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Review

Drug resistance in African animal trypanosomes: A review

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Animal African trypanosmiasis (AAT) is the most important factor contributing to the sub potential performance of livestock. AAT is caused by *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*. Chemotherapy and chemoprophylaxis are the major means of combating the disease. An estimated 17 million cattle are treated with trypanocides annually. The emergence of drug-resistant trypanosome strains is considered a serious problem in trypanosomiasis control particularly for the resource-poor, at-risk populations and farmers in Africa. Trypanocidal drug resistance is the decreased or absence of sensitivity of trypanosome strains to standard quality trypanocidal drugs at the dose recommended in a good veterinary practice. Different resistance mechanisms are acquired independently through exposure to different drugs. A good example for this is cross resistance for diminazene and isometamidium. Trypanosomiasis drug resistance has been officially reported in 21 African countries. Moreover, certain African countries reported the presence of multi drug resistance. Safeguarding the available trypanocidal drugs is mandatory to reduce the devastating impact of the disease.

Key words: Resistance, trypanosome, African animal trypanosomiasis.

INTRODUCTION

African trypanosomes cause human African trypanosomiasis (HAT) and African animal trypanosomiasis (AAT), a debilitating disease of humans and domestic animals in the humid and sub-humid zones of Africa, respectively (Muhanguzi et al., 2015). Most African trypanosomes are transmitted by tsetse flies (Glossinidae), which inhabit in many parts of the continent (Kebede and Animur, 2009). AAT is the most important factor contributing to the sub potential performance of livestock (Chanie et al., 2013).

AAT is caused by *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* species. *Trypanosoma evansi* causes 'Surra' in camels (*Camelus dromedarieus*) (Mbaya et al., 2010). Concurrent infections can occur with more than one species of trypanosome (IICAB, 2009). Even though the name of the disease is called as African trypanosmiasis, *T. vivax* and *T. evansi* by virtue of their transmission by

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haematophagous biting flies also occur in Asia, Central and North and Southern America (Hoare, 1966).

The occurrence of animal trypanosomiasis coincides with the distribution of tsetse fly vectors which includes the regions between latitudes 14°N and 29°S (OIE, 2013). AAT causes a great economic loss in the livestock industry with an estimated 3 million cattle death annually. Estimated direct production losses in cattle to African farmers are between 1 and 4 billion US dollars per year (Swallow, 2000; Abebe and Jobre, 1996).

Infection of cattle by one or more of the three species results in sub-acute, acute or chronic disease. The cardinal clinical sign observed in AAT is anemia, within a week of infection with *T. congolense* and *T. vivax*. Moreover, there is a pronounced decrease in packed cell volume (PCV), hemoglobin and red blood cell count. These manifestations are observed in goats experimentally infected with *T. vivax* (Osman et al., 2012).

Chemotherapy and chemoprophylaxis are the major means of combating the disease. An estimated 17 million cattle are treated with trypanocides annually (Kristjanson et al., 1999). However, effectiveness of these drugs is limited by factors such as parasite resistance (Afework et al., 2000; Melaku and Birrasa, 2013) and unacceptable toxicity (Onyekwelu, 1999). The emergence of drug-resistant trypanosome strains is considered a serious problem in trypanosomiasis control particularly for the resource-poor, at-risk populations and farmers in Africa (Kagira and Maina, 2007). The presence of indiscriminate drug utilization practices and subsequent complaints over the efficacy of the available trypanocidal drugs supplemented the presence of resistant strains (Fiarcloug, 1963).

TRYPANOCIDAL DRUG RESISTANCE

Drug resistance also called drug fastness (Uilenberg, 1998) is the heritable loss of sensitivity of a micro-organism to a drug to which it was sensitive (Sinyangwe et al., 2004). Similarly, trypanocidal drug resistance is defined as the decreased or absence of sensitivity of trypanosome strains to standard quality trypanocidal drugs at the dose recommended by the manufacturer and administered according to the good veterinary practice (Peregrine et al., 1996; Uilenberg, 1998). Information on the extent and significance of the problem of drug resistance is scant (Sinyangwe et al., 2004).

Over the last decades there has been a dramatic slowdown in the development of new antimicrobials, which increases the need to preserve existing antimicrobials (Grace, 2015). Due to this, only small group of chemoprophylactic and chemotherapeutic trypanocidal compounds are currently in use and new compounds are unlikely to be available (Barret, 2004). This is also the major limitation of trypanosomiasis treatment, because there is unwillingness of pharmaceutical companies to invest in development of drugs against trypanosomiasis (Geerts et al., 2010). Therefore, the exiting trypanocidal drug should be safeguarded. Moreover, it is clear that medicinal plants play a prominent role against various human and animal diseases. A variety of medicinal plants and plants extracts have been reported for their significant anti-trypanosomal activity (Assefa, 2017).

