is essential in order to block the parasite and the associated pathogenesis.

Control of the disease should combine restricted movement of diseased animals, treatment of *T. congolense* infected animals, epidemiological monitoring of the distribution and severity of the disease and vector control [10]. Trypanocidal drugs will continue to play an important role in the integrated control of trypanosomosis. However, the development of trypanosome resistance to trypanocides is a continuous threat to their sustainable use in the control of trypanosomosis [12]. Therefore this paper reviews the current knowledge and gaps about *T. congolense* epidemiology, genetic diversity, economic importance and the problems faced in controlling the disease.

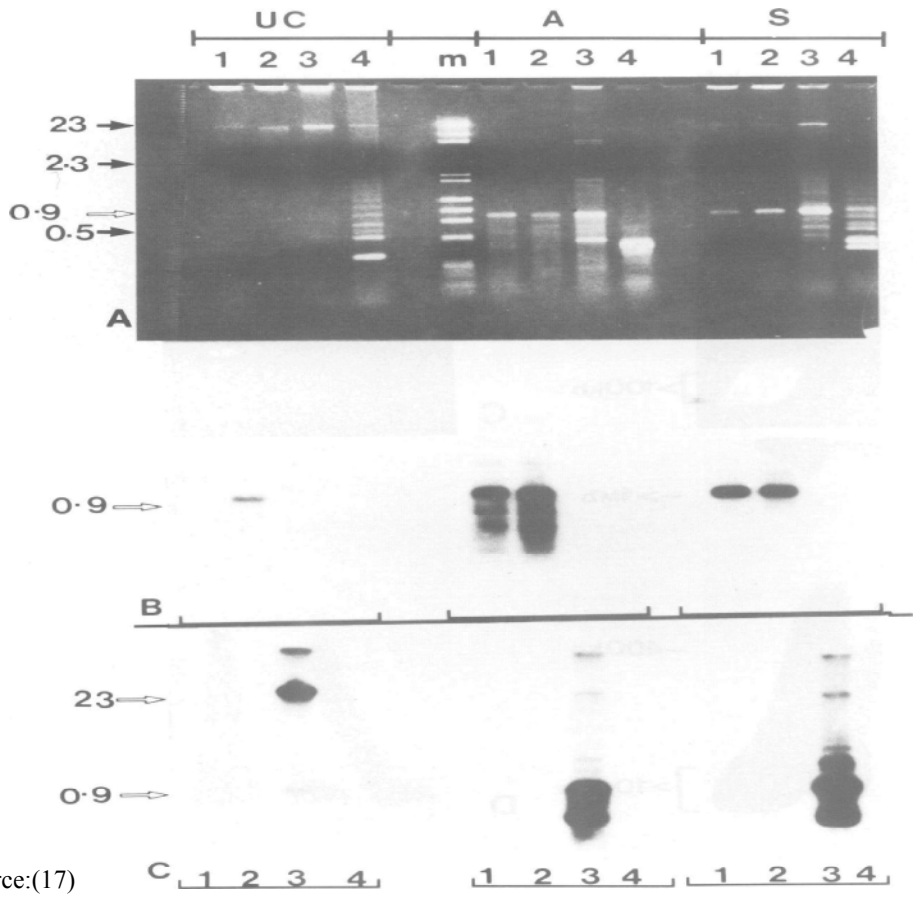
**General Accounts of *Trypanosoma congolense***

**Classification:** Trypanosomes have been classified into taxonomic groups based upon criteria including, morphology, development in the tsetse fly vector and preference for certain vertebrate hosts [13]. By these criteria, *T. congolense* has been proposed to comprise a collection of diverse organisms and not just a simple species within the subgenus Nannomonas. To simplify such diversity another methods of classification were needed. Cytological examination of karyotypes is an effective and reliable method by which many scientists are agreed and many eukaryotic cells can be classified into their respective taxonomic groups [14,15].

Trypanosomes do not condense their chromosomes into discrete structures [16]; thus it is not possible to examine karyotypes of these protozoa by classical cytochemical techniques. Majiwa[17] have found differences in molecular karyotypes of trypanosomes that belong to the species under the genus *T. congolense* by using electrophoretic techniques for the separation of chromosome-sized DNA molecules. The molecular karyotypes were found to be so different between the trypanosome clones from Kilifi isolates and those from elsewhere in East Africa.

The finding of differences in the molecular karyotypes analysis, repetitive DNA sequences and the kinetoplast DNA sequences suggest that there are some trypanosome populations that, on the basis of morphology, are classified together as *T. congolense* but which differ significantly from each other at the molecular level. Different isoenzyme based researches also confirm that there is no correlation between the isoenzyme patterns of the different groups of *T. congolense*. Isoenzymatic differences and molecular techniques recently resulted in a subdivision of the species in several "types''. They are *T. congolense* savannah type, *T. congolense* Tsavo type, *T. congolense* forest type, *T. congolense* Kilifi type (15). Supporting the finding of Majiwa[18], Makhosazana *et al.* [14] also revealed that *T. congolense* is a complex species comprising three distinct genotypic types: [1] the savannah-type [2] the West African riverine/forest-type and [3] the kilifi-type and each genotype of T. congolense, contains different styrains of trypanosoma.

