# TRYPANOSOMOSIS IN DOGS: A REVIEW

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## Abstract

Trypanosomosis is described as a complex, debilitating and often-fatal disease of man and domestic animals caused by infection with one or more of the pathogenic tsetse-transmitted protozoan haemo-flagellate parasites of the genus *Trypanosoma*. Animal trypanosomosis has profound social, economic and biological implications for the affected regions. It has been reported to be a major factor responsible for under development in sub-Saharan Africa (SSA). Dogs are susceptible to *Trypanosoma brucei brucei, T. congolense, T. evansi and T. cruzi.* Dogs are also infected by *Trypanosoma b*rucei *rhodesiense* and *Trypanosoma brucei gambiense* of man. The incubation period for canine trypanosomosis caused by *T. b. brucei* is from 4 - 8days post infection whereas it is about 4 - 24days for *T. congolense*. The *T. brucei* mostly invade tissues whereas, *T. congolense* and to a lesser extent *T. vivax* and *T. cruzi* predominantly restrict themselves to the blood circulation. However, it has been observed that *T. congolense* can invade tissues under certain conditions. Canine trypanosomosis is marked by facial swelling involving the eyelids, lips and the skin beneath the lower jaw. Other clinical signs are weakness, lethargy and keratitis. Parasitological diagnosis could be made by microscopic examination of lymph node aspirates, cerebrospinal fluid (CSF) or blood of the infected dog. Control strategies employed in the control of trypanosomosis include vector control and the use of chemotherapy both for curative and prophylactic purposes. In general, however, the chemotherapeutic approach is used much more widely than vector control because it is easier to kill the trypanosomes than the flies.

**Keywords:** Dogs, *Trypanosoma brucei, T. congolense,T. evansi, T. cruzi,* Tse tse fly

## Introduction

Trypanosomosis of dogs was first described in 1908 (Bevan, 1913). It has been established that dogs are susceptible to various trypanosomes including *T. brucei brucei, T. brucei rhodesiense, T. brucei gambiense, Trypanosoma congolense, Trypanosoma evansi and Trypanosoma cruzi* (Stephen, 1970; Gibson *et al* 1978; Morrison *et al.*, 1983; Matete, 2003; Abenga *et al*., 2005b). Recently, *Trypanosoma caninum* of unknown pathogenicity was isolated from an intact skin of a dog along with *Leishmania* in South Eastern Brazil. Infections with *T. b. brucei* and *T. congolense* are found mainly in sub-Saharan Africa. The infections are relatively common in Nigeria because of the high prevalence of *Glossina spp*. in most parts of the country (Ahmed, 2007). However, dogs also get infected by ingestion of fresh animal carcasses that died from trypanosomosis and through oral experimental infection (Raina *et al.,* 1985; Uilenberg, 1998). All breeds of dogs are

susceptible to trypanosomosis (Annette *et al.,* 2006; Akpa *et al.,* 2008).

**Identification of Canine Trypanosoma Species**

Trypanosoma species that affect dogs can be identified by the following morphological characteristics. *Trypanosoma brucei brucei* may be found in two different forms (WHO, 2006): the long slender and short stumpy forms. The long slender form measures approximately about 17 to 33 µm in length and 3.5 µm in width. The undulating membrane is conspicuous with a free flagellum at the anterior end. Its posterior end is pointed with a small and sub-terminal kinetoplast (Franccois *et al.,* 2005; OIE, 2008; Turnbull, 2001). The short stumpy form measures approximately 17 to 22 µm in lenght and 3.5 µm in width with a conspicuous undulating membrane. This form possesses a free flagellum and a pointed posterior end with a small and sub-terminal kinetoplast (OIE, 2008). *Trypanosoma congolense* has its small forms measuring 8 to 25 µm with an obvious undulating membrane (Uilenberg, 1998). The posterior end is rounded with no free flagellum. The kinetoplast is medium sized and terminal, often laterally positioned. Although considered monomorphous, a degree of morphological variation is sometimes observed which includes savannah, forest, kilifi and tsavo sub groups of *T. congolense* with different pathogenicities (Bengaly *et al.,* 2002; Masumu *et al.,* 2006; OIE, 2009).

*Trypanosoma evansi* is typically represented almost exclusively by thin trypomastigotes comprising slender and intermediate forms corresponding to those in *T. brucei*. The slender forms have a long free flagellum and a narrow posterior extremity, which may be rounded or truncated, with the kinetoplast situated at some distance from the tip. The intermediate forms have a shorter free flagellum and a short, frequently pointed posterior extremity, with the kinetoplast lying near this end (Hoare, 1972). *Trypanosoma cruzi* measures about 10 µm long, is slender, thin with an irregular shaped undulating membrane. Its nucleus is centrally positioned and the kinetoplast is posterior (Uilenberg, 1998; Hunt, 2010). The free flagellum runs through the remainder of the parasite and also extends beyond it. Visualized instained sample the parasite assumes a C or U shape (De Souza, 1999). *Trypanosoma rangeli* has a similar morphology to *T. cruzi* (OIE, 2008). *Trypanosoma caninum* is morphologically distinct from Salivarian trypanosomes. It differs from *T. cruzi* mainly by the size of its trypomastigote forms and kinetoplasts and absence of infectivity for macrophages and triatomine bugs (Madeira *et al.,* 2009). This means that parasite may not be transmitted through tsetse bite.

**Epidemiology of Canine Trypanosomosis**

Infections from *T.b. brucei* and *T. congolense* are found mainly in sub- Saharan Africa and the disease is relatively common in Nigeria because of the high prevalence of *Glossina spp* in most parts of the country (Ahmed, 2007) due to lots of vegetations along rivers and lakes and large wooded savannah landmasses (Nwoha, 2013). Anene *et al* (1999), reported that canine trypanosomosis due to *T. congolense* occurs commonly.

***Host Affected***

Indigenous, foreign and cross breeds of dogs are susceptible to trypanosomosis (Annette *et al.*, 2006; Akpa *et al.,* 2008).

***Intermediate Host***

*Glossina spp.* (tse-tse fly) and

haematophagous flies in the genus *Tabanus*, *Stomoxys* and triatomids bugs are vectors for the transmission of trypanosomosis in dogs (Uilenberg, 1998).

***Distribution***

This is dependent on the distribution of tse-tse flies and triatomine bugs which are the primary vectors responsible for the transmission of African trypanosomosis and triatomine transmitters of American trypanosomosis, respectively in dogs (WHO, 2013; Serap *et al.,* 2003; Hunt, 2010). Tse-tse flies are currently restricted to 14° latitude north and 29° latitude south of sub-Saharan Africa, affecting 10 million square kilometers of landmass (Molyneux, 1997; Serap *et al.*, 2003; WHO, 2010). Canine trypanosomosis has found its way beyond the boundaries of Africa into Europe that is apparently devoid of tse tse flies. This is due to the diverse clinical forms of the different infecting parasites. *Trypanosoma congolense* has three main strains of unequal pathogenicities that cause diverse clinical conditions in the dog (Nwaoha, 2013). The savannah strain of *T. congolense* produces an asymptomatic clinical condition in infected dogs and has been implicated as the cause of the first ever recorded African canine trypanosomosis in the UK (Gow *et al.,* 2007). Unknown to the importer, a six year old neutered male Jack Russell Terrier was harboring the Savanna strain of *T. congolense* and therefore was passed undetected after long days of quarantine. However few days after arrival in the UK, Jack developed signs of anaemia and before long it died (Nwaoha, 2013). Gow *et al.,* (2007) also stated that asymptomatic strains of *T.* congolense enhance the distribution of canine trypanosomosis beyond geographical boundaries. *T. evansi* is found mainly in Northern Africa, Near East, Far East, Central and Southern America. It was spread mechanically by several haematophagous biting flies and vampire bats in Latin America (William and Deborah, 1997; FAO, 2006). *T. rangeli* in dogs is endemic in Latin America as a parasite of man while *T. caninum* has only been identified in South-Eastern Brazil (CBVD, 2010; Basso *et al.,* 2012). American canine trypanosomosis (Chagas disease) is found mainly in the southwestern United States and sporadically in southern United States (Masumu, 2006). The disease spread to Latin American populations following human migration in the last three centuries into natural habitats of triatomine species commonly known as "kissing bugs" (Amora, 2004). The distribution of Chagas disease in dogs beyond triatomine zones is greatly influenced by the rise in canine blood transfusion (Rosypal *et al.,* 2007) and in 1996, Chagas disease was recorded in the municipal areas of Brazil (Maywald *et al.,* 1996).

