Vector-Borne Diseases & Treatment

Chapter 2

Salient features of Trypanosoma congolense in African Animal Trypanosomiasis in the sub-Saharan Africa

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Abstract

Despite the multiple causes, contribution of *Trypanosoma congolense* in African Animal Trypanosomosis (AAT) in the sub-Saharan Africa is great. More than 80% of AAT and losses in domestic animals (cattle, goats, sheep, horses, pigs and dogs) in South, East and Central Africa are due to *T. congolense* infections. In the West, *T. congolense* remains one of the major causes of AAT in livestock. This chapter discusses the biology and disease caused by *T. congolense*, challenges and opportunities for control are highlighted.

Keywords: *Trypanosoma congolense*, African Animal Trypanosomosis

1. Introduction

African Animal trypanosomosis (AAT) or "nagana" is one of the vector-borne diseases that have impact on economic growth and a threat to food security in the sub-Saharan Africa. The disease is caused by salivarian trypanosomes mainly *Trypanosoma congolense*, *T. vivax* and the lesser extent *T. brucei brucei*. *T. evansi* and *T. equiperdum* are responsible for other forms of the disease, Surra and Dourine respectively. The disease is spread by the bite of infected tsetse flies (*Glossina species*). Its distribution in the sub-Saharan Africa corresponds to geographical boundaries of tsetse flies within the latitude 14^{o} N and 29^{o} S. The possibility of mechanical transmission by Tabanidae and Stomoxynae has enabled the disease to spread beyond the tsetse belt of sub-Saharan Africa [1,2]. *T. vivax* is now established cause of the disease in cattle causing high morbidity and mortality in South America [3,4] and to the lesser extent in Asia and Europe [5]. Nevertheless, *T. evansi* is a threat to livestock production particularly cattle, water buffaloes and camels across Asia and South America [5,6].

It is estimated that about 40 million cattle are at risk and 3 million die every year, leading to economic loss of US$ 1.0 – 1.2 billion annually [7]. The total domestic product lost is estimated at US$ 4.5 billion per annum when secondary losses such as reduced manure and draft power are included [7]. Despite the multiple causes, contribution of *T. congolense* in AAT in the sub-Saharan Africa is enormous. More than 80% of AAT and losses in domestic animals (cattle, goats, sheep, horses, pigs and dogs) in South, East and Central Africa are due to *T. congolense* infections [8]. In the West, *T. congolense* is second to *T. vivax* in causing AAT morbidity in livestock [8]. The characteristic features of *T. congolense* to such high prevalence and losses is probably due to host susceptibility, intrinsic factors, virulence and vectorial capacity of the vector tsetse flies to the parasite [9]. This chapter discusses the biology and the various features of the disease caused by *T. congolense*, challenges and opportunities for control approach are discussed.

2. Biology of *Trypanosoma congolense*

2.1. T. congolense as Intravascular Parasite

Trypanosoma congolense was first discovered by Broden in 1904 in the blood of sheep and donkey from then "Leopoldville" which is currently known as Kinshasa in the Republic of Congo [10]. It is a monomorphic (12.1–17.6 μm) salivarian parasite (development take place in the mid-gut and mouthpart of tsetse flies) and lacks a free flagellum at any stage of development [11], and can grow in mice [10]. Unlike *T. vivax* and *T. brucei*, *T. congolense* occurs in the blood vessels only [12] except during development of infection at the site of inoculation where the parasite is found in the skin, extravascularly and localized draining lymphatics [13,14]. In established infection, studies have shown unevenly distribution of *T. congolense* in the host circulation, but mostly localized to the walls of capillaries and small

vessels particularly of brain, heart and skeletal muscles [12,15] and therefore, providing the possibility of passively damaging the attached cells in response to anti-trypanosome antibody and complement fixation [16].

2.2. Trypanosoma congolense Types

T. congolense has been found to comprise three different types that are morphologically identical but genetically heterogeneous types infective to livestock and other mammalian hosts [17,18]. They have been classified as Savannah, Riverine-Forest and Kilifi [19,20]. Savannah and riverine-forest are genetically closely related than do Kilifi with the other two [21]. However, all three varies in their virulence, pathogenicity, drug resistance, vectors and geographical distribution [22]. Studies have associated Savannah type with a number of *Glossina* species (morsitans, forest and fusca groups) and affect a wide range of hoofed mammals and carnivores across savannah ecosystem of sub-Saharan Africa [23]. In contrast, *T. congolense* riverine-forest type is largely restricted to the *palpalis* group of tsetse mainly affecting pigs, goats, cattle, and dogs in the humid forest ecosystem of West, Central and to the lesser extent in East Africa [24–26]. *T. congolense* Kilifi type is restricted to East Africa and to the small extent in South Africa; it is associated with tsetse of morsitans group and mainly reported in cattle, sheep and goats [20,22,27,28].

However, field investigations in many parts has frequently found co-infections of *T. congolense* types in livestock and tsetse flies [23]. For instance, whereas savannah and riverine-forest co-infection are common in West and Central Africa [25,26,29,30], savannah and Kilifi co-infections occur in East and South Africa [31,32]. Nevertheless, Zambia, Kenya and Tanzania are the only countries which have reported co-infections of all three types [28,31,33].

