1 INTRODUCTION

African trypanosomes cause a serious and often fatal disease commonly called nagana in domestic livestock. In cattle, *Trypanosoma congolense*, *T. vivax* and *T. brucei* are the main species responsible for this disease. Most African trypanosomes are transmitted to their mammalian hosts by tsetse flies, which inhabit many parts of the African continent that extend about 15° N and 20° S of the equator (Hoare, 1972; Donelson, 2003). The disease is among the major constraints to increasing livestock production in sub-Saharan Africa. The Food and Agricultural Organization of the United Nations (FAO, 1991) approximated that 60 million cattle are continuously at risk of being infected with trypanosomes. The presence of tsetse flies prevents people from moving cattle into the 7 million square kilometres of land, which apart from the presence of tsetse flies, is suitable for agriculture. This results in overstocking of tsetse-free areas with negative effects on environmental and productivity in these areas. If control of trypanosomosis could be effectively achieved there would be the potential, even at the current low rates of production, to increase both meat and milk supplies by approximately 16% and 17% of the current production level, respectively (De Haan and Bekure, 1991). This would be due to an addition of 33 million cattle in the trypanosomosis controlled areas (ILRAD, 1994). The impacts of this disease complex on the agricultural systems of African nations struggling to overcome the adverse effects of poverty and hunger are enormous.

According to Budd (1999), African farmers spend 35 million US$ per year on trypanocidal drugs in trying to protect and treat their cattle of this disease. Losses of meat production, milk yield, and traction power have been estimated at US$ 500 million annually. Furthermore, if lost potential for livestock and crop production are also considered, then trypanosomosis may be costing Africa as much as US$ 5 billion per year (ILRAD, 1994).

To date, there is no effective vaccine against trypanosomes. Hence, due to the lack of coherent and environmentally friendly and sustainable vector control strategies, the control of trypanosomosis continues to rely principally on chemotherapy and chemoprophylaxis using the salts of just three compounds: diminazene, an aromatic diamidine; homidium, a phenanthridine; and isometamidim, a phenanthridine-aromatic amidine (Leach and Roberts, 1981; ILRAD, 1990).

Unfortunately, drug resistance has developed in trypanosomes and the incidence of such reports appears to be increasing (Peregrine, 1994). So far, resistance to one or more of the trypanocidal drugs in use has been reported from 13 sub-Saharan African countries (Kalu, 1995; Geerts and Holmes, 1998) and in some countries multiple-drug resistance in *T. congolense* has been encountered (Clausen et al., 1992; Codjia et al., 1993; Afewerk et al., 2000). Resource-poor, rural populations and farmers at risk of trypanosomosis in Africa are facing a very serious problem because of this increasing development of resistance coupled with very little hope for the development of new drugs in the foreseeable future. Trypanosome resistance to trypanocides increases costs, reduces the efficacy of production and depletes farmers of effective control tools (Donald, 1994). Particularly, it is a major concern for smallholder crop-livestock farmers in West Africa, where a number of studies have confirmed significant resistance to trypanocidal drugs (ILRI, 2002). Similarly, in the frequently famine affected countries of Africa such as Ethiopia, where crop production is mainly dependent on draught power provided by ploughing oxen, reports on drug resistance in trypanosomes are increasing and alarming (Codjia et al., 1993; Mulugeta et al., 1997; Afework et al., 2000; Tewelde et al., 2004).

Isometamidium is the only recommended prophylactic drug that is widely used in the treatment of trypanosome infections in cattle and small ruminants across sub-Saharan Africa. Despite increasing reports of resistance development to this drug in various parts of sub-Saharan Africa, accurate data on the magnitude, distribution and frequency of emergency of isometamidium resistance are very limited (Geerts and Holmes, 1998; Geerts et al., 2001). Comparative data on the phenotypic and genotypic variations in isometamidium resistance in trypanosomes from different parts of sub-Saharan Africa are required. Therefore, this study was designed to characterise *T. congolense* and *T. brucei brucei* field isolates collected from epidemiologically well-defined areas in East and West Africa for their sensitivity to isometamidium. An attempt was also made to understand the role of the selection pressure of drug use in the development of isometamidium resistance in immunosuppressed animals, through induction of isometamidium resistance in a highly sensitive *T. congolense* clone[1] in mice by progressive sub-curative treatments. This will contribute to our understanding of the bio-mechanism of isometamidium resistance in *T. congolense*. In addition, the isogenic clones derived could be used for further comparative molecular studies on isometamidium resistance.