Generally For the classification of trypanosomes as belonging to *T. (Nannomonas) congolense* subgenus, not only the criteria such asmorphometry, behaviour in the insect vector and host preference of the trypanosome have been used [13] but also different molecular methods like Cytological examination of karyotypes, electrophoretic techniques for the separation of chromosome-sized DNA molecules, repetitive DNA




Source:(17)

Fig. 1: Kinetoplast DNA (kDNA) from *T. (N.) congolense* ILNat 2.1, *T (N.) simiae* clone KETRI 243 1/1 and *T. (N.) congolense* ILNat 5.1 share no sequence homologies. Autoradiograms of these are shown in panels B and C respectively. Restriction enzymes used are A, AluI; S. Sau3AI. DNA fragment sizes (in kb) are given. UC indicates that the kinetoplast DNA networks were not treated with restriction endonucleases before gel fractionation. Blank arrows point to the 900 bp linear unit minicircle of *T. (N.) congolense* in panels A, B and C and to the position of 23 kb molecular weight marker in panel C (17).

sequences and the kinetoplast DNA sequences, different isoenzymes based researchs were used[15]. Thus by the accepted criteria, there are only two recognized species of trypanosomes within the subgenus Nannomonas, namely *Trypanosoma* (*N.*) *simiae* and *T. (N.) congolense*. They differ from each other in two fundamental ways: (i) *T. (N.) simiae* causes fatal disease in pigs, *T. (N.) congolense* does not; (ii) *T. (N.) simiae* does not grow in rodents, *T. (N.) congolense* does [13]. In addition to this Majiwa *et al.*[17] tried to test *T (N.) simiae* clones share any auto radiograms of *T. congolense.* Interestingly and significantly, there was neither gene sequence similarity nor cross-hybridization between kDNA from T. (N.) simiae and T. (N.) congolense even after 48 h exposure of the auto radiograms as shown in Figure 1.

**The Biology of *Trypanosoma congolense:*** A wide range of domestic animals such as cattle, horses, camels, donkeys, mules, water buffalo, pigs, goats and dogs are victim to trypanosome infection (5). According to Makhosazana *et al.,* (14) the severity and course of disease is different between the *T. congolense* types and strains. For example he proved that all of the three types of T. congolense, cause acute disease in inbred Balb/c mice but chronic in Clun sheep and large white pigs.

**Morphology:** *T. congolense* is the smallest of the pathogenic trypanosomes, with a length of 9–22 ìm [4]. The blood forms are monomorphic, in that they lack a *free flagellum* but there is a variation in size and shape between strains. Generally two variants are to be seen,

Table 1: Classification of *T. (N.) congolense*

| Classifications | | References |
|---|---|---|
| Kingdome | Protista | 13 |
| Phylum | Protozoa | 13 |
| Class | Zoomastigophora | 13 |
| Order | Kinetoplastida | 13 |
| Family | Trypanosomatidae | 13 |
| Section | Salivaria | 13 |
| Genus | *Trypanosoma* | 13 |
| Sub genus | Nannomonas | 13 |
| Species | *Trypanosomasimiae* | 17 |
| | *Trypanosomacongolense.* | 17 |
| Type | (1) the savannah-type | 15 |
| | (2)WestAfrican riverine/forest-type | |
| | (3) the kilifi-type | |

Source: Adapted from the above references

Table 1: Pathogenicity of salilvarian trypanosomes in livestock

| Trypanosome subgenus | Trypanosome species | Cattle | Goats | Sheep | Pigs | Horses | Donkeys |
|---|---|---|---|---|---|---|---|
| Trypanozoon | *T. brucei* | + | ++ | ++ | + | +++ | ++ |
| | *T. evansi* | ++ | + | + | ++ | +++ | ++ |
| | *T. equiperdum* | - | - | - | - | +++ | ++ |
| Nannomonas | *T. congolense* | +++ | ++ | ++ | + | ++ | ++ |
| | *T. simiae* | - | + | + | +++ | - | - |
| Duttonella | *T. vivax* | +++ | ++ | ++ | - | ++ | + |
| Pycnomonas | *T. suis* | - | - | - | ++ | - | - |

Source: [10].

+++: highly pathogenic

++: moderately pathogenic

+: low pathogenic

-: not pathogenic

a shorter form (9–18i), the typical *congolense* type and a longer form (up to 25i), with individuals intermediate in length between the two. The proportion of long and short forms varies in different cases and, it has been said, localities of origin. There is evidence which indicates that strains with the longest forms, the so-called "dimorphic" strains, cause a more severe form of trypanosomosis [19].

In stained specimens of *T. congolense* the cytoplasm stains a diffuse, even, pinkish colour and is seldom granular. The nucleus is centrally placed. The *kinetoplast* is of medium size and is usually situated at the margin of the body; just in front of the posterior extremity (*marginal* and *subterminal*). The *undulating membrane* is poorly developed and inconspicuous [19].

