Animal African Trypanosomosis in Sub-Saharan Africa and Beyond African Borders

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Abstract

This chapter mainly focuses attention on trypanosome infections occurring in domestic animal species, with particular emphasis on those in the African continent. Specific trypanosome species cause three major disease syndromes within Africa namely *nagana*, surra and Dourine. *Nagana*, mainly affecting domestic ruminants, pigs and pets in sub-Saharan Africa, is caused by *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma brucei* subspecies as well as *Trypanosoma simiae*, primarily transmitted by tsetse flies (*Glossina* species). Surra, caused by *Trypanosoma evansi*, mainly affects camels, horses, ruminants, pigs and dogs in North and East Africa. Beyond the African borders, surra occurs in the Middle East, Asia and Latin America. Unlike other trypanosome syndromes, Dourine is non-vector borne, but rather transmitted through coitus from stallions to mares and vice versa. It occurs worldwide wherever horses are reared in large numbers. Thus non-vector-borne trypanosomes have a wider geographical distribution beyond the African continent as they may also be spread through international trade.

Although some trypanosome species strictly cause disease to animals, others such as *T. b. rhodesiense* and *T. b. gambiense* cause disease to both animals and humans. In aggregate, trypanosome infections have serious socio-economic implications and significantly contribute to poverty and underdevelopment experienced in the affected regions where livestock production is the main

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livelihood of the local communities. As such, efforts towards effective control of the disease are justified.

10.1 Introduction

Agriculture and particularly livestock production are the main drivers of most of the sub-Saharan African economies. Indeed, the agricultural sector contributes significantly to the gross domestic products and employs the largest part of the populations in the region (Mukhebi and Perry 1992; Swallow 1999). The majority of the livestock is owned by small-scale pastoralists whose livelihoods revolve around their livestock (Mukhebi and Perry 1992; Simuunza et al. 2011). The livestock kept by such traditional farmers include cattle, goats, sheep, pigs, chickens, ducks, guinea fowls, camels and donkeys. Cattle, and to a lesser extent small ruminants, are the principal livestock reared by most of the small-scale pastoralists. Livestock production is critical as a source of the much needed protein (meat, eggs milk and dairy products), income, draught power as well as manure for enhanced crop production. Animal hide and fur are important in manufacturing industries. Livestock is also used as a medium of exchange and may further be kept just for prestige. As such, any level of impediment to livestock production has serious consequences on the livelihoods of local communities in sub-Saharan Africa. Unfortunately, however, livestock production faces several challenges, most of which are as a result of animal diseases caused by viruses, bacteria, protozoa, fungi, ecto- and endo-parasites. Vector-borne diseases, including trypanosomosis, theileriosis, babesiosis and anaplasmosis are among the major livestock diseases in sub-Saharan Africa (Mukhebi and Perry 1992; Simuunza et al. 2011; Kristjanson et al. 1999; Stich et al. 2002). This chapter will address animal African trypanosomosis (AAT), its distribution and the possible impacts on livestock productivity.

10.2 Animal African Trypanosomosis

Despite several decades of research, AAT remains one of the major vector-borne diseases with serious impediment to agricultural and economic advancement in sub-Saharan Africa (Coustou et al. 2012; Morrison and MacLeod 2011). It is caused by various trypanosome species mainly transmitted through the saliva of tsetse flies during a blood meal. Trypanosomes are unusual among the haemoprotozoan parasites in that in the mammalian host, they are completely covered by a thick immunogenic coat called variant surface glycoprotein (VSG) (Borst and Rudenko 1994). Moreover, these parasites constantly modify their VSG by the process of antigenic variation that enables them to evade immune destruction but which is also responsible for the current challenge to design an effective vaccine against trypanosomosis. Infection with trypanosomes is also associated with suppression

of the host lymphocyte blastogenesis in response to parasite-related and -unrelated antigens. Such trypanosome-induced immunosuppression has been amply documented in livestock (Flynn and Sileghem 1991) and mice (De Baetselier 1996). This has been linked with increased susceptibility to secondary infections and decreased responsiveness to vaccination (Sileghem et al. 1994; Namangala et al. 2000; Radwanska et al. 2008). There is cumulative evidence that macrophages of a suppressive phenotype elicited during AAT are the central effector cells in the inhibition of lymphoproliferative responses to antigens and mitogens (De Baetselier 1996; Namangala 2011).

Tsetse flies, only found in Africa, belong to the genus *Glossina* within which three groups are recognised on the basis of their preference for habitat: (1) the riverine (*palpalis*) group (2) the forest (*fusca*) group and (3) the savannah (*morsitans*) group (Manful et al. 2010). AAT is mainly transmitted by *G. morsitans* (savannah species), *G. palpalis* (riverine species) and *G. fusca* (forest species). Other tsetse species that may transmit AAT include *G. pallidepes*, *G. austeni*, *G. swynnertoni* (savannah species); *G. longipennis*, *G. brevipalpalis* (forest species) and *G. tachnoides* (riverine species). Tsetse flies mainly inhabit thickets, particularly in national parks and game management areas where they primarily feed on wildlife habouring a number of different trypanosome species but do not suffer pathogenic consequences of AAT (Anderson et al. 2011). As such, they are referred to as "trypanotolerant" and serve as the major reservoirs of trypanosomes for domestic animals and humans (Namangala 2011). In the same vein, some West African cattle breeds such as the N'Dama, are also trypanotolerant and thus remain productive and gain weight even when they are infected with African trypanosomes (Taylor 1998). Chronically and sub clinically infected domestic animals may also serve as trypanosome reservoirs. During epidemics, tsetse flies and other haematophagous arthropods may also pick trypanosomes from acutely infected individuals with massive parasitosis to susceptible ones within the same vicinity.