***Transmission***

*Trypanosoma b. brucei* and *T. congolense* are transmitted to susceptible dogs through tse-tse flies' bite during their feeding (Luckins, 1973; CBVD, 2010). *Trypanosoma evansi* is transmitted mechanically in South America through vampire bats and by ingestion of infected herbivore meat (Steverding, 2008; Uilenberg, 1998). *Trypanosoma cruzi* and *T. rangeli* are both transmitted by triatomines, such as kissing bugs. Dogs and cats can become infected with *T. cruzi* and *T. evansi* through ingestion of the vector's excreta or ingestion of the entire infected vector (Cohen and Gurtler, 2001; Eloy and Lucheis, 2009). Infection may also be through penetration of dogs' intact or abraded skin by metacyclic forms of *T. cruzi* (Uilenberg, 1998). In-utero and colostral infections rarely occur (Uilenberg, 1998). Infection with *T. rangeli* may be through contamination of tritomine feeding

sites on the body of the dog with infected saliva or through the vectors' excreta (CVBD, 2010). The mode of transmission of *T. caninum* is yet unknown.

**Life Cycle**

Trypanosomes are excellent examples of organisms that display an extreme adaptation to their environment, in many cases because they must evade the immune responses of the host (Baral, 2010). African trypanosomes are transmitted between mammalian hosts by tsetse flies. However, in each host, the parasites undergo many life cycle stages involving forms with discrete morphologies, patterns of gene expression, and proliferation status. In each case, these developmental changes are programmed precisely (Barry and McCulloch, 2001). Infection of the mammalian host begins when the infective stage, known as the metacyclic stage, is injected intradermally by the tsetse fly. The organisms rapidly transform into the blood-stream trypomastigotes (long, slender forms), and divide by binary fission in the interstitial spaces at the site of the bite. The buildup of metabolic wastes and cell debris leads to the formation of a "chancre". In the mammalian host, the metacyclic parasites rapidly undergo cyclical and morphological changes. They exchange the restricted repertoire for antigenic variation that is the characteristic of the metacyclic forms with a more elaborate system of the bloodstream forms (Vickerman, 1965). Once established in the mammalian host, the bloodstream parasite is heterogeneous (Vickerman, 1965; Matthews *et al.*, 2004) comprising the proliferative slender forms during the ascending phase of parasitemia and the nonproliferative stumpy forms at the peak of parasitemia (Vassella, *et al.*, 1997). Transition between the morphological extremes (i.e., the slender versus stumpy forms) involves a progression from proliferation to cell cycle arrest, accompanied by a series of biological and morphological transformations (Tyler *et al.*, 1997; McLintock *et al.*, 1993). Once stumpy forms develop during the course of parasitaemia, the population is pre-adapted for transition to the procyclic forms, which occupy and proliferate in the mid-gut of the tsetse (Baral, 2009). The key features of the stumpy formation are the cell cycle arrest, the elaboration of some mitochondrial activities and a relative resistance to lysis by antibodies (Redpath *et al., 2000*; Sbicego *et al.*, 1999) and to the proteolytic environment that might be encountered in the midgut of tsetse (Sbicego *et al.*, 1999; Ziegelbauer *et al.*, 1993). When the vector-fly (tsetse) bites an infected individual, it takes the parasites with the blood meal. The parasites undergo metabolic changes in the midgut of the fly. They lose their surface coat, which consists of about 107 molecules of the Variant Specific Surface Glycoprotein (VSG), and transform into the proliferative procyclic forms. In this form, they express their own surface proteins called the Procyclic Acidic Repetitive Proteins (PARPs, or procyclins) (Baral, 2009). The defining events of the differentiation from the bloodstream forms to the procyclic forms are the loss of VSG and gain of the procyclins. Variant surface glycoprotein loss occurs very rapidly and involves the combined action of glycosyl-phosphatidyl-inositol-specific phospholipase C (GPI-PLC) and a proteolytic cleavage of the VSG via a zinc metalloprotease (Ziegelbauer *et al.*, 1993; Gruszynski *et al.*, 2003; Matthews, 2005). The transformation to procyclic form also changes the energy generation from being exclusively based on glycolysis in the bloodstream to a mitochondrion-based respiratory system, which requires structural elaboration and the metabolic

activation of organelles (Sternberg *et al.,* 1988). For successful transmission, the parasite undergoes two stages of differentiation in the fly: first, establishment in the midgut and then maturation in the mouthparts or the salivary gland (Baral, 2009). It is generally thought that during normal development in the fly, there are no intracellular stages and the parasites do not cross an epithelial barrier to enter the fly. After proliferation in the tsetse midgut, the parasite migrates to the salivary gland. The epimastigote forms generated there attach to the gland through elaboration of the flagellar membrane. After further multiplication, the parasite undergoes division arrest, re-acquires a VSG coat, and is released into the salivary gland lumen, in preparation for inoculation into a new mammalian host (Sternberg *et al.,* 1988).

If the tsetse flies ingest more than one strain of trypanosomes, there is the possibility of genetic exchange between the two strains, generating an increase in the genetic diversity in an organism that may not have a true sexual cycle. Indeed, it was shown by laboratory crosses that genetic exchange in the African trypanosome is possible (Sternberg *et al.,* 1988; Sternberg and Tait, 1990; Turner *et al.,* 1991; Turner *et al.,* 1990; Jenni *et al.,* 1986; Schweizer and Jenni, 1991). Precisely at which stage of the life cycle this genetic exchange takes place is equivocal, it could be at the midgut stage (Bingle *et al.,* 2001), in the salivary gland of the fly (Van Dan Abbeele *et al.,* 1997), and possibly at the proventriculus and foregut stage (Gibson, 2001). Though there are conflicting results for the stage at which this exchange takes place, it is shown that this is not a compulsory process. The mechanism of genetic exchange in *T. brucei* is still unclear though it appears to be a true sexual process involving meiosis (Van Dan Abbeele *et al.,* 1997). However, no haploid stage has been observed and the intermediates in the process are still a matter of conjecture. The frequency of sex in trypanosomes in nature is also a matter for speculation and controversy, with conflicting results arising from population genetics (Gibson and Stevens, 1999; Brun *et al.,* 1998).

In contrast to tsetse transmitted trypanosomes, *T. evansi* is transmitted mechanically by blood sucking insects. In Asia, transmission is by the horseflies (*Tabanus spp*.) and the stable flies (*Stomoxys spp*.), and in Africa the tsetse fly, like other biting flies, can act as a mechanical vector. In South and Central America, in addition to blood sucking flies, *T. evansi* can also be transmitted by the vampire bats (*Dosmodus rotundus*). Besides mechanical transmission by insects and vampire bats, *T. evansi* can be transmitted through milk or during coitus (Borst *et al.,* 1987). Developmental stages were not observed in any of the vectors mentioned above (Nikolay *et al*., 2012).

