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Ghibe river basin in Ethiopia: Present situation of trypanocidal drug resistance in *Trypanosoma congolense* using tests in mice and PCR-RFLP

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ABSTRACT

A cross-sectional study was carried out in the Ghibe valley from August to October 2010. 411 head of cattle were sampled in eight villages for buffy coat examination (BCE) and blood spots were collected from each animal for trypanosomose diagnosis by 18S-PCR-RFLP and diminazene aceturate (DA) resistance by Ade2-PCR-RFLP. Three villages were selected in a zone where trypanosomosis control operations are currently on-going whereas the other 5 villages were located outside these control operations. Twenty-four samples (5.84%) were diagnosed positive for *Trypanosoma congolense* by BCE and injected in mice for further characterization. Twelve of those isolates successfully multiplied in mice and were tested by an *in vivo* mouse test for diminazene (DA) (10 and 20 mg/kg B.W.) and isometamidium (ISM) (1 mg/kg B.W.) resistance. All were shown to be resistant to both drugs at all doses. The use of the Ade2-PCR-RFLP on these isolates confirmed their DA-resistance profile. Seventy-three of the collected blood spots (17.8%) were diagnosed positive for *T. congolense* by 18S-PCR-RFLP of which 37 (50.7%) gave amplification products with the Ade2-PCR-RFLP. Here, 35 (94.6%) showed a resistant profile, 1 (2.7%) a sensitive profile and 1 (2.7%) a mixed profile. The data were analysed by logistic regression model and the relapsing time in mice tests was assessed using the Cox regression model. There was no significant intervention effect ($P = 0.83$) with odds ratio equal to 1.21 when using the BCE data. 18S-PCR-RFLP test also showed no significant intervention effect ($P = 0.60$) with odds ratio equal to 1.43. The hazard ratio of getting parasitaemic after treatment with DA at 20 mg/kg B.W. compared to the control group was 0.38 which differs significantly from one ($P < 0.001$). Relapsing time after treatment with DA 10 mg/kg B.W. or ISM 1 mg/kg B.W. was also significantly longer than the prepatent period of the control group. The situation of drug resistance in the Ghibe valley is further discussed.

1. Introduction

Tsetse-transmitted trypanosomes are responsible for diseases unique to Africa affecting both humans and animals. They occur in about 10 million km^2 in 37 sub-Saharan countries (FAO, 2002) and constitute a major threat to

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the survival and productivity of domestic livestock in sub-Saharan Africa (Holmes, 1997). Trypanosomoses cause serious economic losses and an estimated annual loss in cattle production alone has been reported to be in the range of 1.0–1.2 billion dollars (FAO, 2002).

The situation of African Animal Trypanosomosis (AAT) has become even worse due to the fact that some drugs have been abandoned due to resistance (Matovu and Mäser, 2010). Practically, only ethidium bromide (Homidium®) and DA (Berenil®) are still available for therapy and ISM (Samorin®, Trypamidium®) for prophylaxis (Holmes, 1997; Geerts et al., 2001; Matovu and Mäser, 2010). The inevitable outcome of continued use of the same compounds for decades has resulted in drug resistance that has been largely responsible for the frequently observed chemotherapeutic failures. At present, trypanocidal drug resistance is reported in 18 countries of sub-Saharan Africa (Delespaux et al., 2008). Although tsetse and trypanosomosis research and intervention in the Ghibe valley of Ethiopia has started a long time ago (Rowlands et al., 1993; Codjia et al., 1993; Mulugeta et al., 1997), the disease persists to exert its deleterious effects on the most vulnerable segment of the society. Resistance to commonly used drugs has been reported from different parts of Ethiopia including Ghibe valley. For instance, Tewelde et al. (2004) reported ISM resistance of trypanosomes (*Trypanosoma congolense*, *T. vivax* and *T. brucei*) infecting cattle in the upper Didessa valley of Western Ethiopia. Mulugeta et al. (1997) documented multiple drug resistance in *T. congolense* at the Ghibe river basin. Chaka and Abebe (2003) reported the existence of *T. congolense* resistance originally isolated from cattle in the Southwest of Ethiopia, namely, Ghibe, Bedelle, Sodo and Arbaminch. In view of today's resistance reports from many parts of Ethiopia including Ghibe valley, intervention needs to be complemented with regular surveillance of the evolution of the trypanocidal drug resistance.

The aim of this study was to explore an easier and faster method of DA resistance diagnosis based on the Ade2-PCR-RFLP (Vitouley et al., 2011). Results from this Ade2-PCR-RFLP DA resistance test were compared with the gold standard, *i.e.*, the mice test (Eisler et al., 2001), to evaluate the newly proposed molecular test. The mice test was also used to diagnose ISM resistance. Based on the comparative results of the two drug resistance tests, the actual situation of drug resistance in the Ghibe valley is discussed.