Domestic animals get infected following encroachment of humans and their animals near or into tsetse-infested areas. Tsetse flies may thus pick bloodstream trypomastigotes from wildlife and transmit them as metacyclics to such domestic animal, usually with fatal consequences. Larger animals such as cattle (mainly adult cows and oxen), possibly due to the larger odour plumes they produce, and indeed their greater availability as compared to others livestock, are more attractive to tsetse flies than smaller animals that are usually kept in fewer numbers (Torr and Mangwiro 2000; Torr et al. 2006; Simukoko et al. 2007). Thus, in a given pasture where several domestic animals graze, their susceptibility to AAT is influenced by their size and age. As such, cattle tend to shield smaller and fewer livestock from AAT in most African communities. AAT is usually debilitating to affected animals. Although there are more reports on the impact of AAT in cattle, being the main livestock reared in sub-Saharan Africa, AAT is also a major constraint to the productivity of several other domestic animals including small ruminants, pigs, horses, camels and dogs (Snow et al. 1996; Matete 2003; Dhollander et al. 2006; Simukoko et al. 2007; Salim et al. 2011).

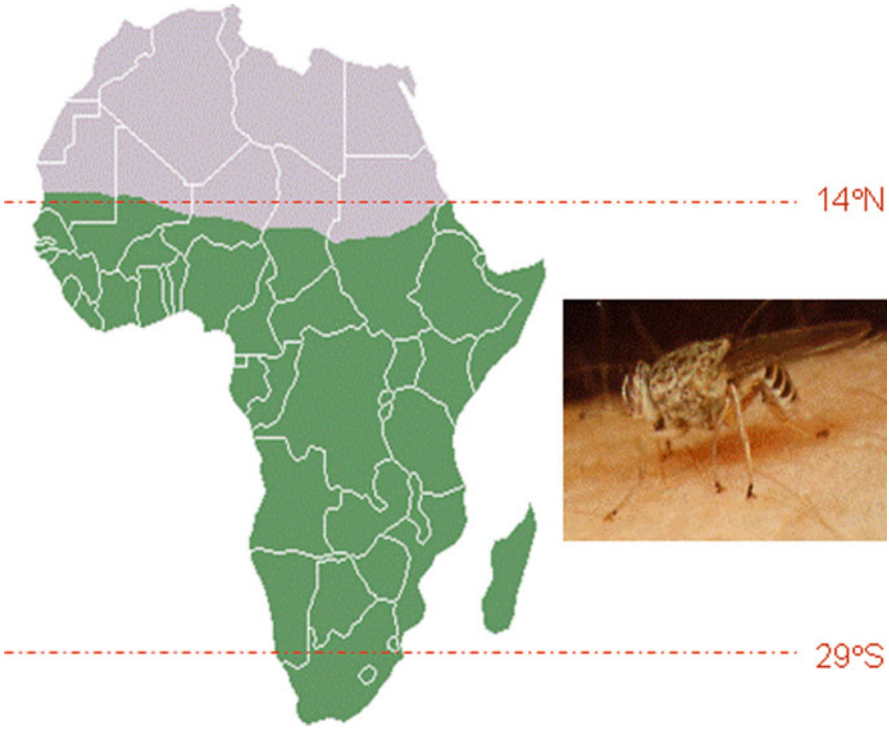

Fig. 10.1 Tsetse-infested sub-Saharan Africa extending between latitude 14° North and 29° South of the Equator (except for Madagascar and most of the Republic of South Africa below Latitude 29° South)

10.3 Aetiology, Transmission and Geographical Distribution

Unlike human African trypanosomiasis that occurs in specific foci within the 20 countries in sub-Saharan Africa, AAT is much more widespread in distribution. AAT occurs literally wherever there is livestock being reared in sub-Saharan Africa within a tsetse-infested region stretching 14° North and 29° South of the equator, affecting about 40 countries (Cattand 2001; Fig. 10.1). Depending on the trypanosome species, there are three major syndromes of AAT: (1) *Nagana*, (2) Surra and (3) Dourine (Table 10.1). Chagas disease, the fourth trypanosomosis syndrome, occurs outside the African continent, in Latin America.

10.3.1 Nagana

Nagana, mainly caused by the tsetse-transmitted *Trypanosoma congolense* (subgenus *Nannomonas*) followed by *Trypanosoma vivax* (subgenus *Duttonella*) and to a lesser extent *Trypanosoma brucei* subspecies (subgenus *Trypanozoon*), is the major

Table 10.1 Major characteristic features of animal trypanosomosis syndromes

	Trypanosomosis syndrome			
Parameter	*Nagana*	Surra	Dourine	Chagas disease
Aetiology	*T. congolense*, *T. vivax*, *T. brucei subspecies*, *T. simiae*	*T. evansi*	*T. equiperdum*	*T. cruzi*
Transmission	Mainly by tsetse flies; other biting flies may play a role	Biting flies	By coitus, non-vector-borne,	Blood-sucking triatomine bugs
Main susceptible domestic animals	Cattle, small ruminants, pigs, horses, dogs	Camels, horses, mules, donkeys, domestic ruminants, Indian elephants, dogs, cats	Horses, donkeys	Dogs, cats, pigs, rodents
Main clinical signs	Intermittent fever, emaciation, anaemia, abortion, lymphadenopathy, oedema of the throat and ventrum, ocular discharge	As for *nagana*	Mucopurulent discharge from genitalia, oedema of the genitalia, surrounding tissues and ventrum, anaemia, hyperaemia, skin plaques, nervous symptoms	Sudden death, lethargy, tachycardia, exercise intolerance, fever, anorexia, lymphadenopathy, diarrhoea
Geographical distribution	Sub-Saharan Africa	North and North-East Africa, Middle and Far East, Mexico, Central and South America	In all the continents wherever large numbers of horses are reared	Mexico, Central and South America