**Locomotion:** The parasitic unicellular trypanosomes prosper in the circulation of all vertebrate classes, because of adaptation to very different bloodstream conditions [20]. Trypanosomes move actively and progress by movement of the undulating membrane and the free flagellum which acts as a kind of propeller. *T. congolense* move by its inconspicuous undulating membrane since it has no free flagella. The pathogens can readily adjust the beating direction of their moving structure in response to purely mechanical cues in a fluid with in blood vessels moving at speeds that are 50–20,000 times faster than the trypanosome's swimming speed. In the blood they exploit the spacing and shape of blood cells for very efficient forward movement that is required for host antibody clearance. When the parasites get trapped with the extracellular matrix, they reverse the beating direction and consequently move backwards [20]. According to FAO [19], in fresh blood preparation *T. vivax*, moves rapidly forward between the blood cells, whereas other species including *T. congolense* often just wriggle around without showing much forward progress.

**The Diversity of *T. congolense* Population:** Epidemiological surveys in West Africa, based on cloned repetitive DNA sequences employing specific oligonucleotides primers and the polymerase chain reaction (PCR), indicated that the savannah-type was predominant in tsetse flies as well as in cattle while the riverine/forest-type was only present in the vectors [21].

De La Rocque [22] uses molecular methods for the identification of different types of *T. congolense*. *T. congolense* forest1/2(TCF1/2) and *T. congolense1/2* (TCS1/2) specific primers for *T. congolense* forest and *T. congolense* savannah respectively were used. Using genotype-specific primers, amplification products for Savannah and Kilifi [14] revealed 26 *T. congolense* isolates, of which 24 (92.3%) belonged to the Savannah and 2 (7.7%) were Kilifi sub-groups. All the strains gave a single molecular band profile following amplification with species-specific primer, confirming that there were no mixed infections. DNA of samples amplified with Savannah-specific primers could not be amplified when Kilifi, *T. brucei* and *T. vivax* specific primers were used. Moreover, when the primer sets for *T. vivax* and *T. brucei* were used, none of the samples were amplified except for the controls [14].

For the characterization of *T. congolense* forest, Gustave *et al*.[23] were used seven microsatellite markers. Of these seven markers, TCM3 showed no amplification for all *T. congolense* forest positive samples and TCM5 amplified only few samples. TCM3 amplified well the *T. congolense* savannah positive samples; thus confirming its capacity to differentiate savannah from *T. congolense* forest strains. For the five remaining markers considered in the study, the polymorphism generated at each locus varies from one marker to the other TCM1 and TCM6 appeared as the most polymorphic markers with each of them showing 9 different alleles. TCM4 was the less polymorphic marker with only three different alleles. For TCM2 and TCM7, 6 and 5 different alleles were observed, respectively. Whatever the microsatellite marker used in their study, the highest number of alleles were identified in pigs (30 alleles for the five markers). For instance, 9 alleles of TCM6 were identified in pigs while only one allele was identified in dogs for the same marker. The smallest number of alleles, whatever the microsatellite marker, was identified in dogs (8 alleles). In sheep and goats, 18 and 14 alleles were identified respectively Some specific alleles like 240 bp of TCM1, 156 and 180 of TCM4, 194 and 225 of TCM2 and 149, 189, 210 and 224 of TCM6 were identified only in pigs [23].

Gustave *et al*. [23 examined the level of diversity in the population of *T. congolense,* multilocus genotypes (MLGs). The MLGs were derived for each isolate from genotyping with the five microsatellite markers and these were used to construct a dendrogram of similarity, by calculating Nei genetic distance for all subsample pairs. A total of 22 distinct MLGs were identified for the 114 *T. congolense* forest positive samples. These results showed the presence of a predominant clonal reproduction of *T. congolense* forest, although the researcher also observed the presence of some variance across loci which come from differences of polymorphism (i.e. different mutation rates) between loci [23].

**Life Cycles and Reproduction:** *Trypanosoma congolense*, have a complex developmental cycle that involves two phases (in the mammalian hosts and in the tsetse vectors) and four developmental forms: the bloodstream forms, procyclic forms, epimastigote forms, metacyclic forms (BSF, PF EMF and MCF). In the mammalian host, bloodstream forms (BSF) are covered with a dense coat consisting of approximately 107 copies per cell of variant surface glycoproteins (VSG) that is famously involved in antigenic variation, allowing the trypanosome population to avoid elimination by the host immune system [25]. BSF bears a single large mitochondrion which almost entirely inactive during this stage of their life cycle. So it relies entirely on glycolysis and substrate level phosphorylation to meet their energy requirements. When BSF trypanosomes are consumed by a tsetse during its blood meal, they enter the insect mid gut and differentiate to procyclic forms (PF) that are adapted to life in the midgut [26]. Differentiation from BSF to PF is characterized by a switch to proline oxidation and oxidative phosphorylation for energy production. So un like BSF PF, use proline as their preferred energy source than glucose, which is absent or at very low levels in the tsetse vector. Once established in the fly, a small subset of the PF migrate from the mid gut and begin to differentiate, becoming non-motile epimastigote forms (EMF) that adhere to the fly's salivary glands (if *T. brucei*)or to the fly's proboscis and mouth parts (*T. congolese*). Finally, the dividing, adherent EMF differentiate into non-dividing, non-adherent, VSG-expressing and infectious metacyclic forms (MCF) and are injected into the host bloodstream when tsetse take a blood meal. The parasites multiply at the site of the fly bite for 5–9 days after which they invade the bloodstream and lymphatic system, becoming BSF and thus completing their life cycle [26].