3. The Life Cycle

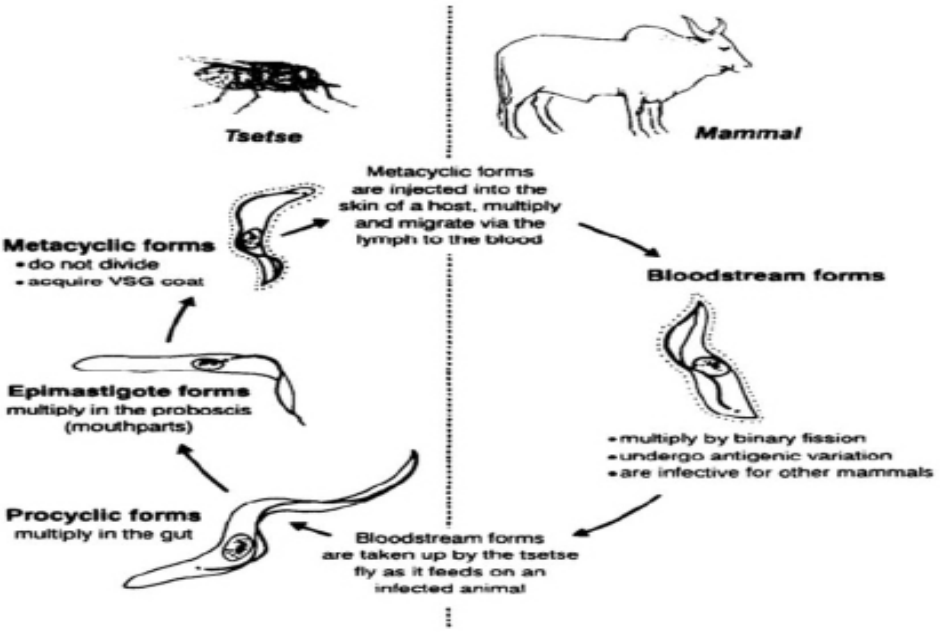
Trypanosomes have a complex life cycle **(Figure 1)**. The bloodstream forms that proliferate in the blood of infected mammalian host are ingested by the insect (tsetse fly) during the blood meal. They differentiate into procyclic forms in the mid-gut and migrate to the salivary glands and proboscis where they attach as epimastigotes forms. They then differentiate into infective metacyclic forms that are transmitted to a new mammalian host during the next blood meal.

More elaborate of the life cycle of *T. congolense* has been described by Hoare [10]. And the recently developmental cycle in tsetse fly outlined by Peacock and colleagues [35]. In fact, four stages are described in tsetse fly ranging from procyclics, trypomastigotes, epimastigotes and the infective metacyclic trypomastigotes. The bloodstream forms taken up by the fly differentiate into procyclics in the fly-midgut and grow in length. The procyclics through

peritrophic matrix penetrate the proventriculus where they cease division and become uniform in size and shape, and hence the name trypomastigotes. These trypomastigotes migrate to the cibarium and proboscis where they differentiate to epimastigotes and eventually metacyclic trypomastigotes, some of these forms have extremely long or truncated posterior ends [35]. The infective metacyclics are very small and do not divide.

Transmission of *T. congolense* into susceptible host is mainly through infected tsetse bites, the reported mechanical transmission have only based on controlled environment [1] and therefore, there is no evidence of mechanical transmission under natural conditions. Following the tsetse bite, few numbers of metacyclic variable antigenic types (M-VATs) will be at the inoculation site leading to raised nodule known as "chancre". Not all isolates of *T. congolense* will lead to formation of chancre. For instance, certain forms of *T. congolense* Kilifi did not induce chancre while isolates from Serengeti where observed to induce chancre formation [36]. Nevertheless, a tremendous multiplication of the parasite do occur with subsequent increase in M-VATs that is essential for survivorship of the parasite and establishment of the infection in the host [13]. After four or five days post infection the trypanosomes will get into the circulation through lymphatic vessels [13].

In the circulation, the parasite continues to multiply until a certain density level where multiplication ceases by the process known as density dependent quorum sensing. Meanwhile, trypanosomes covered by the dominant Variable Surface Glycoprotein (VSG) will be eliminated by antibody mediated immune response. However, residual variable antigenic type (VATs) will give rise to another wave of parasitaemia. This is the mechanism that ensure survivorship and transmissibility by the tsetse fly vector [37].



Source: [34]

Figure 1: The general summary of the life cycle of Trypanosomes showing the stages involved in tsetse and Mammals.

4. Virulence

There is a marked variation in the virulence between and within *T. congolense* types. A study by Authie´ and colleagues [38] showed that cysteine proteinases (CP1 & CP2) were responsible for the pathology and hence virulence in *T. congolense* infection. Later, CP2 was found to be specific for *T. congolense* and was characterised as congopain [39]. Rodrigues and collegues [23] observed a significant variability of congopain among *T. congolense* Savannah, Riverine-forest and Kilifi types with extensive polymorphism within Savannah, moderate polymorphism within Riverine-forest and relative homogeneity within Kilifi. Interestingly, virulence in isolates of the same species was shown to be enhanced by sexual recombination (mating) that give rise to genetic diversity even within a population from a single endemic focus [9].