AAT syndrome affecting cattle, small ruminants, horses, pigs and dogs, in sub-Saharan Africa (Table 10.1). Although AAT affects several domestic animals, it is of major importance in cattle, being the dominant animals reared in sub-Saharan Africa. *Nagana* is a Zulu word that means "to be in low or depressed spirit", describing the debilitation or wasting associated with AAT (Stephen 1986). *Trypanosoma congolense* accounts for more than 80 % of AAT in domesticated animals (cattle, goats, sheep, horses, pigs, dogs) in Southern, East and Central Africa and the losses associated with the disease (Van den Bossche et al. 2006; Simukoko et al. 2007; Laohasinnarong et al. 2011; Tafese et al. 2012). Even in West Africa, *T. congolense* remains one of the major causes of AAT in livestock (Nakayima et al. 2012; Takeet et al. 2012). Clinical signs of *T. congolense*-infected animals are aspecific and include intermittent fever, abortion, cachexia, anaemia, lympadenopathy, lethargy, anorexia, oedema of the throat, ventrum and forelimbs, ocular discharge and death (Taylor 1998).

Trypanosoma congolense is further classified into three different types: Savannah, Forest and Kilifi (Young and Godfrey 1983; Knowles et al. 1988). *Trypanosoma congolense* strains within the Savannah type are regarded to be the most pathogenic/virulent and widespread throughout the savannah ecosystem of sub-Saharan Africa (Bengaly et al. 2002). Whereas *T. congolense* Forest occurs in humid forest ecosystems of West, Central and East Africa, *T. congolense* Kilifi seems to be more restricted to East Africa and to a lesser extent in Southern Africa (Mamabolo et al. 2009). Although *T. congolense* is cyclically transmitted by members of the *Glossina* species, mechanical transmission by other haematophagous arthropods has also been reported (Desquesnes and Dia 2003). The latter is important in that *T. congelense* may still cause AAT in non-tsetse-infested regions inhabited by other biting flies.

In endemic areas where livestock constitute the main reservoir of infection, low pathogenic *T. congolense* parasites circulate, resulting in more chronic AAT and generally low impact of the disease (Van den Bossche and Rowlands 2001; Chitanga et al. 2013). However, epidemics of *T. congolense* AAT may occur with severe impacts (increased animal mortalities and reduced calving rates) especially following recent introduction of livestock to tsetse-infested areas occurring at the livestock/wildlife interface areas. In such situations, tsetse flies or other haematophagous flies transmit high virulent *T. congolense* strains to domestic animals from wildlife reservoirs (Van den Bossche and Rowlands 2001; Chitanga et al. 2013).

Trypanosoma vivax is the second most important cause of *nagana* to ruminants, pigs and horses in sub-Saharan Africa (see also Chap. 11). AAT due to *T. vivax* is generally milder than the one caused by *T. congolense*. In West Africa, *T. vivax* accounts for the majority of AAT cases (Adam et al. 2012; Sow et al. 2012). *Trypanosoma vivax* may be transmitted mechanically by biting *Diptera*. *Trypanosoma vivax* is also a major cause of trypanosomosis (with occasional reports of epidemics), mainly in ruminants, in Latin America (Galiza et al. 2011).

Trypanosoma brucei brucei, cyclically transmitted by tsetse flies, is the most widely distributed African trypanosome, affecting virtually every domestic mammal including ruminants, horses and dogs. Horses and dogs are highly susceptible to *T. brucei* infection, as well as the human-infective *T. b. rhodesiense*, resulting in acute fulminant disease (Stephen 1986; Matete 2003; Dhollander et al. 2006). However, the pathogenicity of *T. b. brucei* and *T. b. rhodesiense* in ruminants is considered to be relatively low. Rather, ruminants are considered to be domestic reservoirs of these trypanosome subspecies (Fevre et al. 2001, 2005). On the other hand, humans are thought to be the main reservoirs of *T. b. gambiense*, although pigs, and to a lesser extent, sheep and goats, are implicated to be domestic animal reservoirs in West and Central Africa (Simo et al. 2006; Cordon-Obras et al. 2009).

Trypanosoma simiae (subgenus *Nannomonas*) causes an acute fatal disease mainly in pigs and to a lesser extent in camels and sheep in the tsetse-infested sub-Saharan Africa. Although the above trypanosome species and subspecies may cause monolytic infections in specific domestic animals, co-infections of a

combination of two or more different trypanosome species and/or subspecies are frequently reported in the field.

10.3.2 Surra

Surra is the trypanosome syndrome caused by *Trypanosoma evansi* (subgenus *Trypanozoon*). *Trypanosoma evansi*, mechanically transmitted by haematophagous *Diptera*, particularly those belonging to the *Tabanidae* family, was first discovered as the causative agent of surra in a horse in India by Griffith Evans in 1880. The local people recognised the disease as an occasional scourge which they termed "surra", denoting anything rotten (Stephen 1986). Surra, with similar clinical manifestations to *nagana*, is now known to mainly affect camels, horses and water buffalos. Donkeys, mules, goats, sheep, cattle, Indian elephants, dogs and cats are also susceptible to surra (Stephen 1986; Delafosse and Doutoum 2004; Dobson et al. 2009) (Table 10.1). The disease tends to be of much more economic importance in camels than in any other animal species. In equine species, horses are more susceptible to surra, followed by mules and donkeys. In dogs, the disease is usually acute with fatal consequences within a few days in the absence of treatment. In Africa, surra is confined to the northern and north-eastern part of the continent. Surra is also endemic in the Middle and Far East, Asia, Mexico, Central and South America (Stephen 1986; Delafosse and Doutoum 2004; Dobson et al. 2009).