Table 2: Unidentified *T. congolese* proteins

| No | Proteins | Specific character |
|---|---|---|
| 1 | TcIL3000.0.22490 | no hit at all |
| 2 | TcIL3000.0.28430 | a 17.9 fold increase when MCF differentiated to BSF and then a 14 fold drop when the BSF trans-formed to PCF. This protein deserves examination as it is so highly expressed in BSF. |
| 3 | TcIL3000.0.38630 | increased 8.5fold during differentiation from EMF to MCF, then decreased 1.6 fold in BSF and 6.7 fold in PCF; thus, it appears to be mainly expressed in meta cyclic forms with lowest expression in PCF. |
| 4 | TcIL3000.0.51090 | Highly expressed in PCF. It dropped 5.3 fold during differentiation from PCF to EMF, dropped 6 fold further in MCF and then raised 5.8 fold in BSF and 5.1 fold more in PCF. |
| 5 | TcIL3000.2.410 | No expression data were obtained |
| 6 | TcIL3000.7.3440 | Highly expressed in EMF (5.44-fold up regulated from PCF) than any other life cycle stage. |

Source;(25)

*T. congolense* is reproducing by binary fission and according to Lori [27], for Reproduction of trypanosome the exchange and recombination of genetic materials may take place in the tsetse fly between two trypanosomes, but it is unknown how frequently this occurs. The kinetoplast divides first. Then the second parabasal body develops, from which a second flagellum develops. The nucleus divides next, followed by the rest of the trypanosome body duplicating all the structures present in the cytoplasm. The body then divides into two daughter cells, beginning at the anterior end. The process is rapid and may result in a vast population in the host within a short period of time [27].

**Proteins Expressed in Life Cycle of *T. congolense***

**Surface Membrane Proteins:** Several of the *T. congolense* surface membrane proteins known to exhibit stage specific expression. In MCF and BSF trypanosomes that are infective for mammals, a total of 11 different variant surface glycoproteins (VSGs) were detected. In addition to VSG expression, other four proteins with unclear function were found by Marcoux *et al*. [28]. Another trypanosome surface protein reported in *T. congolense* is the major surface metallo protease (MSP), also called GP63 which has at least five MSP gene classes (TcoMSP-A to -E) [28]. Latter Brett and his colleges [25] identified six different proteins from *T. congolense* by isobaric tags for relative and absolute quantitation /iTRAQ/ analysis which showed no significant sequence similarity with proteins in the non-redundant database.

**Pathogenesis:** Makhosazana *et al.*[14] proved that neither of the two Kilifi strains was highly or caused moderately virulent as they both fell into the low virulence category. These findings confirm the observations made by Bengaly *et al.* [4] that Kilifi is non-pathogenic. There was no remission or self-cure following the infection in mice infected with the Savannah types, but where infections were by the Kilifi type, the course of the disease was brief and the infection cleared within 2 weeks and remained so throughout the experimental observation. This indicates that the strains were very mild or of non-virulent nature.

High virulence strains (HVS) killed mice rapidly once the parasitaemia was detectable in the blood compared to the mild virulence strains (MVS) and low virulence strains (LVS) categories [14]. This showed that Long Pre patent period (PPPs) and the height of parasitaemia peaks observed in the present study did not influence the outcome of the disease. Bengaly *et al.* [4] and Masumu *et al.* [29] also indicated that there was a shift in the survival time in the present study as a result of longer PPP. Their short PPP was accountable for shorter survival time, whilst, in our case, long PPP resulted in longer survival times. The genetic variation in trypanosomes of the same species from distinct geographic origin may influence the outcome of infection[30].

Biryomumaisho *et al*. [6] stated that anemia has long been established as a significant pathological feature and is a cardinal sign of trypanosomiasis. It is the consensus that the anemia is hemolytic in origin, occurring intra vascularly in the acute phase and also extra vascularly in the sub acute and chronic course of the disease. The cause of this anemia has not been well defined but haemodilution, bone marrow depression, erythro phagocytosis, haemolysis of erythrocytes either by hemolytic factors or by immunological means and reduced life span has been advanced as being responsible [6].

Observations have been made by Ojok(31). In acute bone marrow response (4–8 days post infection) to *T. congolense* infections in multi-mammate rats and he proved that the primary *T. congolense* and *T. brucei* infections caused the increscent of erythrogenesis.

But later, in chronic infection (16–60 days post infection), erythropoietic activity reduced while intra- and extra-vascular erythrophagocytotic activity increased [31]. Since the myloid to erythroid /M:E/ ratios obtained from the *T. congolense* group were higher than those from the *T. brucei* group and anemia was more severe in *T. congolense* infections in experiment held by Biryomumaisho *et al*. [32] it can be deduced that *T. congolense* is associated with more severe clinical pathological effects than *T. brucei* in goats[32].Shimelis [33] also conclude that *Trypanosoma congolense* is considered to be high virulent for cattle than *T. vivax*, mortality rate of over 50% can occur. Generally tissue damage, Biochemical changes (for example Serum proteins, lipids, Enzymes (AST, ALT and ALP)), disturbance of the total erythrocyte counts and the myeloid: Erythroid ratios are observed throughout the course of disease [31-33].