**Pathogenesis**

The pathology associated with canine trypanosomosis depends on the infecting species of trypanosome (Hunt, 2010). However, irrespective of the species, there is formation of chancre within few days of a tsetse fly bite (Nwaoha, 2013). Chancre formation is a local skin inflammatory reaction elicited as trypanosomes gain entry through the skin barrier (Manson-Bahr, 1931; FAO, 1998). The size of chancre is determined by the dog's immune status, the virulence of the infecting *Trypanosoma* species and the inoculation dose (Nwaoha, 2013). Rapidly dividing parasites inside the chancre enter the regional lymph nodes to the afferent lymphatics to the thoracic lymph duct and finally the blood (Mario *et al.,* 1997).

The incubation period for canine trypanosomosis caused by *T. b. brucei* is from four to eight days post infection (Anene *et al*., 1989; CBVD, 2010). From the blood, trypanosomes especially *T.b. brucei* and *T. evansi*, are disseminated to various tissues and organs of the body while other species such as *T. congolense* remain within the blood vessels (Losos, 1986; Abubakar *et al.,* 2005; Mario *et al.,* 1997). However, it has been observed that *T. congolense* can invade tissues under certain conditions (Adah *et al.,* 1992). An infection with *T. evansi* produces similar clinical manifestations as in *T. brucei brucei* (Mario *et al.,* 1997). The presence of parasites in the lymph nodes causes profound enlargement of the tissues due to cellular proliferation in B-cell areas and migration of leucocytes from the chancre. Soon after, there is sequestration of the parasite in several organs such as the heart, liver and spleen (Akpa *et al*., 2008). Splenomegaly is a feature of the acute or parasitemic phase of infection and is mainly the result of red cell and lymphocyte sequestration and an expanded macrophage population (Murray and Dexter, 1988).

In the liver, the Kupffer cells phagocytoze the parasites that are bound by the infected dog's antibodies. The organ may become enlarged and congested with Kupffer cell hyperplasia and periportal mononuclear cell infiltration (Murray *et al.,* 1983). Dogs with pernicious anaemia may have centrolobular necrosis of the liver. The main changes in the bone marrow are a reduction in the cellular components affecting the red blood cells, lymphocytes and platelets (Eloy and Lucheis, 2009).

The heart is consistently damaged in dogs infected with *T. b. brucei*, *T. cruzi* or *T. congolense* producing distinct lesions (Katherine and Edith, 2004; Mario *et al.,* 1997). Pathogenesis of American canine trypanosomosis starts immediately after contamination of feeding site of tritomine. These vectors pass out *T. cruzi* in their faeces during blood meals and the organisms accidentally penetrate the feeding site on the dog. The bite causes itching and the act of scratching facilitates the penetration of parasites into the tissues (Nwaoha, 2013). Acute Chagas disease is usually seen in young dogs between 5 to 6 months of age (Caliari *et al.,* 1996; Hunt, 2010). Such dogs may just die suddenly due to severe inflammatory reactions in the heart. This is often confused with more common causes of heart disease (Eloy and Lucheis, 2009). This condition is rare except in cases of invasion of large numbers of parasites into the heart (Ettinger and Feldman, 1995). Dogs with acute experimental infections of *T. cruzi* showed alterations in the neurons of the Auerbach's plexus and myositis in the lower third of the esophagus (Mario *et al.,* 1997).

Chronic Chagas disease is characterized by myocarditis, as found in man and is associated with remodeling of cardiac structure resulting to right -sided cardiac dysfunction and unusual conduction disturbances such as arrhythmias (Meurs *et al.,* 1998). These conditions are easily detected with electrocardiographic and echocardiographic examinations of the heart (Meurs *et al*., 1998). Such dogs may have alterations in the brain and the peripheral nerves during the acute and chronic phases of the disease (Eloy and Lucheis, 2009). Cardiac lesions associated with African *T. b. brucei* infections show a marked cellular infiltration within the perivascular and interstitial locations. Such infiltrates are composed mainly of lymphoid cells, plasma cells, macrophages and occasionally eosinophils (Andrade *et al.,* 1997). Cardiac lesions

associated with *T. congolense* infection have scanty cellular infiltrations consisting of small lymphocytes and occasionally macrophages and plasma cells (Adah *et al.,* 1992; Murray *et al.,* 1983). Infections with *T.congolense* are mostly vascular with few extravascular parasites (Adah *et al.,*1992).

Oedema of the perivascular and interstitial spaces is often observed in canine trypanosomosis particularly at the terminal stage. The perivascular oedema of the cardiac musculature possibly reflects increased permeability and extensive degeneration of the heart fibers. This is probably due to anoxia caused by the prolonged anemia and immune mediated pathology. Dogs infected with *T. b. brucei* and *T. cruzi* show a severe meningoencephalitis similar to those described in fatal cases of human trypanosomosis.

**Clinical Signs**

Knowledge of the clinical/pathological features in response to trypanosomal infections in dogs has been supplemented by studies in dogs experimentally infected with these pathogens. Experimental infection with canine trypanosomes typically follows three successive stages: acute, sub-acute and chronic forms, though under a natural challenge scenario, it may be more complex (Katherine and Edith, 2004). In dogs *T. b. brucei* is responsible for an acute disease with high parasitaemia. The early acute phase of the disease is marked by the continuous presence of trypanosomes in the blood at detectable concentration ($10^3$ to $10^8$/ml) (OIE, 2008; Nwoha and Anene, 2011a). Pyrexia is highest at the first peak of parasitaemia, thereafter at parasitaemic waves which often corresponds with the development of anaemia (Aquinos, 1997). Anemia is the most prominent feature of canine trypanosomosis (Franciscato *et al.,* 2007; Nwoha and Anene, 2011b). This is easily observed clinically as pallor of the mucous membrane. The virulence of the infecting parasite population and the age, nutritional status and breed of the host influence the severity of anemia.

African canine trypanosomosis is marked by infiltration of the subcutaneous tissues with fluid (oedema) swelling of eyelids, lips and the skin beneath the lower jaw (Nwoha and Anene, 2011a). Some dogs develop keratitis which may result in unilateral or bilateral corneal opacity with moderate lacrimal discharges (Nwoha and Anene, 2011a). A few cases develop the neurological form of the disease usually, post-therapy. This form is similar to rabies and terminates fatally within few weeks (Anene *et al*., 1989a). Emaciation may or may not be seen in dogs with acute infection of the disease. Most dogs show marked weakness and lethargy. In the terminal stage of the disease, animals become extremely weak and are often unable to rise. Death of the infected animal may occur in the first few weeks or months after infection as a result of the acute disease (Nwoha and Anene, 2011a). In contrast to the acute phase of infection, dogs infected with *T. congolense, T. evansi, T. rangeli, T. cruzi* and *T. caninum* often show a chronic form of the disease with ocular signs such as keratitis, uveitis, coagulopathies in *T. evansi* and blepharo conjunctivitis (Amole *et al.,* 1982; Mario *et al.,* 1997). *Trypanosoma evansi* sometimes produces an acute syndrome in dogs manifested as urticarial plaques and ophthalmitis which are transitory and may relapse (Mario *et al.,* 1997). Similarly, less frequently, others cause acute syndromes in imported dogs, with pyrexia, prostration, severe anaemia and death in 2 to 3 weeks post infection (CVBD, 2010; Nwoha and Anene, 2011b).

