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Review on Drug Resistant Animal Trypanosomes in Africa and Overseas

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Abstruct: In Sub-Saharan Africa, about 80% of land is tilled by hand due to the high risk of African animal trypanosomosis that threatens the survival and use of draught animals. In these areas, trypanocidal drugs both prophylactic and curative are the most widely used methods of trypanosomosis control. Resistance of trypanosomes to trypanocidal drugs has been reported in twenty-one African countries, also a report on trypanocidal drug resistance was recorded outside of Africa; in China, Colombia and French Guiana. In addition, the occurrence of multiple drug resistance has also been reported in ten African countries. Under dosing and unsystematic program of treatments are two of the main predisposing factors for the development of drug resistance. At present, symbionts found in the tsetse fly gut make good candidates for delivery of foreign gene products which may provide an avenue for the control of the disease. To conclude, adopting an integrated disease management strategy with legislative reinforcement by way of elaborating a national drug use policy, training livestock owners and further study should be conducted to elaborate the problem of drug resistance. This seminar paper focuses on giving a brief overview on the drug resistance trypanosomes in Africa and overseas.

Key words: Drug Resistant Trypanosomes · Cattle · African Animal Trypanosomosis

INTRODUCTION

African animal trypanosomosis or Nagana ("Nagana" which is a Zulu word that means "powerless/useless"', "to be in low or depressed spirits") is caused by *Trypanosoma congolense*, *T. vivax* and *T. brucei* spp. while *T. evansi* causes Surra in camels (*Camelus dromedarieus*) [1]. Most African trypanosomes are transmitted by tsetse flies, which inhabit many parts of the continent that are restricted to latitude of about 15°N and 29°S of the equator [2]. Tsetse-transmitted trypanosomosis affects cattle production over approximately 10 million km^2 of Africa [3,4]. In Ethiopia, tsetse flies are confined to southwestern and northwestern regions between Longitude 33°and 38°E and Latitude 5°and 12°N an area that covers 220,000 km² [5]. In these areas, trypanocidal drugs are most widely used methods of trypanosomosis control. The control of trypanosomosis through the development of vaccine is hampered because of the unlimited antigenic variation. However, some attempts are still being made at vaccine development using internal non-variable antigens or at immunizing against proteins causing pathogenic effects, instead of against the parasite itself [6].

Trypanocidal drug resistance is defined as a loss of sensitivity of a species of trypanosome to a compound which it had previously been susceptible. Resistance of trypanosomes to the three trypanocidal drugs used in cattle (salts of Isometamidium (ISMM), Diminazene Aceturate (DA) and Homidium) has been widely reported and is considered to be increasing. Under dosing and unsystematic program of treatments are two of the main predisposing factors for the development of drug resistance [7].

Drug resistance would be a difficult problem to solve especially when it occurs in tsetse areas because once the cyclical vectors are infected with resistant strains, they will preserve them as long as they live and pass them to domestic or wild animals. As such if a resistant strain of *T. congolense* or *T. vivax* gets into the cycle of transmission in the tsetse and animals, it would take about 9 months before they are diluted and finally disappear.

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Nevertheless, drug resistance to ISMM is more widespread than to DA; there are increasing reports of multiple drug resistance [8]. Up to now the most important guidelines on the avoidance or delay of the development of drug resistance were considered to be: i) use of the "sanative pair" of drugs (ISMM or Ethidium and DA); and ii) avoidance of the exposure of trypanosomes to sub therapeutic drug concentrations [9].

Strains with cross-resistance to DA and ISMM have been demonstrated in the field and on experimental infection. The origin of multiple resistances to these trypanocides by trypanosomes in the field is unclear, but it has been suggested that it might be associated with cross-resistance between the different compounds as a result of their closely related molecular structures. Thus, drug resistance in trypanosomes poses a serious problem to livestock productivity in countries where it has been reported, unless checked and brought under control [10]. Therefore, the objective of this paper is to review on trypanocidal drug resistance in and outside of Africa with small inference to Ethiopia.

General Overview on African Animal Trypanosomosis: In Sub-Saharan Africa (SSA), tsetse-transmitted animal trypanosomosis is estimated to cause annual losses that run into billions of dollars. African animal trypanosomosis (AAT) is indeed considered one of the root causes of hunger and poverty in most SSA countries where it represents a serious impediment to sustainable agricultural rural development. About 80% of land in SSA is tilled by hand due to the high risk of AAT that threatens the survival and use of draught animals. The fight against the disease is either managed by the control of the vector or of the parasite or a combination of both [11]. However, in poor rural communities, which are mostly affected by the disease, control is mainly relying on the use of trypanocidal drugs.