10.3.3 Dourine

Dourine, a trypanosome syndrome of horses and donkeys, is a unique trypanosome syndrome in that it is non-vector borne. Instead, it is naturally transmitted by coitus from stallions to mares and vice versa (Stephen 1986). In stallions, clinical signs include oedema of the penis, scrotum, prepuce and surrounding skin up to the chest; anaemia; severe loss of weight; inguinal lymph nodes may be swollen; and moderate mucopurulent urethral discharge (Table 10.1). In the case of mares, clinical signs include vulval oedema with profuse fluid discharge; ulceration of vulva mucosa; oedema of the perineum, udder and abdominal floor; hyperemia; anaemia and emaciation. Nervous signs usually follow genital involvement and generalised loss of condition. Unlike in the case of *nagana* and surra, these clinical signs are pathognomonic.

Dourine is also referred to as "Covering Disease" or "Horse Syphilis", denoting its sexual transmission (Stephen 1986). The causative agent for dourine is *Trypanosoma equiperdum* (subgenus *Trypanozoon*). This parasite requires only the vertebrate host to complete its developmental cycle. Because of non-requirement of specific vectors for its transmission, it is widespread in its distribution, occurring in all regions of the world where large population of horses are found, including Europe, USA, Latin America, Africa and Asia (Alemu et al. 1997; Clausen et al. 2003) (Table 10.1).

10.3.4 Animal *T. cruzi* Trypanosomosis

T. cruzi infections or "American trypanosomiasis", referred to in humans as Chagas' disease, is a tropical parasitic zoonosis caused by a *Stercorarian* trypanosome. It is mainly found in the rural areas of Mexico, Central America and South America, where it is mostly transmitted to humans and domestic animals (mainly dogs, cats, pigs and rodents) by the infected faeces of triatomine bugs ("kissing bugs") during blood meal (Table 10.1). Clinical signs of Chagas disease are mainly seen in young animals. In these animals, the disease may be acute or chronic, with symptoms such as sudden death, tachycardia, exercise intolerance, lethargy, fever, anorexia, lymphadenopathy and diarrhoea. The chronic form may be completely asymptomatic. Wild and domestic animals, as well as subclinically and/or chronically infected humans, are natural reservoirs of *T. cruzi* (Bern et al. 2007). Originally, *T. cruzi* only affected wild animals. It later spread to domestic animals and people (Dias et al. 2002). Over 25 million people in Latin America are at risk of contracting Chagas disease (Dias et al. 2002; Bern et al. 2007). The resurgence of Chagas disease and its increasing geographic distribution is mainly due to large-scale population movements from rural to urban areas of Latin America and to other regions of the world. It may also be spread through blood transfusion, vertical transmission or organ donation.

10.4 Wild Animals as Reservoir of African Trypanosomosis

Wild animals are important reservoir of human and animal trypanosomes (Anderson et al. 2011). Infection of wild animals with animal trypanosomes occurs under experimental condition (Olubayo et al. 1991) and in nature (Anderson et al. 2011; Van den Bossche et al. 2011). Natural infections are encountered in African buffalos, duikers, bushbucks, impalas, greater kudus, warthogs, giraffes, elephants, zebras, lions and leopards (Anderson et al. 2011) supporting the occurrence of sylvatic–domestic transmission cycle (Swai and Kaaya 2012). The frequency of transmission is high where buffer zones surrounding game reserves have been turned into grazing ground (Anderson et al. 2011). So far no effective strategy for controlling trypanosomiasis in wild animals exists. Tsetse control is a strategy most applicable in this situation. However, the use of fly traps and insecticides for controlling tsetse vector is not feasible on large area.

10.5 Diagnosis of Anila Trypanosomosis

Unlike Dourine, field diagnosis of *nagana* and surra is difficult mainly because clinical and post-mortem signs of these AAT syndromes are not pathognomonic. Therefore diagnosis must rely on laboratory techniques that confirm the presence in blood of trypanosomes or the presence of anti-trypanosomal antibodies.

10.5.1 Clinical Signs and Post-mortem Findings

The course of trypanosomosis is variable depending on host and parasite factors. Generally clinical signs associated with *nagana* and surra in domestic animals include intermittent fever, wasting, lymphadenopathy, loss of condition, pallor, abortion in pregnant animals and lacrimation. Infected animals are usually weak and lethargic and hence lag behind the herd. The course of the infection may be acute (particularly when animals have just been introduced to a tsetse-infested area), subacute or chronic (mainly in endemic areas), with possible fatal consequences in the absence of intervention. Anaemia is thought to be the most pathogenic consequences of infection with African trypanosomes (Van den Bossche and Rowlands 2001; Taylor 1998). Furthermore, post-mortem examination of an animal that died of AAT reveal generalised carcass emaciation, enlarged lymph node, enlarged liver and petechial haemorrhages of the serosal membranes, especially in the peritoneal cavity. Although these findings could help veterinarians suspect AAT in a herd, they are not fully diagnostic as several other endemic disease conditions including malnutrition, tick-borne diseases, tuberculosis and intestinal helminths exhibit similar clinical signs and post-mortem findings to trypanosomosis.