**Immunity:** The innate immunity followed by secondly, specific, immunity is a usual natural response not only to *T. congolense* infection but also to all other antigens. Cells of the macrophage provide the first line of host defense against infectious diseases and, with dendritic cells, modulate downstream events that impact on the development of acquired immunity [34].

In host defense against infection, macrophages play an important role through their ability to remove specific substances from the blood stream via various receptors, such as complement receptors, Fc-receptors, scavenger receptors and mannose receptors [35]. The control of parasitemia in African trypanosomiasis is mediated by at least two known mechanisms: (1) antibody-mediated phagocytosis [36] and (2) to a lesser degree, by antibody/complement-mediated lysis [37]. In addition to this as a third mechanism, Table *et al*.[36] and Kaushik *et al*.[38] stated that release of nitrogen monoxide (NO) by macrophages in vitro for T. brucei and T. congolense is trypanotoxic. Duleu *et al*. [39] also showed that NO has arole to prevent African trypanosomes to grow in vitro. The role of NO in vivo has been controversial. But it does contribute to control of T. congolense infections[40]. Wei *et al.* [41]also confirm that NO has a role in prevent T. congolense infections in vivo. Wenfa *et al.*[37] found that IgG2a anti-VSG antibody-mediated phagocytosis of T. congolense enhances the synthesis of NO by macrophages, whereas IgM anti-VSG antibody-mediated phagocytosis inhibited synthesis of NO. The ability of macrophages to produce NO upon phagocytosis of trypanosomes makes different species of animals to have different immune response ability against T. congolense infection [37].

Antigenic variation of the VSG and the induction of alterations in the hosts defense system, such as excessive activation of the complement system leading to persistent hypocomplementemia, down regulation of nitric oxide production, polyclonal B-lymphocyte activation and marked immunosuppression are the main mechanisms to survive in the chronically infected host [42]. This is because of available of yet undiscovered proteins in the pathogen, for example the six(6)new proteins in T. congolense by Brett [25].

By switching VSG genes and expressing a new variant antigenic type, trypanosomes evade B and T-cell mediated immune responses. Furthermore, expression of VSG is central to the process of antigenic variation that eventually leads to exhaustion of the host immune system for the benefit of the trypanosome [43, 44].VSG also has several effects on immune elements such as induction of auto antibodies and cytokines, in particular tumor necrosis factor (TNF)-á [40].

*T. congolense* and *T. b. brucei* have been documented to induce a generalized state of immune suppression following infection of cattle or mice [45]. The mechanisms of such immune depression seem to be mediated by both macrophages [46] and T cells [45] with suppressive phenotypes. The production of antibodies to the VSG of *T. congolense* is the major early immune response. The first antibody to the VSG is of immunoglobulin M (IgM) class and is produced independently of T cells [34]. Antibodies to the VSG are able to mediate control of the parasitaemia. O?Gorman *et al* recorded that at the peak of parasitaemia a type 2 helper T cell (TH2) like cytokines were prevalent in the trypanosusceptible Boran cattle with increases in transcripts for the IL6 and IL10 genes in *T. congolense* infection [47].

**Diagnosis and Identification of *T. congolense* Infection:** *T. congolense* infection can be diagnosed by clinical, parasitological, immunological and molecular methods [48].

**Molecular Techniques:** *Trypanosoma* DNA was characterised using universal primer pairs targeting the segment of the 18S ribosomal RNA gene of all trypanosomes [48]. It is possible to characterize the species of trypanosoma with the application of

Table 3: *T. congolense* drug resistance in Ethiopia

| No | Trypanosome species | Resistant to (*) | References |
|---|---|---|---|
| 1 | *T. congolense* | Isomethamedium Diminazineaceturate, Homidium bromide (Ethidium) | 53 |
| 2 | *T. congolense* | Isomethamedium Diminazineaceturate | 54 |
| 3 | *T. congolense* | Diminazineaceturate, Isomethamedium chloride | 55 |
| 4 | *T. congolense* | Isomethamedium Diminazineaceturate | 52 |

(Source: 57: adapted from 33).

oligonucleotide primers amplifying the satellite DNA monomers of *Trypanosoma brucei* [49], *T. congolense* Kilifi type, (eg. OVIKZNTT/7098/07) [50], *T. congolense* Savannah type (eg(IL1180)) (Majiwa&Otieno 1990) and *T. vivax* [50].

Molecular techniques based on nucleic acids and isoenzyme analysis gives more presice detection to *T. congolens*e diagnosis. The principle of molecular tests is the demonstration of the occurrence of sequences of nucleotides, which are specific for a trypanosome subgenus, species or even type or strain. 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 [33]. These tests are not only suitable for detecting parasites in the mammalian host, but also in the insect vector.

**Control and Treatment of Infection:** Prevention and control of tsetse-transmitted trypanosomosis depends on methods directed to the vectors, the host and the parasites including methods like vector control, vaccination and use of trypanotolerant breeds and treatment. Each of these approaches is useful but has important limitations, such as expense, environmental pollution and drug resistance [11]. There is trained in south Africa that aggressive spraying with DDT is successful at controlling the vector and subsequent spreading of *n'agana* and sleeping sickness, but Once infected, domestic and wild animals can remain so for life, providing a constant reservoir of the parasite [51].