The Parasite: Trypanosomes are unicellular protozoan parasites of the phylum *Sarcomastigophora*, order *Kinetoplastida*, family *Trypanosomatidae* and genus *Trypanosoma* [12]. They are haemoflagellated parasites characterized by one nucleus and one flagellum, either free or attached to the parasites body by means of an undulating membrane. They also usually contain a small compact kinetoplast, a disc-shaped DNA-containing organelle, situated within a large mitochondrion [13]. Kinetoplast DNA is arranged into a network of linked circles, grouped into 20,000 minicircles and 20-50 maxicircles. Three principal parasites namely, *T. congolense, T. vivax* and *T. brucei brucei* are known to transmit trypanosomosis in bovines normally via the bite of an infected tsetse. In some rare instances, trypanosome species like *T. vivax* and *T. congolense* are transmitted mechanically [14]. The different trypanosome species differ in morphological characteristics as described by Maudlin *et al* [15].

Trypanosoma congolense is divided into subtypes, with different distributions and pathogenicity: savannah type, forest type, Tsavo type and Kilifi type [16]. *T. congolense* savannah type is the most pathogenic one and is capable of causing severe anemia and even death of infected cattle [17]. *T. vivax* shows variable levels of virulence and distinct pathogenicity in West African isolates, whereas the East African isolates largely cause chronic infection [18]. In East Africa, there are two types of *T. vivax* isolates: the hemorrhagic *T. vivax* that causes an acute hemorrhagic syndrome and the mild strain [19]. Infections with *T. brucei* have been described as being chronic and sub patent, where cattle may act as important reservoirs of human pathogenic *T. brucei* species and can play an important role in the epidemiology of human sleeping sickness [20].

Life Cycle of Trypanosomes in the Tsetse Fly: Bloodstream African trypanosomes ingested by the tsetse fly embark upon a journey fraught with hazards. The aim of this journey is that their descendents should eventually be extruded in the fly's saliva to infect another mammalian host. In order to complete the journey, multiplication and change of parasite form must occur along the route as different environments are encountered. The exact pathway taken by the trypanosomes in the vector depends upon the species. *T. brucei* bloodstream forms on entering the fly undergo active division in the midgut as large procyclic trypomastigotes [21]. These penetrate the peritrophic membrane (PTM) of the gut to reach the ectoperitrophic space where they migrate forward to the proventriculus and cease dividing to become elongate mesocyclic trypomastigotes. In this form the parasites retraverse the PTM and migrate *via* the oesophagus, proboscis lumen and hypopharynx to the vector's salivary glands. Here a second bout of multiplication occurs during which the trypanosomes are anchored by their flagella to the vector's salivary gland epithelium after assuming the epimastigote form. The epimastigotes differentiate into the free, non-dividing metacyclic trypomastigotes that alone among the fly developmental forms can infect a mammal. The entire developmental cycle takes 3-5wk.

T. congolense has a similar developmental cycle except that the epimastigotes multiply attached to the chitinous wall of the proboscis (labrum) and the premetacyclic trypomastigotes swim to the hypopharynx where they mature into metacyclics. While, *T. vivax* omits the fly midgut phase, its procyclic stage occur deep in the foregut (cibarium) and quickly transforming to epimastigotes which invade the proboscis to generate metacyclics in the same manner as *T. congolense.* The time elapsing between trypanosome ingestion and extrusion of metacyclics may be as short as 10 days in this species. Not all flies ingesting trypanosomes produce metacyclics, in many the infection aborts. *T. brucei,* with the longest cycle, may produce metacyclics in only 2-5% of flies [21].

Mechanism of Action of Trypanocidal Drugs: In sub-Saharan Africa, treatment and prophylaxis of trypanosomosis in cattle, sheep and goats is dependent on the use of three compounds: Diminazene aceturate, an aromatic diamidine; homidium, a phenanthridine; and Isometamidium, a phenanthridine- aromatic amidine. Quinapyramine, a quinoline pyrimidine, is recommended for use against trypanosomosis only in camels and horses [22].