Throughout the literature, studies have identified *T. congolense* strains within the Savannah type as the most pathogenic whereas riverine-forest and Kilifi types were indicated as moderate and low pathogenicity respectively [40].

For instance, variations in the virulence have been detected in *T. congolense* Savannah strains isolated from cattle in eastern Zambia, including identification of extremely virulent strains [41]. In another study, all three genetically distinct types produced acute infection in the Balb/c mice but chronic in Clun sheep and large white pigs [42]. In addition, the riverine-forest type resulted in a more severe disease in mice than the two indicating that pathogenicity is attributed to strain variability within *T. congolense* types [42], and the susceptibility of the host species. For example, riverine-forest type is considered to be refractory in cattle. While riverine-forest type was only present in the vectors, savannah type was predominant in tsetse flies and cattle in the epidemiological survey in the West Africa [43]. Furthermore, cattle that were infected with *T. congolense* riverine-forest and kilifi strains were able to clear the parasites without receiving treatment as opposed to those infected with savannah strain [29,44].

Therefore, it can be concluded that virulence within *T. congolense* types could be attributed to polymorphism of congopain, host species susceptibility and genetic recombination of the parasites within a defined population. The higher the virulence in *T. congolense* strains the higher the chances for its transmissibility and hence its maintenance in the field [41]. However, experience has shown that, the virulence of extremely virulent *T. congolense* strains decreases when co-infected with a less virulent strain [45]. Such phenomenon could be attributed to genetic recombination and diversity of the parasite. Interestingly, the severity and infection outcome in animals may depends on the variant circulating in a particular population that is rendered highly pathogenic upon interactions with different hosts and vector [22]. More precisely, at the wildlife-livestock interface where livestock near the park are severely affected

than those at distant [46].

5. Transmissibility

It is clear that virulence of the parasite is positively associated with increased transmissibility [41]. Based on such phenomenon, there is no doubt that *T. congolense* Savannah type has high transmissibility than other types when picked up by tsetse fly [47]. Generally, not all trypanosomes picked up by tsetse fly can successful develop to metacyclic trypomastigotes. It is shown that, despite of high incidence of trypanosomosis in mammals in sub-Saharan Africa; tsetse captured in the wild population have shown to possess a relatively low midgut trypanosomes infection in the range of 2–20 % in different tsetse species and sample sites [48]. In one hand, studies in *T. b. brucei* have shown that stumpy forms can successful survive and grow to infective stage in tsetse whereby the slender forms are digested with proteases [49,50]. Furthermore, stumpy forms are more abundant at peak parasitaemia in *T. b. brucei* infection, indicating that more transmissible forms exist at peak parasitaemia [50]. But *T. congolense* being monormophic does not possess the stumpy forms. Likewise, higher *T. congolense* parasitaemia did not correlate with increased transmissibility in tsetse flies [51]. Interestingly, in contrast to *T. b. brucei* and *T. vivax*, the transmissible forms of *T. congolense* have shown to occur in both ascending and peak parasitaemia [37]. With the exception of few studies, several studies within sub-Saharan Africa have indicated high prevalence of *T. congolense* in tsetse vector [9,31,52]. While *T. vivax* is more transmissible than *T. congolense*, it is hereby suggested that availability of *T. congolense* transmissible forms in ascending and peak parasitaemia could account for its high prevalence in tsetse flies. However, other contributing factors include levels of trypanosome resistance to trypanocidal drugs, stage of infection in the host and bloodmeal type [48]. For instance, it was shown that high infection rates of isogenic clones of *T. congolense* in *G. m. morsitans* was attributed to high level of resistance to isometamidium chloride [53]. On the other hand, a significantly higher infection rate of tsetse midgut was observed when tsetse were fed from mice with acute than in chronic phase independent of parasitaemia level [54]. Nevertheless, *G. m. centralis* fed with infected goat or pig blood had higher rate of infected midgut in contrast to flies fed with blood from other mammals [55]. Therefore, it is suggested that, in a particular ecological environment where different susceptible host species exist for example goats and cattle there could be high transmissibility of *T. congolense* in tsetse flies, however, it might depend on tsetse type. *Morsitans* - group tsetse are more susceptible to *T. congolense* infection than *palpalis* group. Reifenberg and colleagues [56] showed that cyclical development of clones of both *T. congolense* savannah and riverine-forest types were arrested in the midgut of most tsetse belonging to the *palpalis,* completed developmental cycle was acquired in morsitans group.

6. Clinical Presentation

In contrast to other species of trypanosomes, *T. congolense* exhibit different clinical manifestation in infected hosts with severity of disease varying depending on the number of factors. These include host and trypanosomes factors. The host factors include the type of animal species, breed and the immune status [57]. For instance, N'Dama breed were shown to develop less severe form of disease than Boran after sequential challenge with *T. congolense* [58]. Very few studies have been done in small ruminants, however, a study in Sudan showed that *T. congolense* developed a chronic form of disease in goats and in some cases became a source of acute infection in cattle [59].

Nevertheless, the trypanosome factors may include strain virulence and genetic variability within a defined population, and whether co-infected [45,60]. The virulent isolates of *T. congolense* savannah type were shown to induce acute disease and high mortality while less virulent strains caused benign and chronic infection [60].