**Trypanocidal Drug Resistance:** Now a days only small numbers of Trypanocidal drugs for *Trypanosoma* infection are available. For example in Ethiopia isomethamedium (ISM) and diminazineaceturate (DA) are the only alternative drugs for the problem. Maybe due to this narrow drug alternative *T. congolense* showed drug resistance in many countries according to many researcher findings as indicated in the table below. Trypanocidal resistance particularly against *T. congolense* infection is reported in the North West Ethiopia [52] Ghibe Valley (4).

**Conclusion and Recommendation:** *T. congolense* strongly hinders the agricultural development of the sub-Saharan regions including Ethiopia. The parasite has three types each of which bear many strains with a complex characteristics; such as dynamic coat proteins; The different proteins are appeared at different life stage of the parasite and at different time /for example when the parasites are exposed to animal immunity and treatment/. Some of these proteins are still undefined and their function is not well known. The life cycle of this parasite is so complex and very monotonous to understand easily. This makes the pathogen lucky to cope up against protection and treatment mechanisms from both the animal and the technicians. Based on the above conclusion the following recommendations are fore warded.

- Further studding of the different proteins characteristic and role intensively is mandatory for understanding the virulence mechanisms to animals.
- Innovation of new drug is must.
- A repeat understanding of its characteristics such as factors having a role to initiate change of surface protein, the ways of its complex life cycle, the different virulence mechanisms, methods of detection and subsequent protection mode of the parasite is essential in order to block the parasite and the associated pathogenesis.

## REFERENCES

1. James D., 2007. Programme Against African Trypanosomiasis / PAAT/: Tsetse and Trypanosomiasis Information. Bisamberg Austria Vol. 30, Part 2.
2. Mulugeta, D., B. Desta and H. Samuel, 2013. Trypanosome infection rate of *Glossina pallidipes* and trypanosomosis prevalence in cattle in Amaro Special District of Southern Ethiopia; College of Veterinary Medicine, Haramaya University, P. O. Box, 138, Dire Dawa, Ethiopia.

3. Steverding, D., 2008. The history of African trypanosomiasis. Review. Parasite &Vect 1, 3.
4. Bengaly, Z., I. Sidibe, R. Ganaba, M. Desquesnes, H. Boly and L. Sawadogo, 2002. Comparative pathogenicity of 3 genetically distinct types of Trypanosomacongolensein cattle: clinical observations and haematological changes. Veterinary Parasitology, 108: 1–19.
5. Stevens, J.R. and S. Brisse, 2004. Systematics of trypanosomes of medical and veterinary importance. The Trypanosomiasis, pp: 1-23.
6. Biryomumaisho, S., E. Katunguka-Rwakishaya and C. Rubaire-Akiiki, 2003. Serum biochemical changes in experimental Trypanosomacongolense and Trypanosomabrucei infection in Small East Africa goats. Vet. Arhiv., 73(3): 167-180.
7. Abebe, G., 2005. Trypanosomosis in Ethiopia. Ethiopian Journal of Biological Science, 4: 75-121.
8. Oberle, M., O.B. Almer, R. Brun and I. Roditi, 2010. Bottlenecks and the maintenance of minor genotypes during the life cycle of Trypanosomabrucei. PLoSPathog. 6:e1001023 10.1371/journal.ppat.1001023
9. Roditi, I. and M.J. Lehane, 2008. Interactions between trypanosomes and tsetse flies. Curr. Opin. Microbiol. 11: 345-351.
10. FAO (Food and Agriculture Organization of the United Nations) 2004. Production year book.FAO, Rome, Italy.
11. Ermiyas, A. and A. Getachew, 2001. Drug-resistant Trypanosomacongolense in naturally infected donkeys in north Omo Zone, southern Ethiopia.Veterinary Parasitology, 99(31): 261-271.
12. Delespaux, V., D. Geysen, P. Van den Bossche and S. Geerts, 2008. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol., 24: 236-242.
13. Hoare, C., 1972. The Trypanosomes of Mammals, a Zoological Monograph. Blackwell Scientific Publications, Oxford, England, pp: 1-749.
14. Makhosazana Y. Motloang, Justin Masumu, Ben J. Mans and Abdalla A. Latif, 2014. Virulence of Trypanosomacongolense strains isolated from cattle and African buffaloes (Synceruscaffer) in KwaZulu-Natal, South Africa Journal of Veterinary Research, 81, 1.
15. van den Bossche, P., W. Shumba and P. Makhambera, 2000. The distribution and epidemiology of bovine trypanosomosis in Malawi. Vet. Parasitol., 88: 163-176.
16. Vickerman, K. and T.M. Preston, 1970. J. Cell Sci., 6: 365-384.
17. Wahl, G.M., M. Stern and G.B. Stark, 1979. Proc. Natl. Acad. Sci. USA, 78: 3683-3687.
18. Majiwa, Phelix, A.O., Masake, A. Rachael, Nantulya, Vinand M. Hamerst, Raymond and Matthyssenst, Gaston, 1985. Trypanosoma (Nannomonas) congolense: identification of two karyotypic groups International Laboratory for Research on Animal Diseases (ILRAD),The EMBO Journal, 4(12): 3307-3313.
19. Majiwa, P.A.O., M. Mania, J.N. Waitumbi, S. Mihok and E. Zweygarth, 1993. Trypanosome () Nannomonascongolense molecular characterization of a new genotype from Tsavo, Kenya. Parasitology, 106: 151-162.
20. FAO, 2006. Corporate Document Repository. A field 13. Whitelaw, D.D., P.R. Gardiner and M. Murray, 1988.guide for the diagnosis, treatment and prevention of African animal trypanosomosis. Food and Agriculture Organization of the United Nations www.fao.org/DOCREP/2006
21. Heddergott, N., T. Krüger, Sujin, B. Babu, Ai., E. Wei, S. Stellamanns, Uppaluri, P. Thomas, S. Holger and E. Markus, 2012. Trypanosome Motion Represents an Adaptation to the Crowded Environment of the Vertebrate Bloodstream 8(11).
22. Heddergott, N., T. Krüger, S.B. Babu, A. Wei, E. Stellamanns, S. Uppaluri, *et al.*, 2012. Trypanosome Motion Represents an Adaptation to the Crowded Environment of the Vertebrate Bloodstream. PLoSPathog 8(11): e1003023. doi:10.1371/journal.ppat.1003023.
23. De La Rocque, S., Z. Bengaly, J.F. Michel, P. Solano, I. Sidibe and D. Cuisance, 1999. Importance des interfaces spatiales et temporelles entre les bovins et les glossinesdans la transmission de la trypanosomoseanimale in Afrique de l' Ouest. Rev. Elev. Med. Vet. Pays Trop., 52: 21-222.
24. GustaveSimo[a,], Pythagore Fogue Sobgwi[a], Guy Roger Njitchouang[b], FlobertNjiokou[b], Jules Roger Kuiate[a], Gerard Cuny[,c] Tazoacha Asonganyi, 2013. Identification and genetic characterization of Trypanosomacongolense in domestic animals of Fontem in the South-West region of Cameroon, Infection, Genetics and Evolution1., 8: 66-73.

