

Review

A review on trypanosomosis in dogs and cats

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Trypanosoma brucei brucei, *Trypanosoma cruzi* and *Trypanosoma congolense* were initially thought to be the only species of trypanosomes capable of causing diseases in dogs and cats. However, dogs and cats are challenged by diverse species of trypanosomes with varying virulence and pathogenicity. Dogs may develop clinical trypanosomosis by infection with *Trypanosoma evansi* but are refractory to *Trypanosoma rangeli* of man. Recently, a new species, *Trypanosoma caninum*, of unknown pathogenicity and mode of transmission has been reported in dogs. This review describes canine trypanosomosis as an entity of two types, African and American trypanosomosis. It describes the different species involved in each type of the disease condition, the emerging strains, the biological cycle, distribution, clinical symptoms, the pathology and treatment of various species of canine trypanosomes. It also describes different basic diagnostic techniques currently in use and progress towards development of vaccine.

Key words: Dogs, cats, *Trypanosoma brucei*, *Trypanosoma congolense*, *Trypanosoma cruzi*, *Trypanosoma caninum*, *Trypanosoma evansi*, *Trypanosoma rangeli*.

INTRODUCTION

Animal trypanosomosis has profound social, economic and biological implications for the affected regions (WHO, 2006; OIE, 2008; Finelle, 1973). Jones (2000) described canine trypanosomosis as a disease caused by *Trypanosoma cruzi* and *Trypanosoma evansi*. Today different species of trypanosomes have been implicated in causing trypanosomosis in dogs. Hence, canine Trypanosomosis is a disease caused by hemoprotozoan parasites: *Trypanosoma brucei brucei* and *Trypanosoma congolense*. The disease can also be caused by *T. cruzi* the cause of American Trypanosomosis known as Chagas disease in humans (Jimenez-Coelle et al., 2010; Tola and Muniz, 2010; Cohen and Gurtler, 2001; Doyle, 2006). Dogs are also infected by *Trypanosoma b. rhodesiense* and *Trypanosoma b. gambiense* of man (Samdi et al., 2006). It serves as reservoirs and maintenance of infection in humans vice versa. *Trypanosoma rangeli* is a non-pathogenic trypanosome

of humans which also infect dogs (CVBD, 2010). Its importance is mainly for creating confusion during diagnosis in cases of mixed infection with *T. cruzi* (CVBD, 2010). *T. evansi* is found sporadically in dogs causing severe and possibly fatal disease (Uilenberg, 1998; Finelle, 1973). Recently, *Trypanosoma caninum* of unknown pathogenicity has been isolated from an intact skin of a dog along with leishmania in south eastern Brazil (Madeira et al., 2009; Barros et al., 2012). Its mode of transmission is not yet known as efforts to infect triatomids failed (CVBD, 2010).

Canine trypanosomes cause infections of varying severities in dogs. The infection ranges from acute, sub-acute to chronic. 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 vegetation along

Table 1. Species of African and American canine trypanosomosis, predilection sites, therapeutic and management protocols.

| African canine trypanosomosis | Name | Species involved | Predilection site | Treatment (Diminazene aceturate) |
|--------------------------------|---------------|--------------------------|--------------------------|--|
| | | <i>T. brucei brucei</i> | Blood vessels and tissue | 7 mg/kg stat |
| | | <i>T. congolense</i> | Blood vessels and tissue | 3.5 mg/kg stat |
| | Surra/ Nagana | <i>T. b. rhodesiense</i> | Blood vessels | 7 mg/kg stat |
| | | <i>T. b. gambiense</i> | Blood vessels and tissue | 7 mg/kg stat |
| | | <i>T. evansi</i> | Blood vessels and tissue | 7 mg/kg stat |
| | | <i>T. rangeli</i> | Blood vessels | 3.5 mg/kg stat |
| | | <i>T. caninum</i> | Intact skin | 3.5 mg/kg stat |
| American canine trypanosomosis | Chagas | <i>T. cruzi</i> | Blood vessels and tissue | 7 mg/kg stat beta adrenergic blockers eg carvedolol, propanolol and atenolol, (ACEIs) eg enalapril, veno- or iono-dilators like prazosin or pimobendan, |

rivers and lakes and large wooded savannah landmass. The disease in dogs is often as a result of a bite from an infected tse-tse fly. However, dogs can also get the infection by ingestion of fresh animal carcasses that died recently from trypanosomosis and through oral experimental infection (Raina et al., 1985; Uilenberg, 1998). The disease is more common in free roaming dogs than in those housed in tsetse fly net protected kennels.