Diminazene Aceturate: DA is currently marketed under the trade names Azidine®, Berenil®, Ganaseg®, Ganasegur® and Veriben® as both a trypanocide and babesiacide for domestic livestock. Diminazene aceturate is recommended only for use as a therapeutic agent since it is rapidly excreted and therefore thought to have little prophylactic activity [23]. Diminazene binds to trypanosomal kinetoplast DNA and this binding does not occur by intercalation but via specific interaction with sites rich in adenine-thymine (A-T) base pairs. Through this specific interaction in trypanosomes, Diminazene inhibits synthesis of RNA primers, resulting in accumulation of replicating intermediates, thereby inhibiting kDNA replication [24]. Shapiro and. Englung [25], have shown that Diminazene specifically inhibits mitochondrial type II topoisomerase in viable trypanosomes. Thus, inhibition of DNA replication may also occur via this intercalation.

Isometamidium and Homidium: ISMM chloride (Samorin®), marketed since 1961 as a prophylactic and therapeutic drug and homidium (chloride salt; Novidium®; bromide salt or ethidium bromide: Ethidium ®), marketed since 1952 are phenanthridinium compounds. The antitrypanosomal activity of phenanthridinium drugs is blockade of nucleic acid synthesis through intercalation between DNA base pairs, inhibition of RNA polymerase, DNA polymerase and incorporation of nucleic acid precursors into DNA and RNA. Other biochemical reactions that may account partly to their effects include modulation of glycoprotein biosynthesis, lipid metabolism, membrane transport and selective cleavage of kinetoplast DNA minicircles [25].

Alternative Option Through Symbionts Found in the Tsetse Fly Gut: Symbionts with obligate functions in tsetse biology have been termed primary (P)-symbionts, while the more recently established commensal-like organisms, are referred to as secondary (S)-symbionts. Tsetse flies harbor both S- and P-type symbionts primarily in the gut tissue; *Sodalis glossinidius* [26] and *Wigglesworthia glossinidia* [27], respectively, (figure 1). A recent research conducted by Weiss *et al.* [28] showed that Wigglesworthia free (those flies that still house commensal Sodalis and parasitic Wolbachia) and flies that lacked all of their symbiotic microbes throughout their

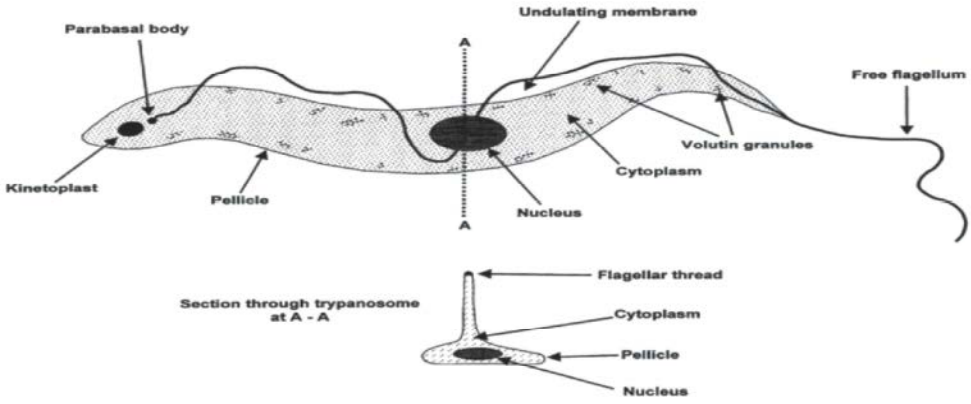

Fig 1: Diagram of a trypanosome showing the fundamental morphological features [71]

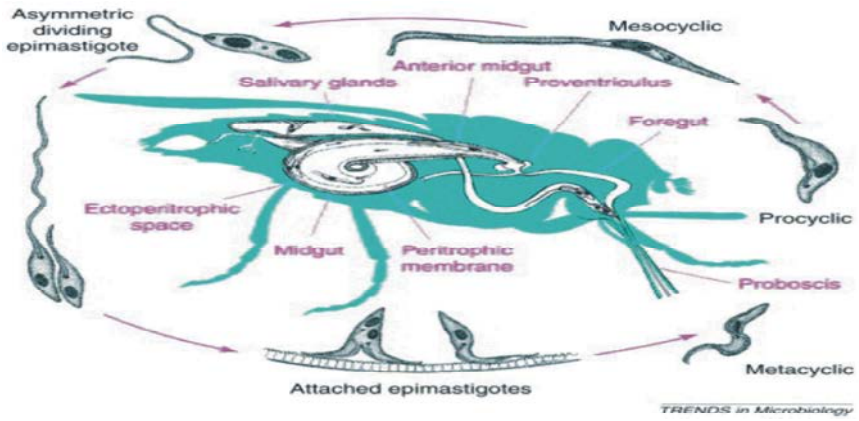

Fig 2: Schematic representation of trypanosomes in the tsetse fly [21]