25. Masake, R.A., P.A. Majiwa, S.K. Moloo, J.M. Makau, J.T. Njuguna, M. Maina, *et al*., 1984. Sensitive and specific detection of Trypanosomavivax using polymerase chain reaction', Experimental Parasitology, 85: 193-205.
26. Brett, A., Eyford, S. Tatsuya, S. Derek, L. Bianca, C. Hertz-Fowler, E. John, N. Donelson, R.J. Connor, P. Van den Bossche, 2011. African animal Trypanosomes. In JAW Coetzer, RC Tustin, Infection Diseases of Livestock, 2nd ed., Vol 1, Oxford University Press, Cape Town, pp: 251-296.
27. Roditi, I. and M.J. Lehane, 2008. Interactions between trypanosomes and tsetse flies. Curr. Opin. Microbiol., 11(4): 345-51.
28. Lori, P., C. Simon, F. Vanessa, B. Mick and G. Wendy, 2012. The life cycle of Trypanosoma (Nannomonas) congolense in the tsetse fly, 5: 109.
29. Marcoux, V., G. Wei, H. Tabel and H.J. Bull, 2010. Characterization of major surface protease homologues of Trypanosomacongolense. Journal of Biomedical. Biotechnology, 41: 57-81.
30. Masumu J., T. Marcotty, D. Geysen, S. Geerts, J. Vercruysse, P. Dorny and P. Van den Bossche, 2006. Comparison of the virulence of Trypanosoma congolense strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. Int. J. Parasitol., 36: 497-501.
31. Taylor, K. and E.M.L. Authié, 2004. 'Pathogenesis of animal trypanosomiasis', in I. Maudlin, P.H. Holmes & M.A. Miles (eds.), The Trypanosomaiases, pp: 331-353. CABI Publishing, Wallingford.
32. Ojok, L., I. Kaeufer-Weiss and E. Weiss, 2001. Bone marrow response to acute and chronic Trypanosomacongolense infection in multimammate rats (Mastomyscoucha). J. Comp. Pathol., 124 (2–3): 149-158.
33. Biryomumaisho, S. and E. Katunguka-Rwakishaya, 2007. The pathogenesis of anaemia in goats experimentally infected with Trypanosomacongolense or Trypanosomabrucei: Use of myeloid: erythroid ratio. Veterinary Parasitology, 143: 354-357.
34. Shimelis, D., B. Tsegaye, G. Terefe, G. Abebe, D. Barry, H. Ashenafi and B.M. Goddeeris, 2015. Prevalence of bovine trypanosomosis and assessment of trypanocidal drug utilization practices in tsetse infested and non-tsetse infested areas of Northwest Ethiopia. The Veterinary Journal (resubmitted as short communication).
35. Mansfield, J.M. and D.M. Paulnock, 2005. Regulation of innate and acquired immunity in African trypanosomiasis. Review article. Blackwell Publishing, Ltd. Parasite Immunol., 27: 361-371.
36. Linehan, SA., L. Martínez-Pomares and S. Gordon, 2000. Macrophage lectins in host defence. Microbes and Infection, 2(3): 279-288.
37. Tabel, H., R.S. Kaushik and J.E. Uzonna, 2000. Susceptibility and resistance to Trypanosomacongolense infections. Microbes and Infection, 2(13): 1619-1629.
38. Wenfa, Lu., Guojian,Wei., Wanling, Pan and Henry, Tabel, 2011. Trypanosomacongolense Infections: Induced Nitric Oxide Inhibits Parasite Growth *in vivo*; Journal of Parasitology Research, 10: 1-10.
39. Kaushik, R.S., J.E. Uzonna, J.R. Gordon and H. Tabel, 1999. Innate resistance to Trypanosomacongolense infections: differential production of nitric oxide by macrophages from susceptible BALB/c and resistant C57B1/6 mice. Experimental Parasitology, 92(2): 131-143.
40. Duleu, S., P. Vincendeau and P. Courtois, 2004. Mouse strain susceptibility to Trypanosome infection: an arginase-dependent effect. Journal of Immunology, 172(10): 6298-6303.
41. Magez, S., M. Radwanska, M. Drennan, L. Fick, T.N. Baral, F. Brombacher and P. De-Baetselier, 2006. Interferon-ã and nitric oxide in combination with antibodies are key protective host immune factors during Trypanosomacongolense Tc13 infections. Journal of Infectious Diseases, 193(11): 1575-1583.
42. Wei, G. and H. Tabel, 2008. Regulatory T cells prevent control of experimental African trypanosomiasis. Journal of Immunology, 180(4): 2514-2521. Guilliamset al., 2007.
43. Pays, E., 2006. The variant surface glycoprotein as a tool for adaptation in African trypanosomes. Microbes Infect., 8: 930-937.
44. Morrison, L.J., L. Marcello and R. McCulloch, 2009. Antigenic variation in the African trypanosome: molecular mechanisms and phenotypic complex. Cell Microbiol., 11: 1724-1734.
45. Namangala, B., P. De Baetselier and L. Brys, 2000. Attenuation of Trypanosomabruceiis associated with reduced immunosuppression and concomitant production of Th2 lymphokines. J. Infect. Dis., 181: 1110-1120.