Practically, treatment is mainly carried out by the livestock owners themselves without any supervision by veterinary personnel (Geerts et al., 2010). Consequently, treatment given by livestock owners is not without serious drawbacks because most farmers do not have adequate knowledge on diagnosis and the appropriate drug to use even in areas of high prevalence of trypanosomiasis. Since trypanocides are frequently used in the absence of diagnosis or used to treat conditions of which they are not effective, emergency of resistance is apparent (Holmes et al., 2004).

When the trypanosome is resistant to more than one drug, it is considered as multidrug resistant. In this case, different resistance mechanisms are selected independently through exposure to different drugs. A good example for this is cross resistance for dimenazene and isometamidium (Black et al., 2001).

Different factors could affect the development of resistance against trypanocidal drugs; some of the major factors are as shown in Figure 1.

MECHANISMS AND GENETICS OF RESISTANCE TO TRYPANOCIDES

Diminazene

Diminazene was introduced onto the market as trypanocide for domestic livestock in 1955. But, in initial experiments, the compound was shown to be highly active against both trypanosome and *Babesia* species. It is the only trypanocidal drug used against *Babesia* spp. It is one of the most commonly used trypanocide drug (Geerts and Holmes, 1998). This might be due to its higher therapeutic index than other trypanocides in most livestock, low incidence of toxicity (Peregrine and Mamman, 1993) and being active against trypanosomes that are resistant to other trypanocides used in cattle (Williamson, 1970).

Even though it has low incidence of resistance, some trypanosomes might have innate resistance subsequent to the first usage of diminazene aceturate in the field. Population of *T. congolense* and *T.vivax* were described in Nigeria that appeared to be innately resistant to diminazene (Jones-Davies, 1968). Furthermore, Wiliamson (1960) suggested that west african popluation of *T. vivax* express a higher level of natural resistance to diminazene than *T. congolense.* However, whether such

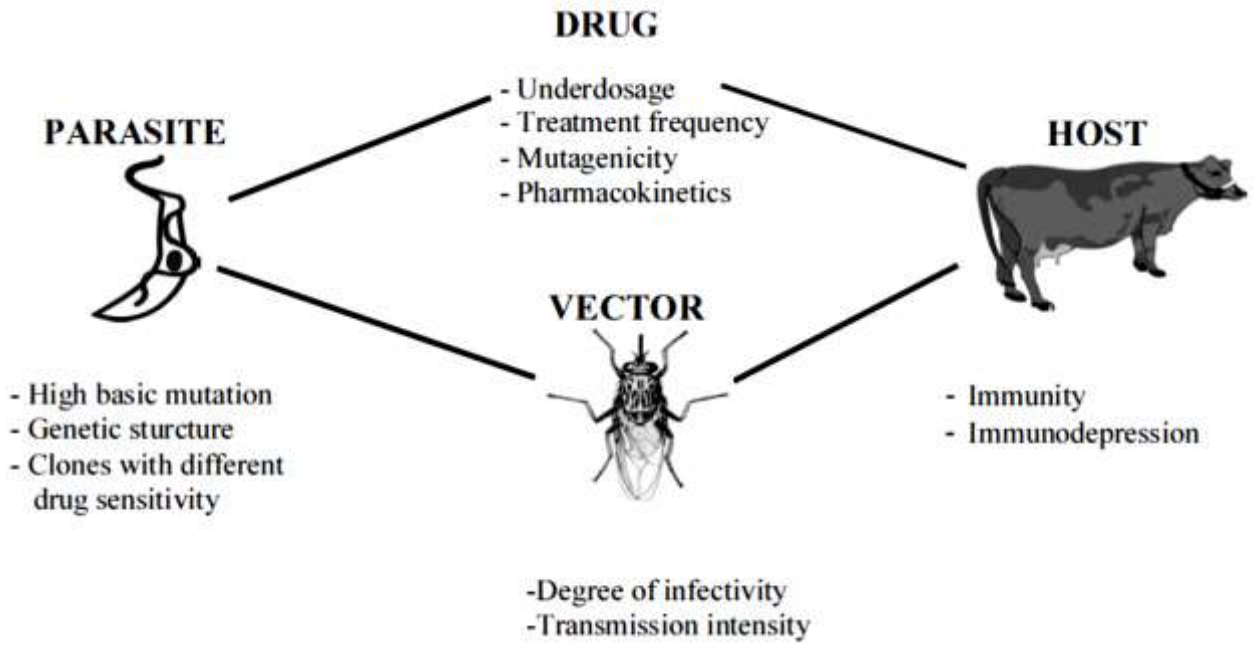

Figure 1. Major factors influencing the development of drug resistance (Geerts and Holmes, 1998).

resistance is a result of cross-resistnace induced by other drugs such as quinapyramine or natural is not clear (Gray and Roberts, 1971).