Generally, the clinical signs of *T. congolense* infected animals are specific and include intermittent fever, abortion, cachexia, anaemia, lymphadenopathy, lethargy, anorexia, oedema of the throat, ventrum and forelimbs, ocular discharge and eventually death [8]. Other symptoms includes the history of premature birth and prenatal losses as such was observed in goats [61]. In some cases, distension of the abdomen in dogs may be a prominent feature particularly as a result of ascites and probably hepato-splenomegaly in advanced stage of the disease [62,63].

On the other hand, the disease caused by Forest type in cattle is of low pathogenicity with mild symptoms, anaemia is present at the earlier stages of the disease but cattle reported to self-cured the infection after three months [44] which means cattle have the ability to eliminate this trypanosome. The same applies for Kilifi type that caused asymptomatic disease and no major alteration in pack cell volume (PCV) and leucocytes count [44].

7. Diagnosis

Precisely, diagnosis is defined as methods for detecting infection through identification of aetiological agent or interpretation of reactions of immunological tests. Normally as rule of thumb, initial diagnosis is based on clinical signs and symptoms, and through demonstration of the causative agent or reactions to diagnostic tests. The demonstration of *T. congolense* in the peripheral blood is readily important during the early infection than in chronic or latter stages of the disease [64]. It is already known that *T. congolense* is an intravascular parasite with much of the parasite occupying the blood capillaries. Therefore, the blood smear should be taken from small veins preferably early in the morning as it may increase chances of detecting the parasites [65] . This is because, the average concentration of *T. congolense* in the ear vessels was inversely proportional to the amount of blood passing through in unit time to the

temperature [65]. On the other hand, although diurnal had little variation, *T. congolense* was detected easily from blood collected from ear than jugular veins [66].

In addition, trypanosomes can be detected through aspiration of chancre several days post tsetse bite [14]. This can be possible if animals are examined in earlier days after introduced in the area known to be tsetse infested. However, this kind of diagnostic procedure may not be reliable in areas where forest type is circulating as some forest strains do not induce chancre formation.

On the other hand, examination of lymph is not a promising efficient tool although in the chronic cases has regarded by some as useful means of diagnosis [64]. More importantly, anaemia is a major clinical sign in AAT caused by *T. congolense* infection, when correlated with ecological conditions might provide a tentative diagnosis.

Immunological methods includes enzyme linked immunosorbent assay (ELISA) which is a very reliable method, and easy to use in the field. However, can only detect anti-trypanosomal antibody and does not determine whether infection is the current or past, this is because antibodies can persist longer even after the parasite has been removed [67].

On the other hand, the molecular method is based on the detection and or amplification of nucleic acid, such technique include polymerase chain reaction and loop-mediated isothermal amplification (LAMP) [24,68–70]. These methods exploit the existence of a 177 bp repetitive sequence in the trypanosomes genome, a set of six primers have been used for differentiation of members of Nannomonas subgenus [68].

Other diagnostic methods that have been developed include restriction fragment length polymorphism (RFLP) [71], randomly amplified polymorphism DNA (RAPD) and amplified fragment length polymorphism (AFLP) [72]. Recently, ITS (Internal Transcribed Spacer) of ribosomal DNA repeating units have been used for the species-specific diagnostics of trypanosomes including *T. congolense*. It allows with one set of primers to distinguish most of African trypanosome species in a single PCR reaction based on the size polymorphism [73].

8. Pathological Findings

Pathological changes of the animal died of AAT caused by *T. congolense* are however not pathognomonic. There is paucity of knowledge on the changes induced by each particular *T. congolense* types in animals. The pathological lesions presented here are from infection due to savannah type in cattle since the other two have low pathogenicity and in most cases undergo self-cured especially in cattle [44]. The carcass is emaciated as evidenced by sunken eyes, prominent vertebrae and ribs and the tuber ischii become prominent with the wastage of the gluteal and crural muscles. The haircoat is lustress and there is starry enlargement of

all body lymph nodes, haemorrhages of superficial lymph nodes particularly prescapular and the mandibular. There is haemorrhagic fluid in the plural cavity and the heart has shown to consistently lose its parenchyma tissue. Other lesions are enlarged liver which may be accompanied with congestion and some signs of necrosis. The kidney size may be normal but with some necrosis in the renal cortex. There might be thick fluid in the bronchus and the trachea.

9. Treatment

Chemotherapy and chemoprophylaxis has been a central component of AAT control and hence *T. congolense* infection for many decades [74]. Two drugs are currently available for control of AAT which are diminazine diaceturate and isometamedium chloride. Diminazene diaceturate has been used therapeutically only due to its rapid metabolism and excretion [75]. The recommended therapeutic dose is 3.5 mg/Kg body weight (7 mg Kg^{-1} may be recommended for resistance strains) administered intramuscular or subcutaneous injections [74]. On the other hand, isometamidium chloride is rapidly cleared from the plasma to very low concentrations and accumulates in tissues from which it can be released slowly to the circulation to exert its activity [76]. The drug is used both therapeutically and prophylactically. Depending on the dosage of drug, species and strains of trypanosomes; it can offer prophylaxis for the period of about 1–5 months [76].