46. Gomez-Rodriquez, J., B. Stijlemans and G. De Muylder, 2009. Identification of a parasitic modulatory protein triggering the development of suppressive M1 macrophages during African trypanosomiasis. J Infect Dis., 200: 1849-1860.
47. O?Gorman, G.M., S.D.E. Park, E.W. Hill, K.G. Meade, L.C. Mitchell, M. Agaba, J.P. Gibson, O. Hanotte, J. Naessens, S.J. Kemp and D.E. MacHugh, 2006. Cytokine mRNA profiling of peripheral blood mononuclear cells from trypanotolerant and trypanosusceptible cattle infected with Trypanosomacongolense. Physiol. Genomics, 28: 53-61.
48. Geysen, G., V. Delespaux and S. Geerts, 2003. 'PCR-RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of Trypanosoma species in cattle', Veterinary Parasitology, 110: 171-180.
49. Sloof, P., L. Bos, A.F.J.M. Konings, H.H. Menke, P. Borst, W.E. Gutteridge, *et al.,* 1983, 'Characterization of satellite DNA in Trypanosomabrucei and Trypanosomacruzi', Journal of Molecular Biology, 167: 1-21.
50. Masiga, K.D., J.A. Smyth, P. Hayes, J.T. Bromidge and W.C. Gibson, 1992. Sensitivity detection of trypanosomes in tsetse flies by DNA amplification, International Journal for Parasitology, 22: 909-918.
51. Gillingwater, K., M.V. Mamabolo and P.A. Majiwa, 2010. Prevalence of mixed Trypanosomacongolense infections in livestock and tsetse in KwaZulu-Natal, SouthAfrica. Journal of theSouth African Veterinary Association, 81(4): 219-223.
52. Shimelis, D., A.K. Sangwan and A. Getachew, 2008. Assessment of trypanocidal drug resistance in cattle of the Abay (Blue Nile) basin areas of Northwest Ethiopia. Ethiop. Vet. J., 12: 45-59.
53. Mulugeta, W., J.M. Wilkes, W. Mulatu, P.A.O. Majiwa, R. Masake and A.S. Peregrine, 1997. Long-term occurrence of T. congolense resistant to diminazene, Isometamidium and homidium in cattle at Ghibe, Ethiopia. Acta. Trop., 64: 205-217.
54. Afewerk, Y., P.H. Clausen, G. Abebe, G. Tilahu and D. Mehlitz, 2000. Multiple-drug resistant T. congolense populations in village cattle of Metekel district, north-west Ethiopia. Acta Tropica, 76: 231-238.
55. Moti, Y., R. Fikru, J. Van Den Abbeele, P. Büscher, P. Van den Bossche, L. Duchateau and V. Delespaux 2012. Ghibe river basin in Ethiopia: present situationoftrypanocidal drug resistance in Trypanosomacongolense using tests in mice and PCR-RFLP. Vet. Parasitol., 189: 197-203.
56. Tewelde, N., G. Abebe, M.C. Eisler, J.J. McDermott, M. Greiner, Y. Afework, M. Kyule, S. Munstermann, K.H. Zessin and P.H. Clausen, 2004. Application of field methods to assess isometamidium resistance of trypanosomes in cattle in western Ethiopia. Acta Trop., 90: 163-170.
57. Delespaux, V., D. Geysen, P. Van den Bossche and S. Geerts, 2008. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol., 24: 236-242.