The accumulation of diminazene has been shown to be markedly reduced in arsenical-resistant *T. brucei*, *T. evansi* and *T. equiperdum* owing to alterations in the P_2-type purine transport system (Carter and Fairlamb, 1993; Carter et al., 1995).

It is also suspected that diminazene resistance is multi-factorial (Delespaux et al., 2006). The T*e*DR40 gene, which is a novel gene encoding protein that appeared to have a ubiquitous cellular localization (Witola et al., 2004), might be a contributing factor to resistance linked to the alteration of the gene coding for the P_2-type purine transporters. It is probable that diminazene resistance is the result of the cumulative effect of these two distinct resistance mechanisms (TeDR40 and P_2-type purine transporter) (Delespaux et al., 2006).

It has been urged that diminazene will not promote the development of resistant trypanosome strains even if sub-therapeutic doses are administered. But, resistance development as a result of the use of sub-therapeutic doses of diminazene and/or intensive use of the drug in the strain of *T. congolense* was reported. Further field studies confirmed the possibility of resistance in sub-therapeutic dose (Mbwambo et al., 1988).

Isometamidium

The amphiphilic cationic phenanthridine, isometamidium chloride, has been used in the field for several decades prophylactically or therapeutically for livestock suffering from trypanosomiasis. It is mainly used against infections with *T. congolense* and other *Trypanosoma* species (Leach and Roberts, 1981).

The first case of resistance to homidium and cross-resistance between homidium and isometamidium was reported in 1967 (Delespaux and De Koning, 2007). Decreased levels of drug accumulation have been observed in drug resistant populations of *T. congolense* (Sutherland et al., 1991). Experimental studies have demonstrated the occurrence of drug resistant trypanosomes to isometamidium (Schonefeld et al., 1987). This has been confirmed and reported by several authors (Codjia et al., 1993; Sinyangwe et al., 2004; Mulugeta et al., 1997).

The transport of isometamidium was known to be energy dependent, as it was reduced in the presence of metabolic inhibitors such as SHAM/glycerol (Sutherland and Holmes, 1993). In *T. brucei*, the P_2 adenosine transporter may be responsible for part of the isometamidium uptake as the drug inhibits P_2-mediated adenosine uptake (De Koning et al., 2005).

The mechanism of resistance to isometamidium chloride, however, is less clear despite certain suggested mechanisms from experimental finding results. Decreased levels of drug accumulation have been observed in drug resistant populations of *T. congolense* (Sutherland et al., 1991) and later work found indirect evidence of an increased efflux of drug from resistant trypanosomes (Sutherland and Holmes, 1993).

Resistance to isometamidium is mostly associated with cross-resistance to homidium (Peregrine et al., 1997),

and it could be speculated that these structurally related compounds might share the same uptake mechanism albeit that their distributions within the trypanosome are slightly different. Isometamidium is mainly concentrated in the kinetoplast, whereas homidium spread much more diffuse throughout the trypanosome (Boibessot et al., 2002).

Homidium

Homidium, which were introduced during 1950s, is active against *T. congolense* and *T. vivax* infections in cattle. Homidium salts are the main therapeutic drugs used in the management of clinical trypanosomiasis in animals (Holmes et al., 2004). It was widely used during the 1960s but due to the spread of resistance and its mutagenic activity, its use has greatly decreased (Holmes et al., 2004). The first report on the resistance of the drug was reported during 1960s (Jones-Davies, 1968).

The resistance mechanism to this drug is still unknown even if some studies suggested that it is similar to isometamidium (Shiferaw et al., 2015). Isometamidium differs from homidium by an additional moiety of *m*-amidinophenyl-*azo*-amine. The resistance mechanism thus seems to be specific for the homidium moiety, not the *m*-amidinophenyl-*azo*-amine functional group common to diminazene (Wragg et al., 1958).