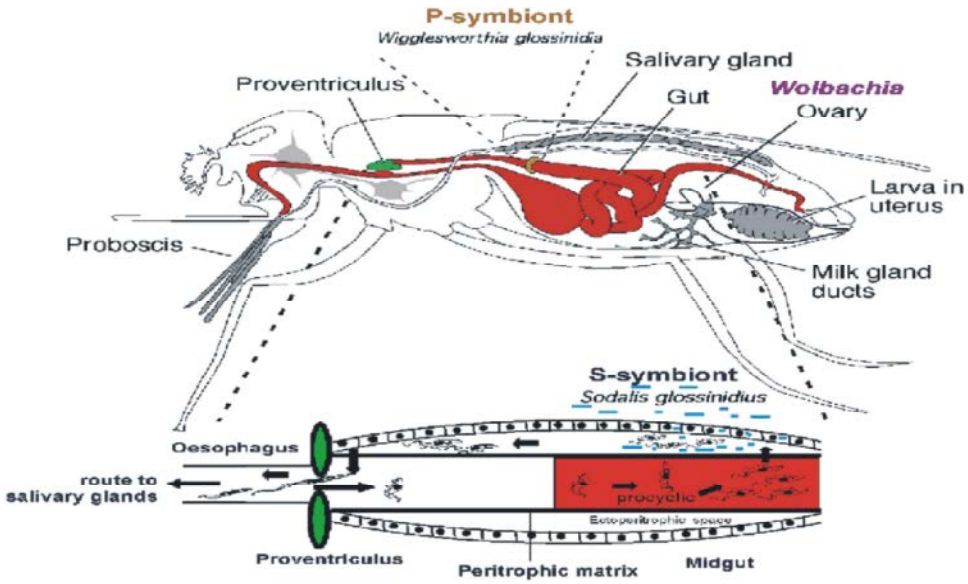

Fig 3: Diagrammatic representation of a female Glossina showing the tissue localization of symbionts and trypanosomes [29].

entire lifecycle flies exhibit a similarly high susceptibility to infection with trypanosomes indicating that obligate Wigglesworthia, as opposed to Sodalis or Wolbachia, is the primary modulator of tsetse's immune response following challenge with pathogenic trypanosomes. Extensive knowledge accumulated on tsetse and its symbionts now provides unique disease management opportunities based on the control of parasite development in its invertebrate host. Their close proximity to the developing trypanosomes in the gut, the ease of prokaryotic transformation systems and gene expression, as well as the soon available genome sequence information make these symbionts good candidates for delivery of foreign gene products [29].

Trypanocidal Drug Resistance

Mechanisms of Trypanocidal Drug Resistance: Trypanosome kinetoplast is the primary site of ISMM accumulation and decreased levels of drug accumulation have been observed in drug resistant populations of *T. congolense* [30] and Sutherland and Holmes [31] found indirect evidence of an increased efflux of drug from resistant trypanosomes. Mulugeta *et al.* [32] showed that the maximal uptake rates (V_{max}) of ISMM in resistant *T. congolense* were significantly lower than sensitive populations. It remains to be shown whether this is caused by a decreased number of protein transporters of ISMM in the plasma membrane and/or by changes in the balance between influx and efflux. The role of nucleoside transporters in resistance to ISMM by *T. congolense*

remains to be examined, although changes in these transporters have been associated with resistance to arsenical drugs in *T. brucei* [33]. More recently, changes in mitochondrial electrical potential have been demonstrated in ISMM resistant *T. congolense* by Wilkes *et al* [34].

Although DA probably exerts its action at the level of the kinetoplast DNA, this has not been proven *in vivo* and other mechanisms of action cannot be excluded [2]. Berger *et al* [35] showed that the accumulation of Diminazene was markedly reduced in arsenical-resistant *T. brucei* owing to alterations in the nucleoside transporter system (P2). Increased resistance to Diminazene was also observed in P2 deficient mutant of *T. brucei* [36] and recently, RNA interference silencing the adenosine transporter-1 gene in *T. evansi* conferred resistance to DA [37]. Those results are confirmed by De Koning *et al* [38], who conclude that the P2/TbAT1 gene mediates Diminazene transport almost exclusively explaining the observed Diminazene resistance phenotypes of TbAT1-null mutants and field isolates.