IDENTIFICATION OF *TRYPANOSOMA* SPECIES THAT INFECT DOGS AND CATS

Trypanosoma species that affect dogs and cats can be (Table 1) identified by the following morphological characteristics. *T. b. brucei* may be found in two different forms (WHO, 2006): the long slender form measuring about 17 to 33 µm long and about 3.5 µm wide, and the undulating membrane which is conspicuous with a free flagellum at the anterior end. Its posterior end is pointed with a small and sub-terminal kinetoplast (Francois et al., 2005; OIE, 2008; Turnbull, 2009). The short stumpy form measures about 17 to 22 µm long and about 3.5 µm wide 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). *T. congolense* have 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, tsavo) sub groups of *T. congolense* with different pathogenicity (Bengaly et al., 2002; Masumu et al., 2006; OIE, 2009). *T. cruzi* measures about 10 µm long, 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 in stained sample, the parasite assumes a C or U shape (De Souza, 1999). *T. rangeli* has a similar morphology to *T. cruzi* (OIE, 2008). *T. caninum* are 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.

Host affected

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

Intermediate host

Glossina spp. (tse-tse fly) and haematophagus 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 which are the primary vectors responsible for the transmission of African trypanosomosis and triatomine transmitters of American trypanosomosis in dogs (WHO, 2013; Serap et al., 2003; Hunt, 2010). Tse-tse flies are currently restricted between 14° latitude north and 29° latitude south of sub-Saharan Africa, affecting 10 million square kilo-

kilometers of landmass (Molyneux, 1997; Serap, 2003; WHO, 2010). African canine trypanosomosis have found its way beyond boundaries into Europe where apparently is devoid of tse tse fly. This is due to the diverse clinical forms of the different infecting parasites. *T. congolense* having three main strains of unequal pathogenicity causes diverse clinical conditions in the dog. The savannah strain of *T. congolense* produces 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. Asymptomatic strains therefore enhance the distribution of canine trypanosomosis beyond geographic boundaries. *T. evansi* is found mainly in Northern Africa, Near East, Far East, and Central and Southern America. It was spread mechanically by several haematophagus biting flies and vampire bats in Latin America (William and Deborah, 1997; FAO, 2007). *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; Barros et al., 2012).

American canine trypanosomosis (Chagas) 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 triatomines species commonly known as "kissing bugs" (Amoro, 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 in Brazil (Maywald et al., 1996).

Transmission

T. b. brucei and *T. congolense* are transmitted to susceptible dogs through tse-tse flies' bite during their feeding (Luckins, 1973; CBVD, 2010). *T. evansi* is transmitted mechanically in South America through vampire bats and by ingestion of infected herbivore meat (Steverding, 2008; Uilenberg, 1998). *T. 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). Infections 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 triatomine feeding sites on the body of the dog with infected saliva or through the vectors' excreta (CBVD, 2010). The mode of transmission of *T. caninum* is yet unknown.

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. 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. 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 is been observed that *T. congolense* can invade tissues under certain conditions (Adah et al., 1992). An infection with *T. evansi* produces similar clinical manifestation 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 the red cell and lymphocyte sequestration and an expanded macrophage population (Murray and Dexter, 1988).