10. Prognosis

T. congolense was reported to became refractory to diminazene treatment when issued on day 19 rather than 24 hours post-infection [75]. Therefore, early detection of the disease and prompt treatment could normally lead to good recovery.

11. Challenges and Opportunities for *T. congolense* Control

Privatization of veterinary services in 1980s and 1990s in most African countries left many livestock keepers to administer chemotherapy in absence of veterinary professionals [77]. This, in some instances has lead to misuse of trypanocidal drugs by farmers and contributing to wide-spread treatment failure [78]. Considering the fact that drug to treat AAT are over than 50 years in the market coupling with the highly needed safe and effective drugs for HAT, there is a need of new trypanocidal agents. One of the opportunities is that *T. congolense* expresses specific surface proteins, lectin-like glycoproteins (TcoClecs) that are involved in its parasitic lifestyle which have shown to be suppressed with trypsin [79], drugs that could target this protein is a most welcome. Nevertheless, plants have always been a frequent source of medicaments either in form of traditional preparations or as active principles. In the recent past, pioneering screening work on various plants [80–82], have shown that many have promising *in vitro* and or *in vivo* trypanocidal activity potential. It is now more than 20 years

of anti-trypanosomal research from plant sources in Africa. There is yet a realistic molecule(s) that is to be subjected to vigorous clinical trials leading to useful agent that can help fight trypanosomosis. Most studies have ended either *in vitro* and or *in vivo* studies. There is a need of conducting more research to turn the promising anti-trypanosomal compounds into useful product(s).

On the other hand, most livestock keepers in the affected regions have limited access to quick diagnostic methods for early detection of the disease. They frequently rely on clinical signs that are often not pathognomonic. Therefore, simple and easy to use field diagnostic tools could play an important part in the control of disease and minimize risks associated with AAT.

12. Conclusion

Although there is distinct variability between genetic types of *T. congolense* with some cause moderate to low disease phenotype. *T. congolense* savannah type is the most important pathogenic trypanosome species in the sub-Saharan Africa. Much is needed in order to control this parasite.

13. Reference

1. Desquesnes M, Dia M. Mechanical transmission of *Trypanosoma congolense* in cattle by the African tabanid *Atylotus agrestis*. Exp. Parasitol. 2003;105(3-4)226-31. 2003;105:226–31.

2. Baldacchino F, Muenworn V, Desquesnes M, Desoli F, Charoenviriyaphap T, Duvallet G. Transmission of pathogens by Stomoxys flies (Diptera, Muscidae): a review. Parasite. 2013;20:26.

3. Batista J, Riet-Correa F, Teixeira, MMG Madruga C, Simões S, Maiae T. Trypanosomiasis by *Trypanosoma vivax* in cattle in the Brazilian semiarid: Description of an outbreak and lesions in the nervous system. Vet. Parasitol. 2007; 43: 174–81.

4. Batista J, Rodrigues C, Olinda R, Silva T, Vale R, Camara A, et al. Highly debilitating natural *Trypanosoma vivax* infections in Brazilian calves: epidemiology, pathology, and probable transplacental transmission. Parasitol. Res. 2012; 110: 73–80.

5. Desquesnes M, Holzmuller P, Lai D, Dargantes A, Lun Z, Jittaplapong S. *Trypanosoma evansi* and Surra: A Review and Perspectives on Origin, History, Distribution, Taxonomy, Morphology, Hosts, and Pathogenic Effects. Biomed Res. Int. 2013; 22 pp.

6. Mekata H, Konnai S, Witola W, Inoue N, Onuma M, Ohashi K. Molecular detection of trypanosomes in cattle in South America and genetic diversity of *Trypanosoma evansi* based on expression-site-associated gene 6. Infect. Genet. Evol. 2009; 9: 1301–5.

7. James D. Programme Against African Trypanosomiasis/PAAT. Tsetse Trypanos. Inf. Vol. 30 Pa. Austria: Bisamberg; 2007.

8. Namangala B, Odongo S. Animal African Trypanosomiasis in sub-Saharan African and Beyond African Borders. In: Magez S, Radwanska M, editors. Trypanos. Trypanos. Springer Science and Business Media; 2013. p. 294.

9. Gitonga P, Ndung'u K, Murilla G, Thande P, Wamwiri F, Auma J, et al. Differential virulence and tsetse fly

transmissibility of *Trypanosoma congolense* and *Trypanosoma brucei* strains. Onderstepoort J. Vet. Res. 2017; 84: a1412.

10. Hoare C. The Trypanosomes of Mammals. A Zoological Monograph. Oxford and Edinburgh: Blackwell Scientific Publications; 1972.

11. Vickerman K. The Fine Structure of *Trypanosoma congolense* in Its Bloodstream Phase. J. Protozool. 1969;16: 54–69.

12. Banks K. Binding of *Trypanosoma congolense* to the walls of small blood vessels. J. Protozool. 1978; 25: 241–5.

13. Luckins A, Sutherland D, Mwangi D, Hopkins J. Early stages of infection with *Trypanosoma congolense*: parasite kinetics and expression of metacyclic variable antigen types. Acta Trop. 1994; 58: 199–206.