Pathogenicity and Interaction of Drug Resistant Trypanosomes with Tsetse Flies: Whether drug-resistant trypanosomes are less pathogenic than susceptible ones remains a controversial issue. Several authors [39, 40] have observed a loss of virulence in drug-resistant trypanosomes. However, transmission by tsetse flies does not appear to affect the sensitivity of trypanosomes. Recent studies at the International Livestock Research Institute (ILRI), used four populations of *T. congolense*, ranging from extremely sensitive to strongly resistant to ISMM and found that no differences in virulence between them; where only the most resistant one showed a reduced viability, i.e. it took longer to establish parasitamia than the other three [41].

Detection of Trypanocidal Drug Resistance

In Vivo Tests: A standardized protocol for the assessment of susceptibility and resistance to trypanocidal drugs in mice or in ruminants has been described [42]. Although there is a good correlation between the tests in mice and in ruminants, the curative dose that must be used in ruminants cannot be extrapolated from the results in mice. Another disadvantage is that *T. vivax* and some *T. congolense* isolates do not develop in mice [43].

In Vitro Tests: In vitro tests using bloodstream or metacyclic trypanosomes can be used to detect resistance in *T. brucei* and *T. congolense* [44]. A major disadvantage of these tests is the slow adaptation of the trypanosomes to the culture conditions. Furthermore, it is difficult to maintain *T. congolense in vitro* [45].Two alternative approaches for *T. congolense* have been evaluated in vitro incubation in the presence of various drug concentrations is sufficient. The first approach is the drug incubation infectivity test (DIIT) where trypanocidal drugs resistance is checked by determining the infectivity of trypanosomes. Under this principle the infectivity of drug resistant trypanosomes will be reduced after being incubated for 4hr in plasma samples derived from cattle treated with trypanocides. The second approach is the drug incubation Glossina infectivity test (DIGIT), where the main limiting factor being the availability of tsetse flies [46].

Trypanocidal Drug ELISA: The use of trypanocidal drug ELISA in combination with parasite detection tests has given promising results for the detection of resistant trypanosomes. The test is both sensitive and specific. It allows the monitoring of drug levels over extended periods and the evaluation of factors influencing drug disappearance rates from the plasma. One interesting finding has been that the drug disappears more rapidly in animals challenged and becoming infected with drug-resistant trypanosome isolates than in those challenged but protected against infection with sensitive trypanosomes [47]. The presence of trypanosomes in animals with an ISM serum concentration >0.4 ng ml^{-1} suggests that parasites are resistant [10].

Conventional Field Tests for the Detection of Trypanocidal Drug Resistance: Resistance to ISMM can be assessed under natural trypanosoma challenge in the field using the 'block treatment' approach [48]. Two groups of infected cattle, either treated with 1 mg kg^{-1} ISMM or untreated are exposed to natural challenge and tested for the presence of trypanosomes in the blood using the phase contrast Buffy coat technique every two weeks for two to three months. If >25% of ISMM-treated cattle become infected within eight weeks of exposure, drug resistance is strongly suspected. This approach can also be used for assessing whether there is suspected resistance to DA by treating the control group at the start of the experiment and all animals that become infected during the trial, with DA and checking for the presence of parasites two weeks after treatment [49]. Furthermore, longitudinal parasitological field data can be suitably analyzed using appropriate statistical techniques to detect problems of resistance to DA [50].

Table 1: Summary on drug resistant trypanosomes in Africa.

Country	Trypanosome species	Resist to (*)	References
Burkina Faso	Tc	I	[56]
		I,D,H	[57]
	Tv	I,D	[49,58]
Mali	Tc	I,D	[59]
	Tv	I	[59]
Mozambique	Tc	I,D	[11]
Kenya	Tc	I	[44]
	Tc	I,D,Q	[22]
Zambia	Tc	I,D	[7]
Zimbabwe	Tc	I,D	[60]
Kenya/Somalia	Tv	I	[61]
		H	[62]
Nigeria	Tv	D, H, I	[63]
	Tb	D, I	[64]
Sudan	Tc, Tv, Tb	H	[65]
Uganda	Tb	D, I	[66]
Ethiopia	Tc	D,H,I	[32]
	Tc	I,D	[9]
	Tc, Tv, Tb	I	[67]
	Tv	I,D	[68]
	Tc	D,I	[69]

(*) D = Diminazene Aceturate; H = Homidium Bromide (Ethidium); I = Isometamidium Chloride; Q = Quinapyramine; Tc = *T. congolense*; Tv = *T. vivax*; Tb = *T. brucei (Compiled Data).*

Genetic Markers for Drug Resistance in Trypanosomes: Due to the vast limitations of the currently available tests to validate drug resistance in trypanosomes, an alternative approach in the future may be made to identify genetic markers for drug resistance, which might be developed into reagents for the identification of resistant trypanosomes using the polymerase chain reaction (PCR). A PCR-based test could provide a rapid and convenient tool, suitable for large-scale epidemiological surveys of livestock. Developments of such tests require the identification of genetic mutations that may be associated with drug resistance in livestock infective trypanosomes [51].