In the liver, the Kupffer cells phagocytose 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., 1980). Dogs with pernicious anaemia may have centrilobular 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 trito-

mine. These vectors pass out *T. cruzi* in their faeces during blood meal which accidentally penetrates the feeding site on the dog. The bite causes itching and the act of scratching facilitates the penetration of parasites into the tissues. Acute Chagas disease is usually seen in young dogs between 5 to 6 months old (Caliari, 1996; Hunt, 2010). Such dogs may just die suddenly due to severe inflammatory reactions in the heart often confused with more common causes of heart disease (Eloy and Lucheis, 2009). Though this condition is rare expect in cases of invasion of large numbers of parasites into the heart (Ettinger et al., 1997). Dogs with acute experimental infections with *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 in man 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 echocardiography examination 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 shows 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 infiltrate consisting of small lymphocytes and occasionally macrophages and plasma cells (Adah et al., 1992; Murray et al., 1980). 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 meningo-encephalitis similar to those described in fatal cases of human trypanosomosis.

Clinical signs

Knowledge of the clinical/pathological features in response to *Trypanosoma* species infections in dogs has been supplemented by studies in dogs experimentally infected with these pathogens. Clinical manifestations of American canine trypanosomosis differ markedly from that of African canine trypanosomosis. 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). Dogs are refractory to infection with *T. rangeli* (CVBD, 2010). In dogs *T. b. brucei* is responsible for an acute disease with high parasitemia. 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 Nwoha, 2011a). Pyrexia is highest at the first peak of parasitemia, thereafter at parasitemic waves which often corresponds with the development of anemia (Aquino, 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 the eyelids, the lips and the skin beneath the lower jaw (Nwoha and Anene, 2011a). Some dogs develop keratitis which may result in unilateral or bilateral cornea opacity with moderate lacrimal discharge (Nwoha and Anene, 2011a). A few cases develop the neurological form of the disease usually in post-therapy. This form is similar to rabies and terminates fatally within few weeks. 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). *T. evansi* sometimes produces 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 syndrome 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 form of the disease is commonly seen in adult dogs after several months of initial infection and is characterized by ventricular arrhythmias and myocardial dilation. The cardiac insufficiency is initially detected on the right side and later progressed to left ventricular insufficiency (Ettinger et al., 1997). Cats may have pyrexia, convulsions and paralysis of the hind limbs (Kirchhoff, 2011).

Anemia in the chronic phase is not strictly associated with the presence of parasite 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 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. General lesions are congestive, inflammatory, coagulopathies, oedematous, degenerative and sometimes haemorrhagic changes 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, oedematous on cut surface, the liver and spleen are swollen and congested, while the kidneys are pale and on 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 become atrophy and sclerose (Katherine and Edith, 2004). There is hydrothorax and hydropericardium containing straw coloured fibrin flaked fluid (Nwoha and Anene, 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 with 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).

Diagnosis

The diagnosis of canine trypanosomosis is dependent on a combination of detailed clinical examination, proper sample selection/collection, sample size, appropriate diagnostic tests, and proper conduction of tests and logical interpretation of results. 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 of 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 at the tip of the ear yield a larger quantity of parasites when compared to venepuncture (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). 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 bubbles. It is then examined microscopically (magnification, 400x) with condenser aperture, phase-contrast or interference contrast for proper visualization (22 x 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. 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, 400x) 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 pathogen that causes 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. 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 μ l and 5 to 10 μ l 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 sometimes referred to as the capillary tube centrifugation technique or as the Woo test, 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, heparinized capillary tubes are three-quarter filled with the suspected blood sample containing 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 min. Trypanosomes becomes 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 low magnification of x100 or x200 for motile parasites. mHCT 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 (QBC): 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

hemoparasites including trypanosomes (Levine et al., 1989; Bailey and Smith, 1992). 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 μ l 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 fluorescing 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 mAECT 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 than most of the other described techniques because of large blood volume (300 μ l) 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 trypanosome transforms into large proliferating procyclic forms detectable within three to four weeks (François et al., 2005). The technique requires sophisticated equipment, it 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 (Truc et al., 1992). However, its effectiveness in the isolation of *T. congolense* and *T. brucei brucei* in dogs is yet to be determined.

Animal inoculation

Mouse inoculation may be used for the detection of posi-

tive cases with sub-clinical infections by the inoculation of specific pathogen free (SPF) mouse with blood sample 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. 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, is the fact that some strains of *T. congolense* do not replicate in the mice and the 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 trypanosome 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. The primer sets available for different *T. 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, a full trypanosome species identification requires that five PCR test can be carried out per sample, and therefore cannot be used as a routine diagnostic technique in dogs (OIE, 2008).