10.5.2 Microscopy

The detection of circulating trypanosomes in host blood, cerebral spinal fluid and lymph biopsy by light microscopy is specific, definitive and is the most reliable applied method for diagnosis of AAT (Chappius et al. 2005). Body fluids may be directly examined as wet smears for trypanosome presence by light microscopy. The trypanosomes are detected by their movement among the blood cells. In addition, trypanosomes may be examined in Giemsa-stained thin or thick smears. Thin blood films preserve the morphology of trypanosomes and are useful in morphological differentiation of species (Büscher et al. 2009). Thick smears are useful for the detection of scanty trypanosomes.

However, the disadvantage of the above techniques is their low sensitivity, mainly in view of the fact that in endemic areas, parasitaemia in domestic animals naturally infected with trypanosomes tends to be low. Various concentration techniques, including body fluid centrifugation, aimed at improving the sensitivity of microscopy, are currently available. Animal blood in a heparinised (or EDTA) capillary tube may be centrifuged and the resultant buffy coat examined for the presence of trypanosomes (Waiswa and Katunguka-Rwakishaya 2004). Centrifugation has further advantages in that packed cell volume (PCV), used for anaemia determination, is simultaneously determined.

10.5.3 Xenodiagnosis

Xenodiagnosis is the inoculation of trypanosome-infected blood into laboratory animals such as mice, rats and rabbits. This technique can be used to detect some *T. congolense* and *T. brucei* (but not the non-rodent-adapted *T. vivax*) infections. The rodent inoculation technique may be more sensitive than microscopy, especially when the parasitaemia is scanty. However, its disadvantage is that not all trypanosome species, including some strains of *T. congolense* and *T. brucei*, become established in the rodents (Duleu et al. 2004).

10.5.4 Immunodiagnosis

Commonly used techniques in AAT serodiagnosis include the enzyme-linked immunosorbent assay (ELISA), complement fixation test (CFT), indirect fluorescent antibody test (IFAT) and card agglutination test (CATT) (Monzon et al. 2003). Serological tests are particularly useful in the diagnosis of Dourine and surra. However, the disadvantages of serodiagnosis are (1) strong cross-reactions among antibodies to various pathogenic trypanosome species (Desquesnes et al. 2001) and (2) no distinction can be made between the past (recovered animals) and present (active infection) infections, restricting their usefulness to measuring exposure. Antigen ELISA for detection of *T. congolense* was developed but later abandoned because of low specificity and sensitivity (Rebeski et al. 1999). The low sensitivity is caused by existence of circulating antigen in complex with host antibodies leaving few epitopes for attachment of monoclonal antibody used in the test (Rebeski et al. 1999). Therefore no commercial antigen ELISA for specific detection of *T. congolense* is available.

10.5.5 Molecular Diagnosis

Molecular techniques such as polymerase chain reaction (PCR) have significantly improved the sensitivity and accuracy of trypanosome diagnosis compared to the traditional parasitological methods (Chappius et al. 2005). For regular diagnosis, the sensitivity of PCR has increased to reach a level of 1 trypanosome/ml of blood, which may be up to thrice the sensitivity of microscopic observation of the buffy coat. PCR differentiates between trypanosome species and subspecies using specific primers (Desquesnes and Dávila 2002). However, the cost implications and requirement for highly skilled manpower are obstacles to their wide application in clinical settings in resource-limited sub-Saharan Africa. Advances in molecular methods of diagnosis have led to the development of a multispecies PCR capable of distinguishing all the major pathogenic trypanosomes of domestic animals in a single test, hence reducing the overall cost (Desquesnes and Dávila 2002). The internal transcribed spacer (ITS) region of rDNA is a preferred target for such a universal trypanosome test because of its highly conserved flanking regions and

size variability among trypanosome species and subspecies (Desquesnes et al. 2001; Desquesnes and Dávila 2002). Thus the ITS1-CF/ ITS1-BR and the KIN1/ KIN2 primers, both targeting the ITS1 of rDNA, offer promise in the routine diagnosis of pathogenic trypanosomes in clinical specimens from infected animals (Njiru et al. 2005).

Loop-mediated isothermal amplification (LAMP) is a novel strategy which amplifies DNA with high sensitivity and rapidity under isothermal conditions (60–65 °C), producing large quantities of DNA within 30–60 min (Notomi et al. 2000). LAMP has the advantage over PCR of being cheaper and simpler/easier to perform as it only requires a heating block or water bath and may hence be performed even in the field. Compared to PCR, the LAMP assay is also rapid; the whole process, including DNA extraction, can be done within an hour and the LAMP products can be visualised by naked eyes or through measurement of turbidity or fluorescence (Notomi et al. 2000; Thekisoe et al. 2007; Namangala et al. 2012) and may thus be more practical for resource-limited communities in sub-Saharan Africa where AAT is endemic.

10.6 Anaemia During Animal African Trypanosomosis

Anaemia is reportedly the most pathogenic consequence of infection with animal-infective trypanosomes (Logan-Henfrey et al. 1992). Thus all the three trypanosomosis syndromes (*nagana*, surra and Dourine) are associated with the development of anaemia in the affected animals. In particular, intravascular haematic trypanosomes such as *T. congolense*, *T. vivax* and *T. simiae* are associated with induction of severe anaemia (Losos and Ikede 1972). Although anaemia also occurs during infection with extravascular humoral trypanosomes (*T. brucei* complex and *T. evansi*), tissue degeneration and inflammation are the main pathologies associated with these species.