Clinical signs in American canine

trypanosomosis often present asymptomatic to chronic disease states in dogs and cats (Teixeira *et al.,* 1990). The acute phase often is seen in young dogs characterized by generalized myocarditis and extensive degeneration of the central nervous system. Such dogs exhibit signs of lethargy, splenomegally, enlarged lymphnodes, diarrhea, myocarditis and sudden death. Diseased hearts may slowly deteriorate in function and resultant symptoms may be confused with those of other heart diseases (Kirchhoff, 2011). Chronic forms of the disease are commonly seen in adult dogs after several months of initial infection and are characterized by ventricular arrhythmias and myocardial dilation. The cardiac insufficiency is initially detected on the right side and later progresses to left ventricular insufficiency (Ettinger and Feldman, 1995). Cats may havepyrexia, convulsions and paralysis of the hind limbs (Kirchhoff, 2011).

Anemia in the chronic phase is not strictly associated with the presence of parasites in the blood, but is as a result of exhaustion of the limited pluripotent stem cells of the bone marrow from constant assaults by waves of parasitemia (Manson-Bahr, 1931). Dogs may be intermittently parasitemic at this period. Dogs having chronic canine trypanosomosis are weak, cachexic and debilitated towards the terminal stage of the disease. Regardless of their weakened condition, some dogs continue to eat, and this may last for months before their death (Nwoha and Anene, 2011a).

**Pathology**

There are no pathognomonic lesions in trypanosome infected dogs (Nwaoha, 2013). General lesions are congestive, inflammatory, oedematous, degenerative and sometimes haemorrhagic coagulopathies in various organs such as the heart, central nervous system, (CNS), eyes, testes, ovaries and the pituitary gland (Eloy and Lucheis, 2009). There is usually oedema of the head, thorax and forelimbs. The carcass shows muscle wasting and gelatinous appearance of cutaneous fat (Nwoha and Anene, 2011a). Congestive heart failure is an important cause of death in chronic cases and is related to the combined effects of prolonged anemia, myocardial damage and increased vascular permeability (Katherine and Edith, 2004). The superficial lymph nodes are slightly enlarged and oedematous on cut surface. The liver and spleen are swollen and congested, while the kidneys are pale and the cut surface shows hemorrhages especially along the corticomedullary junction (Nwoha and Anene, 2011a). In chronic cases, lymphnodes and spleen frequently return to normal size and in some cases, they eventually atrophy and sclerose (Katherine and Edith, 2004). There is hydrothorax and hydropericardium containing straw coloured, fibrin flaked fluid (Nwoha andAnene, 2011a). The pericardial fats are gelatinous and the lungs are emphysematous with haemorrhages in the trachea. The meninges of the brain are haemorrhagic. The urine shows deviation from the normal amber colour with a pH of 6.0. There is evidence of an increased number of leukocytes in urine. This could be the reason for the slight change in specific gravity and increased turbidity of the urine (Katherine and Edith, 2004; Nwoha and Anene, 2011a).

**Differential Diagnosis**

Diseases which can be confused with clinical cases of trypanosomosis in dogs include:

1. Canine babesiosis, canine anthrax, canine

anaplasmosis, canine haemorrhagic septicaemia. These can be confused with acute trypanosomosis with pyrexia.

2. Canine ancylostomosis, canine ascariasis, malnutrition and other helminthosis (Nwaoha, 2013). These can be confused with chronic trypanosomosis showing anaemia and emaciation

**Diagnosis**

Diagnosis of canine trypanosomosis is based on a combination of detailed clinical examinations, proper sample selection/collection, appropriate diagnostic tests, proper conduct of tests and logical interpretation of results (Nwaoha, 2013). In canine trypanosomosis where disease prevalence is high, some tests of low diagnostic sensitivity may suffice (OIE, 2008). Parasitological diagnosis could be made by microscopic examination of either the lymph node aspirates, blood, or cerebrospinal fluid (CSF) of infected dogs (François *et al.*, 2005). Blood samples should be examined as soon as possible to avoid immobilization and subsequent lysis of trypanosomes in the sample. Often blood samples collected from the tip of the ear yields a larger quantity of parasites when compared to venipuncture (Uche, 2010). The collected blood sample should be preserved in an ice pack container away from sunlight because trypanosomes are rapidly destroyed by sunlight (OIE, 2008).

***Wet Mount Method***

In preparation of wet blood films, a drop (about 2 µl) of blood is placed on a clean slide and covered with a cover slip to eliminate air bubble. It is then examined microscopically (magnification, X40) with condenser aperture, phase-contrast or interference contrast for proper visualization (22 × 22 mm). A detailed procedure of this test is seen in WHO Trypanosomosis Control Manual (1983).

Although, this technique has a very low detection power of 10,000 parasites in 200 microscopic fields, it is the most commonly used test in trypanosomosis (François *et al*., 2005). Microscopic examination enhances detection of trypanosomes darting across the microscopic field in positive *T. brucei* while *T. congolense* parasites move sluggishly and thus allows a definite diagnosis to be made. The movement of the surrounding erythrocytes often attracts attention to the presence of trypanosomes in the blood. Due to fluctuations in parasitemia, blood samples should be collected every other day to check for a peak in parasitemia when the parasites will be easily detected. The sensitivity of this technique may be significantly improved by lysis of the RBCs before examination using a haemolytic agent such as sodium dodecyl sulfate (SDS) (OIE, 2008).

***Lymph Node Aspirate***

Examination of lymph aspirates from prescapular lymph nodes detects up to 80 % of the infection (Robson and Ashkar, 1972). Lymph is aspirated from enlarged cervical lymph nodes and one to two drops of the fresh aspirate is expelled onto a slide, and a cover slip is applied to spread the sample and prepare a smear. The wet preparation is mounted immediately and viewed under the microscope (magnification, X40) for the presence of motile trypanosomes (François *et al.*, 2005). The sensitivity of this procedure varies between 40 and 80 % depending on the parasite strain, the stage of the disease (sensitivity is

higher during the acute stage), and concurrent infection with pathogens that cause lymphadenopathy (Simarro *et al.,* 2003*;* Van Meirvenne, 1999).

***Thin and Thick Blood Smears***

Thin/thick blood smear is another parasitological technique that can be used in the diagnosis of trypanosomosis (Nwaoha, 2013). This technique is not tedious and can be carried out easily by an experienced technician. Giemsa- or Field's-stained thin blood films are made by placing a drop of blood (about 5 µl and 5 to 10 µl for thick blood) film at one end of a slide, the edge of another slide is placed just close enough to the drop of blood for it to spread along the edge. Then, with a swift movement blood is spread on the slide. Ideally, thin films should be prepared so that the RBCs are fairly closer to each other but with no overlapping. The slide is air dried and then fixed in methanol. The fixed slide is later stained with Giemsa stain in phosphate buffered saline at a pH of 7.2. A more detailed technique can be found in WHO trypanosomosis control manual. After preparation, the stained slide is allowed to dry and then examined under a phase contrast microscope. This technique helps in the identification of the particular infecting trypanosome species and is often used where there is no centrifuge (Lumsden *et al.,* 1979). Sensitivity of this test may be improved by increasing the thickness of stained slides. A fixed smear should be kept dry and protected from dust, heat, flies and other insects that may feed on them (OIE, 2008).