14. Luckins A, Gray A. An extravascular site of development of *Trypanosoma congolense*. Nature. 1978; 272: 613–4.

15. Maxie M, Losos G. Release of *Trypanosoma congolense* from the microcirculation of cattle by Berenil. Vet. Parasitol. 1977; 3: 277–81.

16. Banks K. Injury induced by *Trypanosoma congolense* adhesion to cell membranes. J. Parasitol. 1980; 66: 34–37.

17. Godfrey D. Types of *Trypanosoma Congolense* II.—Differences in the Courses of Infection. Ann. Trop. Med. Parasitol. 1961; 55: 154–66.

18. Godfrey D. Types of *Trypanosoma Congolense* I.—Morphological Differences. Ann. Trop. Med. Parasitol. 1960; 54: 428–38.

19. Young C, Godfrey D. Enzyme polymorphism and the distribution of *Trypanosoma congolense* isolates. Ann. Trop. Med. Parasitol. 1983; 77: 467–81.

20. Knowles G, Betschart B, Kukla B, Scott J, Majiwa P. Genetically discrete populations of *Trypanosoma congolense* from livestock on the Kenyan Coast. Parasitology. 1988; 96: $61-474.

21. Garside LH, Gibson WC. Molecular characterization of trypanosome species and subgroups within subgenus Nannomonas. Parasitology. 1995; 111: 301.

22. Auty H, Torr SJ, Michoel T, Jayaraman S, Morrison LJ. Cattle trypanosomosis: the diversity of trypanosomes and implications for disease epidemiology and control. Rev. Sci. Tech. Off. Int. Epiz. 2015; 34: 587–98.

23. Rodrigues AC, Ortiz PA, Costa-Martins AG, Neves L, Garcia HA, Alves JMP, et al. Congopain genes diverged to become specific to Savannah, Forest and Kilifi subgroups of *Trypanosoma congolense*, and are valuable for diagnosis, genotyping and phylogenetic inferences. Infect. Genet. Evol. 2014; 23: 20–31.

24. Masiga D, McNamara J, Laveissière C, Truc P, Gibson W. A high prevalence of mixed trypanosome infections in tsetse flies in Sinfra, Côte d'Ivoire, detected by DNA amplification. Parasitology. 1996; 112: 75–80.

25. Simo G, Silatsa B, Flobert N, Lutumba P, Mansinsa P, Madinga J, et al. Identification of different trypanosome species in the mid-guts of tsetse flies of the Malanga (Kimpese) sleeping sickness focus of the Democratic Republic of Congo. Parasit. Vectors. 2012; 5: 201.

26. Simo G, Sobgwi P, Njitchouang G, Njiokou F, Kuiate J, Cuny G, et al. Identification and genetic characterization of *Trypanosoma congolense* in domestic animals of Fontem in the South-West region of Cameroon. Infect. Genet. Evol. 2013; 18: 66–73.

27. Mamabolo M, Ntantiso L, Latif A, Majiwa P. Natural infection of cattle and tsetse flies in South Africa with two genotypic groups of *Trypanosoma congolense*. Parasitology. 2009; 36: 425–31.

28. Njiru Z, Makumi J, Okoth S, Ndungu J, Gibson W. Identification of trypanosomes in *Glossina pallidipes* and *G.*

longipennis in Kenya. Infect. Genet. Evol. 2004; 4: 29–35.

29. Seck M, Bouyer J, Sall B, Bengaly Z, Vreysen M. The prevalence of African animal trypanosomoses and tsetse presence in Western Senegal. Parasite. 2010; 17: 257–65.

30. Nakayima J, Nakao R, Alhassan A, Mahama C, Afakye K, Sugimoto C. Molecular epidemiological studies on animal trypanosomiases in Ghana. Parasite & Vectors. 2012; 5: 217.

31. Mekata H, Konnai S, Simuunza M, Chembensofu M, Kano R, Witola W, et al. Prevalence and source of trypanosome infections in field-captured vector flies (*Glossina pallidipes*) in southeastern Zambia. J. Vet. Med. Sci. 2008; 70: 923–8.

32. Gillingwater K, Mamabolo M, Majiwa P. Prevalence of mixed *Trypanosoma congolense* infections in livestock and tsetse in KwaZulu-Natal, South Africa. J. S. Afr. Vet. Assoc. 2010; 81: 219–23.

33. Malele I, Magwisha H, Nyingilili H, Mamiro K, Rukambile E, Daffa J, et al. Multiple Trypanosoma infections are common amongst *Glossina* species in the new farming areas of Rufiji district, Tanzania. Parasite & Vectors. 2011; 4: 217.

34. Authié E. Trypanosomiasis and trypanotolerance in cattle: A role for congopain? Parasitol. Today. 1994; 10: 360–4.

35. Peacock L, Cook S, Ferris V, Bailey M, Gibson W. The life cycle of *Trypanosoma* (Nannomonas) *congolense* in the tsetse fly. Parasit. Vectors. 2012; 5: 109.

36. Paling R, Leak S, Katende J, Kamunya G, Moloo S. Epidemiology of animal trypanosomiasis on a cattle ranch in Kilifi, Kenya. Acta Trop. 1987; 44: 67–82.