Quinapyramine

Liao and Shen (2010) tried to look for physical changes, including proteins and genes, involved in the quinapyramine-resistance. Based on the confirmation made by other authors regarding the involvement of some enzymes in the production of drug-resistance in trypanosomes (Venegas and Solari, 1995; Iten et al., 1997), Liao and Shen (2010) detected for some isoenzymes. The enzymes analyzed were isoenzyme bands of hexokinase, glucose-6-phosphate dehydrogenase, alanine transaminase and aspartate aminotransferase, but they showed that the four isoenzymes were not involved in the quinapyramine resistance. And the soluble proteins of the 7 *T. evansi* lines was studied, their study implied that the two protein bands of 15.79 kDa in the lines, R1, R2, R4, R6 and R7, and 19.76 kDa in the three lines with higher levels of the antrycide resistance.

THE IMPACT OF RESISTANCE

The impact of drug resistant trypanosomes on livestock productivity has been assessed by fewer studies. But, it is imperative to assess not only the distribution of the problem, but also the constraints it encounters on effective control of the disease and economic impact (Melaku and Birrasa, 2013).

Increasing drug resistance increased trypanosome prevalence in cattle three-fold and in tsetse two-fold at a treatment frequency of once-a-month. The benefits of increased trypanocide treatment frequency diminish rapidly with increasing resistance (McDermott et al., 2000).

Previous study showed that trypanocidal drug resistance had no significant impact on the PCV and body weight losses of goats infected by *T. vivax* except for a few highly virulent strains. This conclusion contradicts the general believe that the development of resistance against trypanocides would leave farmers helpless in trypanosome infested areas (Vitouley et al., 2012).

A study to assess the impact of drug-resistant trypanosomes on the productivity of the local cattle was carried out by Codjia et al. (1993) in the Ghibe valley, Ethiopia. The area had a high prevalence of multiple drug resistance. In their study, the authors indicated that calf mortality was rather high, incidence of abortion was increased and the financial and economic returns were also affected.

DISTRIBUTION OF TRYPANOCIDAL DRUG RESISTANCE

The problem of drug resistance in animal trypanosomes is highly spreading geographically to many regions in which trypanosomiasis occurs (Grace et al., 2009; Geerts and Holmes, 1998). Decades after first case of drug resistance in trypanosomes, Clausen et al. (1988) confirmed multiple resistance trypanosomes isolates in the pastoral area of Samorogouan in Burkina Faso. Moreover, resistance developed by trypanosomes to trypanocidal drug, has been reported from East Africa (Mulugeta et al., 1997).

There is a report on a five-fold increase in the prevalence of dimenazene resistance over a seven year period in the Eastern Province of Zambia, suggesting, there might be a worsening of the problem. Trypanosomiasis drug resistance has been officially reported in 17 African countries (Burkina Faso, Chad, Ivory coast, Ethiopia, Kenya, Mali, Somalia, Sudan, Tanzania, Uganda, Zimbabwe, Zambia, Mozambique, Cameroon, Nigeria, Guinea, and Central African Republic) (Delespaux et al., 2008). But recently, this number is increased to 21 African countries (Tsegaye et al., 2015).

In addition, in 10 African countries including Ethiopia (Tsegaye et al., 2015), Sudan (Mohammed-Ahmed et al., 1992), Nigeria and Kenya, multiple drug resistance trypanosomes that decreased sensitivity to the common trypanocidal drugs (dimenazene, isometamidium and homidium) have been reported (Mulugeta et al., 1997;

Table 1. Report on drug resistant trypanosomes.

Country	Trypanosmes	Resistance to	References
Zambia	Tc	I	Sinyangwe et al. (2004)
	Tc	ID	Chitanga et al. (2011)
Mali	Tv	I	Mungube et al. (2012)
	Tc	ID	Mungube et al. (2012)
Burkina Faso	Tv	ID	Sow et al. (2012)
Mozambique	Tc	ID	Jamal et al. (2005)
Ethiopia	Tc	ID	Moti et al. (2012)
Zambia	Tc	I, D, ID	Sinyangwe et al. (2004)
Tanzania	Tc	I, D	Mbwambo et al. (1988)
Uganda	Tb	D, I	Kazibwe et al. (2009)
Zimbabwe	Tc	D	Joshua et al. (1995)
Kenya	Tc	I	Gray et al. (1993)

Tc: *T. congolense*; Tb: *T. brucei*; Tv: *T. vivax*; I: isometamedium; D: dimenazene; ID: both isometamedium and dimenazee.

Delespaux et al., 2008).