***Microhematocrit Centrifugation Technique (MHCT)***

This technique is sometimes referred to as the capillary tube centrifugation technique or as the Woo test. It was developed more than 30 years ago and is still used in the diagnosis of trypanosomosis in man and animals (Woo, 1970; 1971). In this procedure, heparinzed capillary tubes are three-quarters filled with the suspected blood sample containing an anticoagulant. The dry ends of the capillary tubes are sealed with plasticine or heat (OIE, 2008). The capillary tubes are centrifuged at 3000 rpm for 6 to 8 minutes. Trypanosomes become concentrated at the level of the white blood cells, between the plasma and the erythrocytes. The centrifuged capillary tubes can then be examined under the microscope at a low magnification of x10 or x40 for motile parasites. Microhematocrit Centrifugation Technique is a more sensitive technique than wet mount, and the sensitivity of mHCT is increased with the number of tubes examined (OIE, 2008).

***Quantitative Buffy Coat***

The quantitative buffy coat or Murray method (QBC; Beckton-Dickinson) was initially developed for the rapid assessment of differential cell counts, but is now being applied to the diagnosis of hem parasites including trypanosomes (Levine et *al.*, 1989; Bailey and Smith, 1992; Nwaoha, 2013). It is a widely used improved method of diagnosis of trypanosomes involving the staining of trypanosome kinetoplasts and nuclei with acridine orange for easy differentiation from the white blood cells at the buffy coat level (François *et al.*, 2005). About 1500 to 2000 µl of blood in heparinized capillary tubes containing acridine orange is centrifuged at 3000 rpm to allow separation. The buffy coat is aspirated into a microhaematocrit capillary tube and re-centrifuged. Motile trypanosomes can be identified by their

fluorescencing kinetoplasts and nuclei in the expanded buffy coat. The flourescented trypanosomes are best appreciated in a dark room using ultraviolet light generated by a cold light source connected by a glass fiber to a special objective containing the appropriate filter. The QBC has about 95 % sensitivity and can detect positive cases of low parasitemia (François *et al.*, 2005).

***Mini-Anion-Exchange Centrifugation Technique (MAECT)***

The Mini-Anion-Exchange Centrifugation Technique was introduced by Lumsden *et al.* (1979) based on a technique developed by Lanham and Godfrey (1970). An updated version has been described by Zillmann *et al.* (1996). The technique is based on the ability of the negatively charged RBCs to be held back in the anion column, and the less negatively charged trypanosomes to pass through with the solution. The trypanosomes are concentrated in the solution by low-speed centrifugation (François *et al.*, 2005). The concentrate is then examined in a special holder under the microscope for the presence of trypanosomes. This technique is highly sensitive compared to most of the other described techniques because of large blood volume (300 µl) used, which enables the detection of less than 100 trypanosomes/ml (OIE, 2008).

***In-Vitro Cultivation***

*In-vitro* cultivation of *T. brucei* has been described over the years but with varying degrees of success (McNamara et al. 1995; OIE, 2008). About 5 to 10 ml of blood is cultured in the laboratory and blood stream forms of trypanosomes transform into large proliferating procyclic forms detectable within three to four weeks (François *et al.*, 2005). The technique requires sophisticated equipment, is time consuming and not suitable for large scale or routine diagnosis. KIVI kit can be used *in-vitro* in the isolation and amplification of all species of *T. brucei* in humans, domestic and game animals.

***Animal Inoculation***

Mouse inoculation may be used for the detection of positive cases with sub-clinical infections by the inoculation of specific pathogen free (SPF) mice with blood samples from animals suspected to be infected with trypanosomosis, and allowing for establishment of infection and screening them for parasitemia (WHO, 1998). The immunity of the test mouse can be suppressed by administration of corticosteroids or by irradiation in order to increase their chances of developing parasitemia and isolating the parasite (Nwaoha, 2013). The SPF mice are bled thrice a week for at least two months until detection of parasitemia. Factors such as chronic infections of low parasitemia, the fact that some strains of *T. congolense* do not replicate in the mice and animal welfare regulations may influence the use of this technique (OIE, 2008).

***DNA Amplification Tests***

A polymerase chain reaction (PCR) technique could be used as a diagnostic tool in cases of canine trypanosomosis as it can be applied on any patient sample that contains trypanosomes DNA (OIE, 2008). The technique involves the amplification of specific DNA of different trypanosome species. Samples to be analyzed should be protected from sunlight to avoid DNA degradation (François *et al.*, 2005). Currently, the technique has been applied on *T. brucei* for detection of its three species and three types of *T. congolense* with success. Other species of trypanosomes that affect dogs can undoubtedly be tested with it. This test is

important in the detection of possible new strains of trypanosomes that may affect dogs (Nwaoha, 2013). The primer sets available for different *T. brucei brucei* subgenus, species and types are referred to as follows: Trypanozoon subgenus - TBR1 and TBR2; *T. congolense* (savannah type) - TCN1 and TCN2; *T. congolense* (forest type) - TCF1 and TCF2; *T. congolense* (Kenya Coast type) TCK1 and TCK2. Due to the multiplicity of these taxon-specific primers, afull trypanosome species identification requires that five PCR tests can be carried out per sample, and therefore cannot be used as a routine diagnostic technique in dogs (OIE, 2008).

***Antibody-Detection Enzyme-Linked Immunosorbent Assay (Indirect Serological Assay)***

The technique of antibody ELISA has been developed for use in the diagnosis of trypanosomosis in animals (Lumsden, 1979) and has been used in large-scale surveys of bovine trypanosomosis (Desquesnes, 1997; Hopkins *et al.*,1998). It could also be employed in canine trypanosomosis. The standard antigen for trypanosomosis antibody tests is derived from purified bloodstream-forms of trypanosomes and the procedure can be obtained from OIE Terrestial manual (OIE, 2008).

In bovine trypanosomosis, ELISA, using *T. congolense* or *T. vivax* pre-coated microtitre plates, has been developed for diagnosis of bovine trypanosomosis (OIE,2008). Similar pre-coated microtitre plates can also be produced for diagnosis of canine trypanosomosis especially as it has an advantage of providing a standardized denatured antigen that can be preserved for a long time at room temperature (Nwaoha, 2013). The suspected test serum is reacted with trypanosomal antigens present in the ELISA microtitre plate, after which the resulting antigen/antibody complex is then incubated with an enzyme-conjugated antiglobulin IgG fraction of the suspected dog. The reaction is then visualized by the addition of enzyme substrate and chromogen, with the resulting colour change allowing a photometric interpretation (Luckins,1973).

The absorbance of each ELISA-sample tested is expressed as a percentage (percentage positivity: PP) of the strong positive reference standard or the positive and negative reference standard results (OIE, 2008). The cut-off value is determined using known positive and negative field or experimental samples. Both antibody-detection tests have high sensitivity and genus specificity. Their species specificity is generally low, but may be improved by using a standardized set of the three species-specific tests (Desquesnes, 2004) or by fractionation of crude trypanosomal antigen extract which will enable discrimination between infecting species (Ijagbone *et al.*, 1989).

***Laboratory Assays Essential For Confirmation of Trypanosomosis***

Most of the immune-histochemical techniques are of high sensitivity and little specificity such as antigen- or antibody-ELISA test described above which often detects the presence of IgM during acute infection and IgG in chronic cases. The reduced specificity encountered in the use of these techniques is because of cross reactivity between trypanosomal species and with concurrent infections such as microfilaria and Leishmania (Nwaoha, 2013). The invention of modified ELISA technique (Cellabs Elisa *T. cruzi* and Hemagen Chagas kit) used in diagnosis of *T. cruzi* infection in humans gives a 100 % sensitivity and specificity (Annette *et al.*, 2006). This modified ELISA technique may also give

similar result in the diagnosis of canine trypanosomosis (Nwaoha, 2013). Indirect fluorescent antibody test (IFAT) has been used extensively in the diagnosis of typanosomosis in both man and animals. The original method for this test has been replaced by a new technique for the preparation of trypanosomal antigens. This involves fixation of live trypanosomes using a mixture of 80 % cold acetone and 0.25 % formalin in normal saline. The use of IFAT in the diagnosis of bovine trypanosomosis has proven to be both specific and sensitive in detecting trypanosomal antibodies in infected cattle (Wilson, 1969) and camels (Luckins, 1973).