Potential New Tests for Detection of Resistance to Isometamidium: Other tests that are still in the experimental trial or that are not used frequently. These tests based on the mitochondrial electrical potential (MEP) and the ISMM-ELISA technique. It has been suggested that variation of the MEP might be the primary factor determining the rate of ISMM accumulation in the trypanosome kinetoplast. Initial studies using a limited number of *T. congolense* populations have shown that an increased or decreased MEP might be a candidate quantitative marker for ISMM susceptibility or resistance [10].

Distribution of Trypanocidal Drug Resistance: The first case of drug resistance in trypanosomes was reported in 1967 in northern Nigeria [52]. At present, there are twenty-one African countries (e.g. Burkina Faso, Chad, Côte d'Ivoire, Ethiopia, Kenya, Nigeria, Somalia, Sudan, the United Republic of Tanzania, Uganda, Zimbabwe, the Central African Republic, Zambia, Cameroun, Mozambique, Benin, Ghana and Togo) in which trypanocidal drug resistance has been reported [7, 53]. In addition, the occurrence of multiple drug resistance to DA, ISMM and homidium has been reported in trypanosome populations in ten African countries [53], some of them include Nigeria, Kenya, Burkina Faso, Sudan and Ethiopia. Even more worrying are the recent reports of multiple drug resistance (to ISMM and DA) because this is threatening the last stand to overcome drug resistance through the use of the sanative pair [32]. The control of *T. vivax* in South America relies heavily on drug therapy, principally based on DA, with ISMM used in some areas. Since antitrypanosomal drugs are freely available, there is indiscriminate use by farmers and such practice is thought to be a major factor encouraging the appearance of drug resistant populations and resistance to DA has been reported in Colombia and French Guiana [54]. In China, *T. b. evansi* infection is a major health problem for many domestic animals and is at epidemic

level causing significant economic damage in about eight provinces, including Yunnan, Guangdong, Zhejiang and Jiangxi, during the 1980s and 1990s. Quinapyramine is a main drug that veterinarians use for treatment of trypanosomosis in local areas because of its low price, but many treated animals are not cured at the dosage recommended by the pharmaceutical supplier because of the emergence of drug-resistance [55]. Summary on the distribution of the different trypanosome species in the different countries of Africa is shown below (Table1).

Resistance to trypanocidal drugs has been reported from different parts of Ethiopia including Ghibe valley. Tewelde *et al* [67] reported ISMM resistant *T. congolense*, *T. vivax* and *T. brucei* in cattle in the upper Didessa valley of Western Ethiopia. Mulugeta *et al* [32] documented multiple drug resistance in *T. congolense* in the Ghibe river basin. Chaka and Abebe [70] reported the existence of resistant *T. congolense* originally isolated from cattle in the Southwest of Ethiopia, namely, Ghibe, Bedelle, Sodo and Arbaminch. Afewerk *et al* [9] also showed that clones of *T. congolense*, which were derived from primary isolates collected from relapsed cattle in the field after treatment with 1 mg/ kg bw of Isometamidium, were resistant to both DA and ISMM when tested in mice; this indicates the appearance of a multiple drug resistant *T. congolense* population in northwestern Ethiopia. The occurrence of *T. vivax* resistant population due to indiscriminate and frequent use of DA and ISSM was also reported in the Tselemti Woreda [68].

CONCLUSION

It can be concluded that when there are indications of drug resistance, it is essential to try to maintain the efficacy of the currently available drugs. The most important and most efficient measure is to adopt an integrated disease management strategy which includes the control of the agent and vector. Finally, some measures which can be adopted to delay the development of drug resistance and to control drug resistance when it occurs are recommended: adopting an integrated disease management strategy with legislative reinforcement by way of elaborating a national drug use policy is required to address the indiscriminate drug usage and to stop illegal traders, community participation is essential on the situation of trypanosomosis in the area on the characteristics and consequences of the occurrence of drug resistance strains of trypanosome as well as on the factors that lead to drug resistance.

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