Serological techniques

Antibody-detection enzyme-linked immunosorbent assay (indirect assay)

The technique of antibody ELISA has recently been developed for use in the diagnosis of trypanosomosis in animals (Lumsden, 1977) and has been used in large-scale surveys of bovine trypanosomosis (Desquesnes, 1997; Hopkins et al., 1998), though 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 Terrestrial manual (OIE, 2008).

In bovine trypanosomosis, ELISA, using *T. congolense* or *T. vivax* precoated microtitre plates, has been developed for diagnosis of bovine trypanosomosis (OIE, 2008). Similar precoated 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. 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 to 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 absorbances 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 test 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 immunohistochemical techniques are of high sensitivity and little specificity such as (Ag, Ab) 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*. Recently, 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. 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; Luckins and Mehlitz, 1978) and camels (Luckins et al., 1979).

Thus, it may show similar sensitivity and specificity in the diagnosis of canine trypanosomosis. 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 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 min 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 have been conjugated to fluorescein isothiocyanate is added and then slide washed and incubated as aforementioned. A clear detailed procedure could be seen in OIE Terrestrial 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 which include high cost of the technique involving sophisticated microscope and cross reactivity between trypanosomal species. Therefore, IFAT cannot be used for routine test diagnosis of canine trypanosomosis.

Biochemistry 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, aspartate amino phosphotase ASP and LDH in the acute phase of Chagas disease in dogs. Eloy and Linchein (2009) observed hyperproteinaemia which contradicts the findings of Barr et al. (1991) on TP. The hyperproteinaemia was attributed to high antigenic stimulation associated with trypanosomosis (Aquino, 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).

However 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). Several workers have recorded decreases in TP 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 biochemistry changes in canine trypanosomosis are function of the diagnostic technique, expertise and physicochemical dynamics in the dog.

Treatment

Treatment of African canine trypanosomosis is an area that has been under a lot of challenges especially as regards to the availability of effective trypanocides in the market. There have been development of several compounds with efficacy against canine trypanosomosis, however none of these products have been produced in a large commercial scale or even available in the market. The apparent unavailability of new trypanocides in the market have remained a great challenge to the treatment of the disease. Diminazene aceturate have shown efficacy when used to treat canine trypanosomosis used at the dose of 3.5 mg/kg in *T. congolense* infection; 7 mg/kg in *T. brucei brucei* and *T. evansi* (Aquinos, 2007). Usually parasitaemia disappears after 48 h post treatment. The constant use of diminazene aceturate over time has lead 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 results to repeat treatment of infected dogs and constant relapses (Doyle, 2009; Nwoha and Anene, 2013; Chigozie et al., 2012). 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, propranolol and atenolol could be beneficial to reduce the blood volume and cardiac out. This helps to reduce the stress on an ailing heart, low doses of angiotensin conversion enzymes 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 HDs to HF in infected dogs (Sisson, 1994; Wolley et al., 2007) and therefore could be of some clinical benefit in cases of Chagas disease in dogs.

Vaccination

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 of dogs by its expansion of B-cells clones bearing the appropriate id without specific antigen stimulation. Mice immunized against trypanosomiasis with anti-id antibodies gave a partial to complete immunity to infection (David et al., 1982) and this may be tried in dogs.

Recently, dogs were vaccinated with a fixed *T. rangeli* against canine trypanosomiasis (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 reservoir of Chagas disease in man, advances in this area could reduce the rate of infection of kissing bug which will in turn aid in the control of the disease in man (Basso et al., 2007).

Differential diagnosis

Some diseases can be confused with clinical cases of trypanosomiasis in dogs and these include:

1. Acute trypanosomiasis with pyrexia: canine babesiosis, canine anthrax, canine anaplasmosis, canine haemorrhagic and septicaemia.
2. Chronic trypanosomiasis with anaemia and emaciation: canine ancylostomiasis, canine ascariasis, malnutrition and other haemoparasitosis.

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