Thus, it may show similar sensitivity and specificity in the diagnosis of canine trypanosomosis (Nwaoha, 2013). The technique involves the preparation of a thin smear from a suspected blood sample which is allowed to dry and is later fixed in acetone for a few minutes. About 5 mm diameter circles are marked on glass slides using nail varnish. A 1: 40 diluted test sample's serum is pipetted into each circle, ensuring that the area in each circle is completely covered. The antigen/test serum preparation is incubated at 37 °C for 30 minutes in a humid chamber. Afterwards, the preparations are washed thrice in PBS for 5 min each time at 4 °C with gentle agitation and then air-dried.

Rabbit or goat anti-bovine IgG conjugate that has been conjugated to fluorescein isothiocyanate is added and then slide washed and incubated as afore mentioned. A clear detailed procedure could be seen in OIE Terrestial manual (OIE, 2008).

The slides are further rinsed in distilled water and air dried. The dried slides are mounted in PBS or buffered glycerol and examined for fluorescence. However, this technique has some limitations such as the high cost of the technique which involves use of a sophisticated microscope and cross reactivity between trypanosomal species. Therefore, IFAT cannot be used for routine test diagnosis of canine trypanosomosis (Nwaoha, 2013).

***Biochemical Analysis***

Though there is so much inconsistency in the biochemical changes observed in canine trypanosomosis, there are still some parameters that are somewhat consistent from the literature. Barr *et al.* (1991) recorded elevated serum liver enzymes, alanine transferase ALT and aspertate amino phosphotase (ASP) in the acute phase of Chagas disease in dogs. Eloy and Lincheis (2009) observed hyperproteinaemia which contradicts the findings of Barr *et al.* (1991) on trypanosomosis. The hyperproteinaemia was attributed to high antigenic stimulation associated with trypanosomosis (Aquinos, 2002). African canine trypanosomosis caused by *T. brucei brucei, T. congolense* and *T. evansi* are mostly characterized by elevated liver enzymes, blood urea nitrogen (BUN), creatinine and bilirubin concentrations (Aquinos, 2002; Nwoha *et al.,* 2013). Under field infection ASP has been the only liver enzyme found above the normal range in the serum and has been attributed to either hepatic or muscular damage (Franciscato *et al.,* 2007). However, several workers have recorded decreases in total proteins in experimental African canine trypanosomosis and attributed it to loss of albumin in urine (Franciscato *et al.,* 2007; Nwoha *et al.,* 2013). The discrepancies in biochemical changes in canine trypanosomosis are function of the diagnostic techniques, expertise and

physicobiochemical dynamics in dogs (Nwaoha, 2013).

## Immunology of Trypanosomosis

### *Humoral immunity*

Trypanosomes cause diseases with variable symptoms depending on the infected host, trypanosome species and serodeme. Generally, trypanosomosis is characterised by fever, anaemia, cachexia, reduced productivity, infertility and, if left untreated, animals often die from heart failure or opportunistic infections. The infected animals tend to exhibit persistent fluctuating parasitaemia comprising a series of trypanosome waves expressing different variable surface glycoproteins (VSG) (Vickerman, 1978; Barbet and McGuire, 1982). This pattern of parasitaemia exposes the host to a series of antigenically distinct surface antigens of the parasite. The VSG genes encode a family of proteins that exhibit extensive heterogeneity at the N-termini, but are fairly similar at the C-termini (Rice-Ficht *et* al., 1981; Shak *et* al., 1988). The C-terminus is covalently bound to di-myristyl-phosphatidylinositol, which is responsible for anchoring it to the membrane (Ferguson *et al*., 1985). Cleavage of the VSG phosphatidylinositol by endogenous phospholipase C leads to subsequent exposure of a cross-reactive determinant, a cryptic epitope, formed in part by the terminal inositol phosphate (Shak *et* al., 1988). It has been shown that the host mounts a humoral immune response to both the N and C termini of VSG. The antibodies directed to the N terminus are specific to the particular VSG, and therefore responsible for the elimination of the parasites displaying on their surface the particular VSG. These parasites are eliminated through opsonisation by macrophages (Urquhart and Holmes, 1987). Despite the effectiveness of the anti-VSG-specific antibodies, complete elimination of trypanosomes is hampered by the rapid appearance of those with different variable surface antigens to which the host has not mounted an immune response. The persistence of parasites in circulation leads to continuous stimulation of the host immune system, as evidenced by a marked increase in size and activity of germinal centres, with a concomitant increase in proliferating lymphocytes in the medullary cords and paracortex of lymph nodes, periarteriolar regions and peripheral follicular areas of the spleen (Masake and Morrison, 1981). Despite the apparent overstimulation of the immune response organs, the elevated levels of IgM and IgG immunoglobulins occurring in African trypanosomosis are specific to the infecting serodeme/strain in view of the fact that infecting trypanosomes can absorb 85-100% of the generated immunoglobulins (Musoke *et al*., 1981).

Antibody response to non-VSG invariant antigens also occurs during trypanosome infection (Authie *et al*., 1993a). Although these responses have no direct correlation to the control of parasitaemia, it has been shown that a predominant IgG response to a cysteine protease, heat-shock protein (hsp 70/BIP) and cryptic VSG epitope could be associated with tolerance to trypanosome infection (Authie *et al*., 1993b). This would suggest a role for invariant antigens in modulating the infection (Masake and Musoke, 1998).

### *Cell-mediated immunity*

African trypanosomosis and, to a lesser extent, *T. evansi* infection in water buffaloes, are associated with profound suppression of host immune responses to heterologous antigens introduced following the establishment of infection

(Rurangirwa *et* al., 1979; Rurangirwa *et* al., 1983). B cells appear to be one of the targets of immunosuppression as evidenced by greatly reduced IgG1 and IgG2 responses in cattle following vaccination against *Brucella abortus* (Rurangirwa *et* al., 1983). Nevertheless, the immunosuppression observed in trypanosomosis varies depending on the strain of parasite and animal breed. In mice, the immunosuppression has been attributed to polyclonal activation (Urquhart and Holmes, 1987). This polyclonal activation has been linked to the appearance of antibodies to foreign antigens, increased serum IgG concentration, massive plasma cell response in the lymph node and spleen and a relative reduction in specific antibody responses to vaccines (Hudson *et al*., 1976). Studies on the causes underlying immunosuppression have clearly demonstrated the role of macrophages, as removal of cells expressing Mac-1 (macrophages, CD5+ B-cell lineage and granulocytes) from *in vitro*-cultured lymph node cells led to 100% restoration of the proliferative reaction, while the depletion of the Thy-1+ fraction (T cells) failed to restore proliferation (Sileghem *et al*., 1994). The contribution of macrophage lineage to immunosuppression had been demonstrated earlier by Borowy *et al*. (1990) when the authors abrogated immunosuppression in mice through treatment with L-leucine methyl ester. Moreover, supplementation of cultures of spleen cells obtained from infected mice with accessory cells from uninfected ones restored proliferative activity (Grosskinsky and Askonas, 1981; Grosskinsky *et* al., 1983). Interaction of trypanosome-activated macrophages and T cells lead to up-regulation of interferon-gamma (IFN-γ) secretion by CD8+ T cells with subsequent suppression of interleukin-2R (IL-2R) expression on both CD8+and CD4+ T cells. This effect can be reversed by a 40-45 kDa protein derived from *T. brucei brucei* (Olsson *et al*., 1991; Olsson *et al*., 1992). Apart from modulatory immune responses in the bovine host, activated macrophages also play a key role in the removal of erythrocytes coated with immunoglobulins and even those exhibiting distortion of surface membranes. Erythrophagocytosis by the activated macrophages may be a major factor in the induction of extravascular anaemia (Murray and Dexter, 1988). Additionally, macrophages produce tumour necrosis factor-alpha (TNF-α), the secretion of which has been observed in *T. vivax* infection in which severe erythrophagocytosis and anaemia were evident (Sileghem *et al.*, 1993).