Although the exact mechanisms of anaemia during trypanosomosis largely remain unknown, they may involve interactions between the parasite molecules and host immune system, resulting in immunopathology. During trypanosome infection, a number of trypanosome stimuli and host immune mediators synergize to produce the haemophagocytic syndrome and the resultant anaemia (Naessens 2006). The aggressive parasitaemic waves occurring during early-stage trypanosomosis result in massive quantities of trypanosome molecules including soluble and membrane-bound VSG molecules in circulation, hyperactivation of macrophages (and concomitant production of tumour necrosis factor and interferon gamma) and other phagocytes which in turn lead to massive erythrophagocytosis in the spleen, liver and lymph nodes (Taylor 1998; Namangala 2011; Stijlemans et al. 2007). During chronic trypanosomosis, anaemia may be the result of insufficient haemopoiesis. Current evidence suggests that both non-specific erythrophagocytosis by hyperactivated phagocytic system and specific phagocytosis of damaged host cells contribute to anaemia (Taylor 1998). Erythrophagocytosis may

occur following deposition of immune complexes, together with C3 molecules, on erythrocytes or following generation of erythrocyte autoantibodies.

What could lead to the induction of auto-reactive antibodies during trypanosomosis? Firstly, this may be possible if trypanosomes share certain antigens with host erythrocytes. Members of the *Trypanosoma brucei* complex possess proteins that are similar to spectrin, a highly conserved structural protein occurring on erythrocyte membranes (Schneider et al. 1988). Evidence also suggests the existence of autoreactive antibodies that do not only recognise VSG antigens, but also human, murine and bovine epitopes (Müller et al. 1996). Furthermore, an increase in the levels of antibodies that bind to sheep and chicken erythrocytes has been observed in *T. vivax*- and *T. congolense*-infected cattle as well as *T. vivax*-infected mice (Musoke et al. 1981; Tabel et al. 1981; Mahan et al. 1986).

Secondly, current evidence suggests that *T. vivax* and *T. brucei* complex secrete enzymes including neuraminidases, proteases and phospholipases that may damage red blood cell membranes, exposing epitopes that are normally hidden (Taylor 1998). The latter will be recognised as "foreign" by the host immune system, resulting in the production of autoantibodies to the erythrocytes.

10.7 Impacts of Animal African Trypanosomosis

AAT is a major constraint to livestock productivity in about 40 sub-Saharan African countries, adversely affecting millions of people in rural communities who depend on livestock for their livelihoods (Coustou et al. 2012). Up to 11 million km^2 of potential grazing land in sub-Saharan Africa (nearly 50 % of total land in the region) inhabited by up to 300 million people is rendered unsuitable for livestock rearing (Mcdermott and Coleman 2001). AAT also has direct impacts on the number of livestock kept by farmers, the breed and species composition of the livestock herd and the grazing pattern of livestock (Swallow 1999). The potential benefits of improved AAT control on the continent, in terms of meat and milk productivity alone, are $700 million annually (Kristjanson et al. 1999). In aggregate, AAT significantly contribute to poverty and under-development in sub-Saharan Africa. The exact impact of AAT may not be measured accurately because of the many indirect effects the disease has to the affected communities and their livestock and the difficulty in carrying out such assessment. The following are some of the impacts AAT has on agricultural productivity mainly in rural sub-Saharan communities:

Livestock Mortality. AAT is detrimental to livestock productivity as it puts up to 50 million cattle and 70 million goats and sheep at risk, costing livestock producers and consumers up to $5 billion annually (Coustou et al. 2012). However, this is an under-estimation considering the numerous indirect losses encountered. Such loss does not only contribute to the continent's economic under-development but also deprive the affected communities of the much needed proteins from meat and milk.

Thus AAT reduces the availability of the animal draught power required for general transportation and particularly for crop cultivation, and further reduces the availability of cattle manure, both of which result in reduction in crop yield (Swallow 1999).

Reduction of Calving Rate. Recent data suggest that the largest and most consistent impacts of AAT are on birth rates and mortality of young animals (Swallow 1999). Compared to animals kept in AAT free areas, animals kept in areas of moderate risk of AAT have lower calving rates, lower milk yields and higher rates of calf mortality. Because of the fever associated with AAT, affected pregnant animal usually abort. In susceptible cattle breeds, AAT may reduce calving by up to 20 %, and causes the deaths of another 20 % of calves that are born alive (Swallow 1999). Current evidence suggests that AAT induces a reduction in the calving rates of even the so-called trypanotolerant cattle, sheep and goats in West Africa (Trail et al. 1993; Swallow 1999). Accordingly, AAT reduces calving rates by up to 12 % and increases calf mortality by up to 10 % in trypanotolerant cattle. Furthermore, AAT has been shown to reduce milk offtake by up to 26 % and lambing and kidding rates by as much as 37 % in trypanotolerant sheep and goats in West Africa (Swallow 1999).

Debilitation and Reduced Productivity. AAT is usually chronic and debilitating. As such affected animals are weak and lethargic, further contributing to the reduction in the availability and work efficiency of draught animals (oxen and donkeys) used for preparing land for crops. Thus the most important indirect impact of AAT on crop production is through compromising the availability and health of animals that provide animal traction (Swallow 1999). Additional traction capacity can allow farmers to expand the area that they cultivate, increase yields of existing crops, grow a different mix of crops or allocate labour, land and fertiliser more efficiently. Furthermore, in tsetse-infested sub-Saharan Africa, AAT reduces the offtake of meat and milk by at least 50 %, resulting in lower income from milk and meat sales and less access to liquid capital (Swallow 1999). Moreover, farmers are unable to successfully keep high-yielding exotic livestock breeds in such AAT-endemic areas, which have great economic consequences (Stich et al. 2002).