The common techniques currently in use to identify drug resistance in trypanosomes (tests in ruminants; tests in mice; and *in vitro* assays) suffer from a number of drawbacks. For example, requirements for large numbers of experimental animals, long periods in performing the tests, and difficult adaptation of the parasites to grow in tissue culture or laboratory rodents (Geerts and Holmes, 1998). Simple, more sensitive, fast and reliable methods for typing drug-resistant trypanosomes are undoubtedly required. These would help avoid further increases in resistance levels and the waste of resources by continued use of ineffective drugs.

Trypanosomes lack the ability to synthesize purine bases *de novo*. They therefore depend on purine bases or nucleosides pre-formed by their mammalian hosts for survival (Hassan and Coombs, 1988). Two classes of nucleoside transporters have been identified in *T. brucei*, the P1 type transporters that promote the uptake of adenosine and inosine and the P2 type transporters that mediate the uptake of adenosine and the purine base adenine. The P2 permease also transports such drugs as the pentamidine and melarsoprol (Carter and Fairlamb, 1993; De Konig, 2001). These investigators have linked the resistance to melaminophenyl arsenicals and diamidins to the loss/alteration of the P2 transporter activities. They have suggested that cellular uptake of these agents occur through the transport system specific to adenosine and adenine. The gene that encodes for the P2 transporter, TbAT1, has recently been cloned and studies have revealed several mutations in the gene in a laboratory-derived melarsoprol-resistant stock[2] of *T. brucei*. When expressed in yeast, this mutated P2 transporter could no longer import melarsoprol, and also isometamidium (Mäser et al., 1999). In these trypanosomes, resistance resulted from a reduced net drug uptake. By exploiting two simultaneously occurring mutations that generate a restriction fragment length polymorphism (RFLP) for Sfa NI, Mäser et al. (1999) devised a means of distinguishing between the arsenical-sensitive *T. brucei* stock and its resistant laboratory derivative. This technique has been evaluated by using *T b. gambiense* isolates from melarsoprol refractory patients in north-western Uganda, a *T. b. rhodesiense* isolate from south-eastern Uganda and a *T. b. gambiense* isolate from Angola (Matovu et al., 2001a). These authors reported similar Sfa NI RFLP patterns.

[1] Trypanosome derived from a single individual. The genetic uniformity of a clone cannot be expected to be conserved by continuous passage *in vitro* or *in vivo* (WHO, 1998).
[2] A population derived by serial passage *in vivo* and/or *in vitro* from a primary isolate, without any implication of homogeneity or characterization. Stocks derived at different times from a single primary isolate may differ (WHO, 1998).

This study aimed at validating the findings by Mäser et al. (1999) on the laboratory-derived melarsoprol-resistant *T. brucei* stock using *T. b. brucei* field stocks with known drug sensitivity phenotypes. Consequently, attempts were made to investigate if similar links exist between mutations in TbAT1-gene and isometamidium resistance in *T. b. brucei*, using known isometamidium sensitive field stocks from Uganda and sensitive and resistant reference clones. Furthermore, the study was conducted with the hypothesis that a similar transporter gene involved in Melaminophenyl arsenicals resistance in *T. brucei* (TbAT1) could also be present in *T. congolense*, and its presence could possibly play a role in isometamidium resistance in this parasite, as isometamidium uptake in *T. brucei* was found to be mediated by TbAT1 (Mäser et al., 1999). Thus, investigations were made for identifying a similar transporter gene in *T. congolense*.