While cellular immune responses have been shown to play a role in immunity to murine trypanosomosis, there is no clear evidence that they perform similar functions in bovine trypanosomosis. It has been demonstrated that trypanosome-infected cattle exhibit an increase in the percentage of CD8+ and γ $^{TM}$T cells, while there is a decrease in CD2+ and CD4+ T cells (Lutje *et al*., 1995). Even though CD8+ T cells increase during trypanosome infection, recent data generated following depletion of this subset of cells indicated no modulating effect on the level of parasitaemia or anaemia.

The T cells produce a variety of cytokines which, when bound to specific cell surface receptors, modulate the growth, differentiation or function of the receptor-bearing cells. Interleukin -2(IL-2) and Interferon- gamma (IFN- γ) are secreted by the proliferative lymph node cells during infection (Sileghem and Flynn, 1992; Sileghem *et al*., 1994; Lutje *et al*., 1995). Interferon- gamma (IFN- γ) is known to stimulate macrophage activity and to promote surface expression of major histocompatibility complex (MHC) class I

and II on various cell types. However, the role of IFN- γ in immunity to bovine trypanosomosis remains unclear (Masake and Musoke, 1998).

From these observations it is evident that there is an urgent need to identify molecules responsible for the induction of protective immune responses. Identification of these molecules would greatly facilitate the development of an effective vaccine against trypanosomosis (Masake and Musoke, 1998).

**Control And Eradication**

Fly eradication and drug prophylaxis are the only effective trypanosomiasis control methods now available (Baral, 2009). Several approaches to fly control have been used with varying degrees of success. Discriminative bush clearing, extensively used in early tsetse fly eradication campaigns, has been locally useful because it eliminates the breeding places of the tsetse. But, to be completely effective, bush clearing requires ecologically unacceptable destruction of vast areas of bushes and forest. It is still a useful procedure when used locally in conjunction with other control methods. Game elimination, and thus elimination of the main source of blood meals for the tsetse, was used in early eradication campaigns. This was an ineffective and wasteful procedure. Application of the sterile male technique (as used in screwworm eradication in the United States) received considerable attention in the 1980's. Early problems with breeding of the male flies have been overcome, and field trials have been done in both east and West Africa to determine the effectiveness of this approach in vector control. In limited trials, this procedure has reduced fly populations (Baral, 2009).

Ground and aerial spraying with insecticides and the use of synthetic pyrethroids on cattle have lowered fly densities in some areas, but widespread use would require considerable international co-operation and expense. Widespread application of insecticide has the tremendous disadvantage of also eradicating many other arthropods, several of which are desirable. The recent introduction of odor-baited targets impregnated with insecticides is proving promising as a means of reducing the tsetse fly (Baral, 2009).

**Vaccination Attempt in Animal Trypanosomosis**

Attempts have been made by some workers to produce a protective vaccine against trypanosomosis both in humans and animals. One which seems to provide hope in this direction is the administration of anti-idiotypic (anti-id) antibodies to infected animals. Anti-id induces lymphocytes and antibodies of complementary specificity under certain experimental conditions (Benca *et al.,* 1980, Miller *et al.,* 1981). Injection of minute amounts of anti-id antibodies induces antigen-specific helper T-cells and enhances the expression of the corresponding id in subsequent antibody response (Kelsoe *et al.,*1980).

Administration of the anti-id produces antigen-binding id positive molecules in the absence of exposure to antigen, and therefore may be used to regulate the immune system by its expansion of B-cells clones bearing the appropriate id without specific antigen stimulation. Mice immunized against trypanosomosis with anti-id antibodies gave a partial to complete immunity to infection and this may be tried in dogs (Nwaoha, 2013).

Recently, dogs were vaccinated with a fixed *T. rangeli* against canine trypanososmosis (Basso *et al.,* 2007). Experimental infections of the vaccinated dog produced disease of low parasitaemia apparently from vaccine induced

immunity. Furthermore, feeding of the vaccinated dogs with the nymph stage of triatomine reduced the rate of infection in the bugs. Since dogs are the reservoirs of Chagas disease in man, advances in this area could reduce the rate of infection of kissing bugs which will in turn aid in the control of the disease in man (Basso *et al.,* 2007).

**Trypanotolerance**

Trypanotolerance is a relative rather than absolute trait, which is severely affected by heavy challenge, malnutrition, stress, breed, age, season, and concurrent disease (Feldmann and Hendrichs, 2001; Kalu, 1995). Trypanotolerant breeds are poorly utilized and accepted in husbandry practice because of their size, productivity and traction power compared with the large zebu breeds (Chater, 2002; Shaw and Hoste, 1987). Only 200,000 out of the 10 to 14 million cattle in Nigeria are trypanotolerant (Adeniji, 1993). The trypanotolerant breeds of animals in Nigeria include; N'dama and Muturu cattle, West African Dwarf (WAD) Sheep and Goats (Enwezor and Lawal, 2003). Recently Abenga *et al*., (2005) described tolerance in local dogs infected with *T. congolense* (Abenga and Lawal, 2005). Trypanotolerant cattle serve as reservoir hosts of trypanosomes (Kalu, 1996). The absence of practical reliable markers of resistance or susceptibility is a constraint in selection for breeding. Trypanotolerance is not only a breed characteristic but a heritable trait that is not valued for all association but lost rather quickly with combinations of crosses which are economically inferior to improved breeds (Samdi *et al.,* 2010). The importation of trypanotolerant animals from other countries is further hampered by cost, susceptibility to local strain of trypanosomes and difficulty of importation permit. The absence of centers to subsidize , supply and adapt trypanotolerant animals by a pastoralist as a means of control has further hindered the use of this method as a control strategy against trypanosomosis in Nigeria (Samdi *et al.,* 2010).

**Treatment**

Treatment of African canine trypanosomosis is currently under a lot of challenge especially as regards the availability of effective trypanocides in the market. There have been developments of several compounds with efficacy against canine trypanosomosis, however none of these products have been produced in a large commercial scale or are even available in the market. The apparent unavailability of new trypanocides in the market has remained a great challenge to the treatment of the disease. Diminazene aceturate has shown efficacy when used to treat canine trypanosomosis at the dose of 3.5 mg/kg in *T. congolense* infection; 7 mg/kg in *T. brucei brucei* and *T. evansi* infections (Aquinos, 2007). Usually parasitaemia disappears after 48hrs post treatment. The constant use of diminazene aceturate over time has led to the development of resistant strains of canine trypanosomes. There are abundant strains of canine trypanosomes especially *T. brucei brucei T. congolense* and *T. evansi* which are refractory to diminazene thus resulting in repeated treatments of infected dogs and constant relapses (Doyle, 2009; Nwoha *et al.,* 2013). Treatment of American trypanosomosis is equally as difficult, as infected dogs often develop remodeling of the heart which gradually leads to heart failure.