37. Silvester E. Do different African trypanosome species share quorum-sensing signal responses? The University of Edinburgh; 2016.

38. Authie´ E, Boulange´ A, Muteti D, Lalmanach G, Gauthier F, Musoke A. Immunisation of cattle with cysteine proteinases of *Trypanosoma congolense*: targetting the disease rather than the parasite. Int. J. Parasitol. 2001; 31: 1429–1433.

39. Authié E, Duvallet G, Robertson C, Williams D. Antibody responses to a 33 kDa cysteine protease of *Trypanosoma congolense*: relationship to “trypanotolerance” in cattle. Parasite Immunol.1993;15: 465–74.

40. Abrham A, Shemeles D, Takele A, Berhanu D. Review on Characterization of *Trypanosoma congolense* ; A Major Parasite of Cattle in Africa. Acta Parasitol. Glob. 2017; 8: 39–49.

41. Masumu J, Marcotty T, Ndeledje N, Kubi C, Geerts S, Vercruysse J, et al. Comparison of the transmissibility of *Trypanosoma congolense* strains, isolated in a trypanosomiasis endemic area of eastern Zambia, by *Glossina morsitans morsitans*. Parasitology. 2006; 133: 331.

42. Garba M. The comparative pathogenicities of genetically defined trypanosomes of the subgenus Nannomonas, with especial reference to a new species. Univesity of Bristol; 1991.

43. Solano P, Argiro L, Reifenberg J, Yao Y, Duvallet G. Field application of the polymerase chain reaction (PCR) to the detection and characterization of trypanosomes in *Glossina longipalpis* in Cote d’Ivoire. Mol. Ecol. 1995; 4: 781–5.

44. Bengaly Z, Sidibe I, Ganaba R, Desquesnes M, Boly H, Sawadogo L. Comparative pathogenicity of three genetically distinct types of *Trypanosoma congolense* in cattle: clinical observations and haematological changes. Vet. Parasitol. 2002; 108: 1–19.

45. Masumu J, Marcotty T, Geerts S, Vercruysse J, Van den Bossche P. Cross-protection between *Trypanosoma congolense* strains of low and high virulence. Vet. Parasitol. 2009; 163: 127–3.1

46. Motloang M, Masumu J, Mans B, Latif A. Virulence of *Trypanosoma congolense* strains isolated from cattle and

African buffaloes (*Syncerus caffer*) in KwaZulu-Natal, South Africa. Onderstepoort J. Vet. Res. 2014; 81: 679.

47. Reifenberg J, Solano P, Duvallet G, Cuisance D, Simpore J, Cunny G. Molecular characterization of trypanosomes from naturally infected domestic animals in Burkina Faso. Vet. Parasitol. 1997; 71, 251–26: 251–62.

48. Dyer N, Clair R, Ejeh N, Acosta-Serrano A. Flying tryps: survival and maturation of trypanosomes in tsetse flies. Trends Parasitol. 2013; 29: 188–96.

49. Turner C, Barry J, Vickerman K. Loss of variable antigen during transformation of *Trypanosoma brucei rhodesiense* from bloodstream to procyclic forms in the tsetse fly. Parasitol. Res. 1988;74: 507–11.

50. Rico E, Rojas F, Mony BM, Szoor B, MacGregor P, Matthews KR. Bloodstream form pre-adaptation to the tsetse fly in *Trypanosoma brucei*. Front. Cell. Infect. Microbiol. 2013; 3.

51. Akoda K, Harouna S, Marcotty T, De Deken R, Van den Bossche P. Investigations on the transmissibility of *Trypanosoma congolense* by the tsetse fly *Glossina morsitans morsitans* during its development in a mammalian host. Acta Trop. 2008; 107: 17–9.

52. Isaac C, Ciosi M, Hamilton A, Scullion K, Dede P, Igbinosa I, et al. Molecular identification of different trypanosome species and subspecies in tsetse flies of northern Nigeria. Parasit. Vectors. 2016; 9: 301.

53. Van den Bossche P, Akoda P, Kubi C, Marcotty T. The transmissibility of *Trypanosma congolense* seems to be associated with its level of resistance to isometamidium chloride. Vet. Parasitol. 2005; 135: 365–7.

54. Masumu J, Akoda K, Van Den Bossche P. Transmissibility, by *Glossina morsitans morsitans*, of *Trypanosoma congolense* strains during acute and chronic phases of infection. Acta Trop. 2010; 113: 195–8.

55. Aksoy S, Gibson W, Lehane MJ. Interactions between tsetse and trypanosomes with implications for the control of trypanosomiasis. Adv. Parasitol. 2003; 5: 1–83.

56. Reifenberg J, Cuisance D, Frezil J, Cuny G, Duvallet G. Comparison of the susceptibility of different *Glossina* species to simple and mixed infections with *Trypanosoma* (Nannomonas) *congolense* savannah and riverine-forest types. Med. Vet. Entomol. 1997; 11: 246–52.

57. Tabel H, Kaushik R, Uzonna J. Susceptibility and resistance to *Trypanosoma congolense* infections. Microbes Infect. 2000; 2: 1619–29.