Compared to animals kept in AAT-free areas, animals kept in areas of moderate risk of AAT also require more frequent treatment with prophylactic and curative doses of trypanocidal drugs. African farmers and governments bear the increasing cost of treating cattle exposed to AAT. As such, at least $30 million is spent on administration of up to 30 million trypanocidal drugs and payments for veterinary services (Swallow 1999).

10.8 Treatment and Control

Because of the lack of an effective vaccine against AAT, current control measure against the disease is achieved by targeting either the parasite or the tsetse vector.

10.8.1 Targeting the Trypanosome

Early and accurate diagnosis is essential for successful treatment of AAT. Continuous surveillance of AAT is an important ingredient for successful treatment and ultimate control of the disease. Effective treatment contributes to disease control by reducing the number of infected individuals that may otherwise act as a source of infection to susceptible livestock within their vicinity. The main drugs used for AAT treatment are diminazene aceturate (berenil) and isometamidium (samorin) (Chitanga et al. 2011). Berenil, with only curative properties, has high activity against *T. congolense* and *T. vivax*, particularly those strains that are resistant to other trypanocides, and is hence effective in treating *nagana*. Furthermore, berenil has low toxic side effects. *Nagana* may also be treated curatively or prophylactically with isometamidium chloride (samorin). Berenil, samorin and suramin are also effective against surra (Tuntasuvan et al. 2003). However, although suramin and berenil may be used for treatment of Dourine, these drugs do not completely eliminate the parasites such the treated animals become carriers (Gillingwater et al. 2007). Thus if the aim is to eradicate Dourine, treatment should not be recommended.

Drug Resistance. Currently, single or multiple trypanocidal drug resistant has been reported in at least 18 African countries where the problem is increasing and rapidly spreading (Delespaux et al. 2008). Mechanism of resistance varies with drugs; resistance to diminazene aceturate is caused by low drug uptake resulting from of mutation P2-type purine transporter (Delespaux et al. 2008), whereas isometamidium chloride resistance is attributed to active drug efflux (Sutherland and Holmes 1993) or reduced uptake (Delespaux et al. 2005). Emergence of strains that are resistance to drug is attributed to inappropriate drug use (Delespaux et al. 2002; Clausen et al. 2010).

Detection of Drug Resistance. Without carrying explicit experiment, it is difficult to differentiate parasitaemia due to drug resistance from re-infection (Glover 1948) and drug under dose. Direct and indirect approaches are being used for detection of drug resistant strains. Direct approach involves incubation of trypanosomes in various concentration of trypanocidal for a defined time period followed by establishment of treatment effect (Delespaux et al. 2008). Such direct tests are akinetoplastic induction (Chitambo et al. 1992), drug incubation infectivity test (Kaminsky et al. 1990; Kaminsky and Brun 1993) and drug incubation *Glossina* infectivity test (P. H. Clausen et al. 1999). An approach close to this is where mice or cattle is inoculated with field isolates and the patent animals are treated with specified curative dosage of trypanocidal (Whiteside 1963; Chitambo and Arakawa 1991; Eisler et al. 2001). Use of mice limits drug sensitivity test only to those isolates that able to grow in them.

Indirectly bio-markers can be used to detect drug-resistant strains and where there is doubt the test is performed in combination with a suitable direct in vitro method. Tests relying on detection of marker are PCR (Gall et al. 2004) and PCR-RFLP

(Vitouley et al. 2011; Moti et al. 2012). *Mbo*II-PCR-RFLP is used for detection of resistance to isometamidium chloride in *T. congolense*. With this technique resistant strains are distinguishable from sensitive strains based on size polymorphism of a putative gene. The gene in resistant and sensitive strains is 384 bp and 381 bp, respectively (Delespaux et al. 2008). It is also possible to differentiate diminazene aceturate-resistant strains with a variant of PCR-RFLP which uses *Bcl*I enzyme for digestion of amplified gene coding for P2-type purine transporter *Tco*AT1. In this case restriction patterns of resistant and sensitive strains differ because there is a mutation on the gene where guanine is substituted with adenine (Delespaux et al. 2008).

Wide-scale longitudinal survey for presence of drug-resistant trypanosomes in endemic is done (Wilson et al. 1976; Gall et al. 2004). In this survey trypanosome-free herd is administered chemoprophylactic treatment and continuously followed for development of parasitaemia for a given time period. Concurrently, level of isometamidium chloride in circulation is monitored by ELISA (Whitelaw et al. 1991). This approach distinguishes parasitaemia due to drug resistance from re-infection or under dose.

Targeting the Tsetse Vector. Elimination of the tsetse vector in the transmission cycle is critical and more sustainable in the reduction of the prevalence/incidence of tsetse-transmitted AAT. Current methods for tsetse population control methods include aerial application of ultra-low volume insecticide (such as pyrethroids), the use of insecticide-laced targets and traps, pour-on application on livestock and sterile insect technique (Torr et al. 2006). Chemical control depends upon sufficient contact between the tsetse fly and the insecticide for the fly to pick up lethal dose. Preference for vector control is now given to elaborate combination of techniques (Krafsur 2009). These combinations may include selective aerial and/or ground spraying to obtain an initial knock down of the flies, followed by deployment of targets, traps and screens and the use of insecticide (pyrethroid)-treated livestock and possibly achieving localised eradication through the use of the sterile insect technique (Krafsur 2009). Most of the earlier control methods including ground spraying of insecticides using dichlorodiphenyl-trichloroethane (DDT), the clearing of bush and extermination of native mammals that provide tsetse fly blood meals and act as trypanosome reservoirs, are no longer extensively applied due to environmental considerations (Krafsur 2009). The limitation of vector control in the control of AAT is its heavy reliance on the use of insecticides which are liable to inducing resistance to the targeted insects as well as the high cost of insecticides.