Hence, treatment does not provide complete

recovery but only sustains the life of the dog for some reasonable period (Amoro, 2004; Desquenes *et al.,* 2001). The use of beta adrenergic blockers such as carvedolol, propanolol and atenolol could be beneficial to reduce the blood volume and cardiac output. This helps to reduce the stress on an ailing heart, low doses of angiotensin conversion enzyme inhibitors (ACEIs) and in particular enalapril, veno- or iono-dilators like prazosin or pimobendan, calcium transport and utilization of modifiers singly or in various combinations may be useful in attenuating the progression of heart disease to heart failure in infected dogs (Sisson, 1994; Wolley *et al.,* 2007) and therefore could be of some clinical benefit in cases of Chagas disease in dogs (Nwaoha, 2013).

**Drug Resistance in Animal Trypanosomosis**

Drug resistance, also called drug fastness, may be defined as a loss of sensitivity by a strain of an organism to a compound to which it had previously been susceptible. It implies failure of treatment and prevention, and if no other active drugs are available the animal has to rely on its immune defences alone to combat the disease (Uilenberg, 1998).

Until recently, diminazene aceturate and isometamidium chloride were considered the best therapeutic and prophylactic trypanocides, respectively. The former was reputed as the only drug to which trypanosomes do not easily develop resistance because of its rapid elimination from the system when compared with the more persistent prophylactic drugs such as isometamidium (Rushigajiki *et al.,* 1986). Unfortunately, this view is no longer accepted, as trypanocide resistance has been demonstrated conclusively under laboratory conditions by inoculation of trypanosome stocks into bovines and treating with correct drug dosage regimens, or by administering prophylactic drug dosages and then challenging with tsetse, infected with well-characterized trypanosome populations at regular intervals. Resistance has been reported with some field isolates of trypanosomes requiring up to 45 mg/kg diminazene aceturate as the minimum required dose to achieve cure (Chitambo and Arakawa, 1992; Peregrine and Mamman, 1993). Similarly, isometamidium treatment failures and shortened prophylactic intervals have been attributed to infections with drug-resistant trypanosome species (Sutherland *et al*., 1991; Peregrine *et al*, 1991). Widespread development of resistance by trypanosomes to homidium, another trypanocide that was extensively used as a prophylactic drug, has been reported from East and West Africa (Clause *et al*., 1992; Codja *et al*., 1993; Mulugeta *et al*., 1997).

Drugs used for animal diseases are generally subject to lower standards of quality control than those used for human diseases. Multiple generic variants of the drugs, including isometamidium and diminazene, are available and the variability in quality of these products is striking. The release of preparations that contain low quantities of active drug provokes ideal conditions for the selection of drug resistance as well as leading directly to therapeutic failure (Barrett *et al.,* 2012). This problem of drug resistance in trypanosomes appears to be spreading geographically to many regions in which trypanosomosis occur. So far, resistance to one or more of the three trypanocidal drugs used in cattle has been reported in at least thirteen countries in sub-Saharan Africa (Geerts and Holmes, 1998). More worrying however, are the reported incidences of field stocks that have developed multiple resistances to these trypanocidal drugs (Leach and Roberts, 1981;

Moloo and Kutuza, 1990; Ainanashe *et al*., 1992; Clause *et al*., 1992; Codjia *et al.*, 1993; Mulugeta *et al*., 1997; Afewerk *et al*., 2000). For instance, Codjia *et al*. (1993) isolated 11 stocks in 1989 and 10 stocks in 1993 from cattle in Ghibe valley, southwest Ethiopia, which were shown to be resistant to diminazene, isometamidium and homidium. Afewerk *et al*. (2000) also showed that clones of *T. congolense,* which were derived from primary isolates collected from relapsed cattle in the field after treatment with 1 mg/kg bw of isometamidium, were resistant to both diminazene and isometamidium when tested in mice. This indicated the appearance of a multiple drug resistant *T. congolense* population in northwestern Ethiopia. Recently, there there has been a demonstrated presence of isometamidium resistance in trypanosomes in cattle in Western Ethiopia and a recent study in the eastern province of Zambia indicated the presence of trypanosomes resistant both to isometamidium and diminazene (Sinyangwe *et al*., 2004).

There are a number of evidences to the increase in the prevalence and incidence of drug resistance in trypanosomes in sub-Saharn Africa. The first resistant trypanosome population identified in Burkina Faso was a *T. congolense* stock derived from a cattle primary isolate in Samorogouan, which showed resistance to isometamidium (Pinder and Authie, 1984). Subsequently, Clause *et al*. (1992) identified *T. congolense* isolates from the same area that were resistant to isometamidium, diminazene and homidium, such that trypanosomosis in cattle in Samorogouan was often not cured by some trypanocidal drugs administered at maximum dose rates. In a corresponding chemotherapeutic trial by these authors in previously unexposed Zebu bulls and Sahelian goats infected with one primary *T. congolense* isolate from Samorogouan, the parasite demonstrated a high level of resistance to all three drugs in addition to quinapyramine sulphate at 5mg/kg body weight in goats. McDermott *et al*. (2003) have recently reported a widespread resistance to both isometamidium and diminazene by trypanosomes in the Samorogouan region (Kenedougou Province) of Burkina Faso.

Before quinapyramine ceased to be manufactured (Holmes and Scott, 1982), it was widely used in livestock as a therapeutic and prophylactic drug (Fiennes, 1953; Ndoutmia *et al*., 1993). It was withdrawn not only because resistance to it in trypanosomes appears to develop quite easily and rapidly (Wilson, 1949; Unsworth, 1954; Newton, 1964; Leach and Roberts, 1981) but also because resistance is always associated with high levels of multiple resistance to diminzene, homidium and isometamidium (Mwambu and Mayende, 1971; Whiteside 1960). This has been confirmed experimentally by repeated treatments of infected mice with sub-curative doses of quinapyramine sulphate (Ndoutmia *et al*., 1993). Similarly, resistance to isometamidium was induced and increased 94-fold in *T. congolense* by repeated sub-curative treatment of infected mice with isometamidium, which was associated with different levels of cross-resistance to diminazene, homidium and quinapyramine (Peregrine *et al*., 1997). Nevertheless, it is known that cross-resistance between diminazene and isometamidium rarely occurs in trypanosomes in the field, thus they are used as a "sanative" combination to curtail the development of resistance to either drug (Whiteside, 1960; Moloo *et al*., 1987). However, strains with cross-resistance to diminazene and isometamidium have been demonstrated in the field and experimentally (Chitambo and Arakawa, 1991, 1992), although it is possible that cross-resistance

in these studies occurred because the stock may have consisted of two phenotypically distinct populations, since heterogeneity of drug resistance to diminazene and isometamidium has been demonstrated (Peregrine *et al*., 1991). The origin of multiple resistances to these trypanocides by trypanosomes in the field is unclear, but it has been suggested that it might be associated with cross-resistance between the different compounds as a result of their closely related molecular structures (Whiteside, 1960; Williamson, 1970).

Thus, drug resistance in trypanosomes poses a serious problem to livestock productivity in countries where it has been reported, unless checked and brought under control. The development and spread of drug resistance to the point where drugs become ineffective over large areas of Africa is probably the greatest risk to the future use of the existing three trypanocides. It is also possible that the market will shrink and manufacturers will become uprofitable because of the risk of drug resistance. Additionally, the spread of generic products, some of which are of doubtful quality, may undermine farmers' confidence in trypanocides (Holmes *et al*., 2004).

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