58. Paling R, Moloo S, Scott J, Gettinby G, Mcodimba F, Murray M. Susceptibility of N'Dama and Boran cattle to sequential challenges with tsetse-transmitted clones of *Trypanosoma congolense*. Parasite Immunol. 1991; 13: 427–45.

59. Mahmoud M, Elmalik K. Trypanosomiasis: goats as a possible reservoir of *Trypanosoma congolense* in the Republic of the Sudan. Trop. Anim. Health Prod. 1977; 9: 167–70.

60. Masumu J, Geysen D, Bossche P Van den. Endemic type of animal trypanosomiasis is not associated with lower genotype variability of *Trypanosoma congolense* isolates circulating in livestock. Res. Vet. Sci. 2009; 87: 265–9.

61. Gutierrez C, Corbera J, Morales M, Büscher P. Trypanosomosis in Goats: Current Status. Ann. N. Y. Acad. Sci. 2006; 1081: 300–10.

62. Harrus S, Harmelin A, Presenty B, Bark H. *Trypanosoma congolense* infection in two dogs. J. Small Anim. Pract. 1995; 36: 83–6.

63. Gow AG, Simpson JW, Picozzi K. First report of canine African trypanosomosis in the UK. J. Small Anim. Pract. . 2007; 48: 658–61.

64. Losos G, Ikede B. Review of Pathology of Diseases in Domestic and Laboratory Animals Caused by *Trypanosoma congolense*, *T. vivax, T. brucei, T. b. rhodesience and T. b. gambiense*. In: Dodd D, Philadelphia P, L.-C. Schulz H, editors. Vet. Pathol. Supplement. Basel: S. Karger AG, Verlag fur Medizin und Naturwissenschaften; 1972. p. 71.

65. Hornby H, Bailey H. Diurnal variation in the concentration of *Trypanosoma congolense* in the blood-vessels of the ox's ear. Trans. R. Soc. Trop. Med. Hyg. 1931; 24: 557–64.

66. Greig W, Murray M, Murray P, Mclntyre W. Factors affecting blood sampling for anaemia and parasitaemia in bovine Trypanosomiasis. Br. Vet. J. 1979; 135: 130–41.

67. Van den Bossche P, Chigoma D, Shumba W. The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs and in the absence of tsetse challenge. Acta Trop. 2000; 77: 263–70.

68. Masiga D, Smyth A, Hayes P, Bromidge T, Gibson W. Sensitive detection of trypanosomes in tsetse flies by DNA amplification. Int. J. Parasitol. 1992; 22: 909–18.

69. Solano P, Michel J., Lefrançois T, de La Rocque S, Sidibé I, Zoungrana A, et al. Polymerase chain reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkina Faso. Vet. Parasitol. 1999; 86: 95–103.

70. Thekisoe O, Kuboki N, Nambota A, Fujisaki K, Sugimoto C, Igarashi I, et al. Species-specific loop-mediated isothermal amplification (LAMP) for diagnosis of trypanosomosis. Acta Trop. 2007; 102: 182–9.

71. Geysen D, Delespaux V, Geerts S. PCR–RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of *Trypanosoma* species in cattle. Vet. Parasitol. 2003; 110: 171–80.

72. Thompson R, Constantine C, Morgan U. Overview and significance of molecular methods: what role for molecular epidemiology? Parasitology. 1999; 117: 161–75.

73. Desquesnes M, McLaughlin G, Zoungrana A, Dávila AM. Detection and identification of *Trypanosoma* of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. Int. J. Parasitol. 2001; 31: 610–4.

74. Giordani F, Morrison L, Rowan T, de Koning H, Barrett M. The animal trypanosomiases and their chemotherapy: a review. Parasitology. 2016; 143: 1862–89.

75. Peregrine A, Mamman M. Pharmacology of diminazene: a review. Acta Trop. 1993; 54: 185–203.

76. Kinabo L, Bogan J. The pharmacology of isometamidium. J. Vet. Pharmacol. Ther. 1988; 11: 233–45.

77. Gauthier J, Siméon M, de Haan C. The effect of structural adjustment programmes on the delivery of veterinary services in Africa. Proc. Reg. Conf. Off. Int. des Epizoot. Africa, Dakar Senegal, Paris. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO); 1999.

78. Geerts S, Holmes P. Drug management and parasite resistance in bovine trypanosomiasis in Africa. Issue 1 of. Food and Agriculture Organization of the United Nations; 1998.

79. Thonnus M, Guérin A, Rivière L. A multigene family encoding surface glycoproteins in *Trypanosoma congolense*. Microb. Cell. 2017; 4: 90–7.

80. Freiburghaus F, Jonker S, Nkunya M, Mwasumbi L, Brun R. *In vitro* trypanocidal activity of some rare Tanzanian medicinal plants. Acta Trop. 1997; 66: 79–83.

81. Freiburghaus F, Kaminsky R, Nkunya M, Brun R. Evaluation of African medicinal plants for their *in vitro* trypanocidal activity. J. Ethnopharmacol. 1996; 55: 1–11.

82. Freiburghaus F, Ogwa E, Nkunya M, Kaminsky R, Brun R. *In vitro* antitrypanosomal activity of African plants used in traditional medicine in Uganda to treat sleeping sickness. Trop. Med. Int. Heal. 1996 ;1: 765–71.