10.9 Going Forward

While progress has been made on fighting AAT, there are challenges which continue to hinder complete eradication of the disease. For instance, when compared to diseases of livestock such as Foot-and-Mouth Disease, Rinderpest and Anthrax, AAT is unknown by majority of people in communities where the disease

occurs (Machila et al. 2007). Lack of public awareness campaign and community involvement is an obstacle undermining AAT control programmes. For effective control of AAT control, more attention should be paid to community education. Where stakeholders are fully aware of the disease, instituting and implementation of control programme will become easy. Another important challenge is the increase in human population which has led to encroachment of tsetse-infested areas by livestock keepers in search for pastures. The dilemma can be prevented by promoting rangeland management to ensure availability of pastures. Another crucial factor which has contributed to the spread of AAT beyond its known boundaries is climate change caused by global warming. The first impact of global warming on livestock keeping is inadequate pastures and water for animals. This has lead to migration of livestock keepers in search for pastures and water, resulting in the spread of AAT. Secondly, some regions in Africa were too cold but with the global warming the climate became conducive for survival of tsetse vector and biting flies. The two situations have aggravated the spread of infection.

Much as our own failure is impeding AAT control, African trypanosomes are continuously evolving by means of genetic exchange (Tait and Turner 1990; Morrison et al. 2009). Genetic exchange could have serious negative impact on the control of African trypanosomes because it underlies emergence of strains with undesirable phenotypes such as drug resistance, high host diversity, high virulence, absence of usual diagnostic markers and unknown mode of transmission. Therefore to keep pace with ever-evolving trypanosomes, where feasible, research should be tailored to field isolates of trypanosomes than the usual laboratory strains.

Besides challenges being experienced, hope for stumping out AAT is in sight. Novel technologies have solved some hurdles which had delayed progress on vaccines, trypanocidal drugs and diagnostic tools research. In order to design effective tools for controlling African trypanosomes, an in-depth knowledge of the parasites biology is a requirement. To-date unravelling the biology of African trypanosomes is being aided by technologies such as RNA interference, microarray, mass spectrometer, in vitro culture system of trypanosomes and availability of full genome sequences of most pathogenic animal trypanosomes. Application of these technologies synergistically has proven useful for identification of virulent factors of trypanosomes and drug transporters boosting pursuit for anti-disease vaccine and trypanocidal drug development.

Regarding advance on development of screening test tools for African trypanosomes, there is a current shift from the conventional laboratory based methods to rapid test kits such as immunodiagnostic lateral flow test (Sullivan et al. 2013). This is a simple test which is suitable for field situation and interpretation of result does not require expertise. Cost of producing antibody is high and in the end this cost will be met by the poor farmers making the test kit unaffordable. Once fully established, Nanobody® (Nb) will be a suitable substitute for antibody because it is cheap and easy to produce. This thermo-stable recombinant protein is derived from cloning a gene which codes for the antigen-binding fragment of camelid IgG2 and IgG3 (Hamers-Casterman et al. 1993; Lauwereys et al. 1998). Moreover, Nb has robust finger-like complementary determining region three

(CDR3) domain used for antibody–antigen interaction and has got the ability to reach hidden epitopes usually inaccessible to the conventional IgG CDR 3 (De Genst et al. 2006). Recognition of unique epitopes by Nb is advantageous to circumvent low sensitivity encountered with monoclonal antibody-based tests where hosts' IgGs form immune complex with antigen, thereby depriving monoclonal antibody of binding to the shared exposed epitopes (Rebeski et al. 1999). Thus availability of a user-friendly and affordable on-farm diagnostic test kits will strengthen disease surveillance.

While the search for good vaccine candidate against AAT is on-going, breeding of livestock with natural resistance to trypanosomiasis is encouraged. The short comings of these indigenous trypanotolerant breeds include their low quality in terms of meat and milk yield. With genes responding to trypanosome infection under investigation (Noyes et al. 2011), in future, a more precise selection of breeding parents for desirable traits will be made possible.

African Union, through its technical arm, Pan Africa Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), in partnership with Foundation for Innovative New Diagnostics (FIND), is dedicated to eradicating African trypanosomiasis and tsetse vectors (Kabayo 2002). A strategic plan for action was drawn by the member states and progress is evaluated yearly. The programme success has been reported in Botswana and Namibia. The success story of these two countries is indeed an indication of tremendous achievement towards eradication of African trypanosomiasis worth emulation by other African states.

Finally, climate change, industrialization, population pressure and inherent parasite characteristics have rendered obsolete some strategies that were previously effective in controlling African trypanosomiasis. Thus current control methods against AAT should be re-evaluated and updated in order to achieve the desired goal. Furthermore, AAT is trans-boundary disease whose effective control requires a multi-disciplinary approach, targeting both the insect vectors and the trypanosomes; and active involvement by nations where the disease is prevalent. To design and effectively implement tsetse flies control; agro-foresters, wildlife scientists, entomologists and ecologists should be involved because they are conversant with the flies' habitat. Countries where trypanosomiasis is endemic should be at the frontline to fight the infection through continuous surveillance of the disease. In the end successful control of the disease will ultimately improve the livelihood of the communities where AAT is endemic.

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