As it is suggested by Delespaux et al. (2008), it is suspected that in several other African countries, resistance is present but is yet to be demonstrated, because in several countries surveys for resistance have not yet been carried out or cases of resistance have not been published (Melaku and Birrasa, 2013).

Eze et al. (2015) conducted a study on trypanocidal resistance in trypanosomes isolated from animals to diminazene and isometamidium, in sub humid tropical zone of Southeastern Nigeria. Their results of the sensitivity tests showed that out of the ten isolates tested, nine were resistant to diminazene aceturate at the dose of 7.0 mg/kg out of which four were slightly resistant whereas all the isolates were sensitive to diminazene at 28 mg/kg. On the other hand, only two isolates were resistant to isometamidium chloride at the dose of 1 mg/kg out of which one was slightly resistant.

Clausen et al. (1988) and Grace et al. (2009) reported for the presence of multiple drug resistant *T. congolense* in tsetse infested areas of Burkina Faso. Trypanocidal drug resistance appears to be widespread in the Adamaoua, Cameroon. Apparently, the problem is more serious in *T. congolense* than in *T. brucei*, which is unfortunate because the former is more pathogenic to cattle than the latter.

Qadeer et al. (2015) tested the sensitivity of trypanosome isolates from Nigeria in experimentally induced trypanosomiasis, and their result showed the presence of resistant *T. vivax* strains to a particular dosage of 3.5 mg/kg diminazene aceturate and to all dosages of isometamidium. Some reports on resistant trypanosomes are shown in Table 1.

The two important drugs (dimenazene and isometamidium) have been used for more than 40 years in Ethiopia (Dagnachew et al., 2015). Some studies conducted on the few isolates of *T congolense* in Ethiopia have shown the prevalence of drug resistance that might pose a higher risk for the tsetse infested areas of the countries (Abebe and Jobre, 1996). Moreover, Afework et al. (2000) and Tewelde et al. (2004) have conducted a large scale survey demonstrating area-wide resistance in at least one region of the country.

The magnitude of drug resistant trypanosomes across Ethiopia is not well documented (Chaka and Abebe, 2003). The dynamic nature of the epidemiology of drug resistant infection in the Ghibe valley, which was reported to be 6% in 1986 and i1989, is increased to 14% (Rowlands et al., 1993). In addition, Mulugeta et al. (1997) indicated a long-term occurrence of *T. congolense* resistant to diminazene, isometamidium and homidium in cattle of Ghibe, Ethiopia.

Afework et al. (2000) detected for the presence of resistance in mice using clones of *T. congolense* which were obtained from the isolates collected from relapsed chattels treated with ismetamidium, in northern western Ethiopia. Those clones found were resistant to both diminazene and isometamidium.

In Tigray, Ethiopia, *T. vivax* has developed resistance to dimenazene and iseomtamidum (Wlyohannes et al., 2010). According to the study, relapse occurred to diminazene aceturate and isomethamidum chloride at 3.5 and 0.25 mg/kg of body weight, respectively.

Different authors have reported the development of drug resistance trypanosomes in Gibhe valley (Codjia et al., 1993; Shimelis et al., 2008).

The four isolates of *T. congolense* from Ghibe, Bedelle, Sodo and Arbaminch regions of Ethiopia were found to be resistant to the curative action of diminazene (in mice and cattle) and isometamidium (in cattle) at a dose rate of

3.5 and 0.5 mg/kg body weight, respectively (Chaka and Abebe, 2003).

Recently, Hagos et al. (2014) tested sensitivity of *T. congolense* field isolates to isomethamidium and diminazene in Konso district, Southern Ethiopia. The demonstration of resistance to diminazene was about 33% from their study, which also could be to isometamidium. Current *T. congolense* isolates resistance was unsurprisingly an expected outcome.

Dagnachew et al. (2015) also conducted *in vivo* testing of two isolates of *T. vivax* from tsetse infested and non-infested areas of Ethiopia. The authors concluded that for the presence of drug resistance *T. vivax* (more than 20 percent) against recommended doses of both the available trypanocidal drugs.

CONCLUSION

Treatment of trypanosomiasis is currently facing a number of problems including toxicity of trypanocidal drugs and development of resistance by the parasites. These limitations have prompted the search for alternative active substances (such as of natural origin). Different factors can lead to the development of trypanocidal resistance including the sub-curative dose utilization and mutagenic ability of some of the drugs .Calf mortality can be high, with increased incidence of abortion and the financial and economic returns were also affected due to the prevalence drug resistant strains of trypanosomes. Safeguarding of the currently available drugs is mandatory to preserve the effectiveness of the drugs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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