2. Materials and methods

2.1. Study area

The Ghibe valley is 183 km away from Addis Ababa and located in Southwest Ethiopia. Farmers in and around the valley make their living by mixed farming systems and cattle production using traditional village management (Leak et al., 1993) for subsistence. The area receives a mean annual rainfall of 900 mm and the mean monthly maximum temperature ranges from 29.8 to 44.0 °C (Leak et al., 1993; Rowlands et al., 2001). The Livestock Research Institute (ILRI) is running interventions against trypanosomosis using monthly DA (Vetiben®) administrations (3.5 mg/kg B.W.) and monthly deltamethrin (cypermethrin®) pour-on application (20 ml/adult animal) at the expenses of the community since 1990. The intervention covers two sites namely: upper and lower Ghibe and more than 20 villages benefit from this activity. Our study involved both ILRI-intervention and non-intervention sites around the Ghibe river valley. Intervention was decided in priority zones were the impact of the disease was considered by the farmers as the highest based on their own perceptions. A total of eight kebeles (smallest administrative unit of Ethiopia) were selected to include three from ILRI-priority zones (Abelti, Yatu and Keta Bosso) and the other five from non-priority zones (Weira, Jatu, Wedesa, Shumoro and Gomsho). The vegetation coverage and thus, the tsetse habitat in the entire region are spotty and limited to the vegetation of the river banks or to protected forest zones because of the intensive agricultural expansion in the area. The choice of the kebeles was based on expert opinion taking into account a comparable vegetation of the river banks and equal distance between grazing zones and rivers to harmonize the tsetse challenge between the 8 study sites. In the non-priority zones, veterinary drugs including trypanocidal drugs are available freely in all rural shops or local markets and the farmers do not have the habit of presenting sick animals to veterinarians for proper diagnosis of diseases. Thus, the administration of drugs is usually done by the breeders often without veterinary advice. No information is available on the treatment frequencies, type of trypanocide, doses injected due to this unsupervised use of the drugs out of any legal sanitary framework.

2.2. Cross-sectional study

The cross-sectional study was carried out from August to October 2010. To obtain a 95% confidence interval for the prevalence of trypanosomosis that has width equal to 10%, a sample size of 377 animals is required, assuming that the real prevalence equals 50% to get the maximum sample size (Thrusfield, 1995). A total of 411 whole blood samples were collected from East African Zebu cattle in the study area.

2.3. Animals

A heterogeneous group of East African Zebu cattle in different age and sex categories were used for this study (198 males, 213 females, aged between 1 and 15 years old). Accordingly, all owners in every kebeles were informed to gather their animals the following day at the proposed sampling site. These 411 Zebu cattle were selected randomly using the lottery method.

2.4. Blood collection

From each cattle, 9 ml of whole blood was collected from the jugular vein into heparinized Venosafe tubes (Terumo) for further analysis.

2.5. Parasitological tests

2.5.1. Buffy coat examination (BCE)

The BCE was performed as described by Woo (Woo, 1970). Briefly, about 70 μl of blood was collected in a microhaematocrit capillary tube and sealed on one end. The capillary tube was centrifuged at 15,000 rpm for 5 min to concentrate the trypanosomes in the buffy coat layer. Then, the capillary tube was placed in a Woo viewing chamber and a cover slip of 24 mm × 24 mm was placed on the capillary tube hereafter the space between the tubes and the cover slip was filled with a drop of water to reduce light diffraction. The buffy-coat plasma junction was examined for the presence of trypanosomes and the Packed Cell Volume (PCV) was measured.

2.5.2. Isolation of trypanosomes

About 0.5 ml of the samples BCE-positive for the presence of *T. congolense* (simple or mixed infections) were injected intraperitoneally into three mice aged from 5 to 8 weeks and weighing on average 30 g each. When the parasitaemia reached a minimum of 7.1 on the Herbert and Lumsden scale (Herbert and Lumsden, 1976), the blood was collected by cardiac puncture under final anaesthesia.

2.5.3. Stabilate preparation

The cryomedium was prepared in advance by mixing one part of egg yolk to three parts of phosphate saline glucose buffer (PSG) and to three parts of Triladyl® (Minitube). The ingredients were carefully mixed and divided in aliquots of 0.5 ml in Sarstedt microtubes (Sarstedt) and stored at −20 °C until use. Stabilates were prepared by gently mixing 0.5 ml volumes of blood with confirmed presence of trypanosomes with an equal volume of thawed cryomedium in a Sarstedt microtube that was suspended in the vapor phase of liquid nitrogen for 1 h before being immersed in the liquid phase for long term storage.

2.5.4. Drug resistance tests in mice

The protocol described by Eisler et al. (2001) was used with minor modifications. In this study, use was made of male and female Swiss-white mice aged between 8 and 10 weeks and weighing between 25 and 30 g. They were housed in clean cages at room temperature in fly-proof conditions, fed with commercial pellets and water *ad libitum*. The mice were screened for the presence of blood parasites using wet and Giemsa-stained thin blood films prior to the beginning of the experiment.

Parasitaemic blood of each *Trypanosoma* isolate was diluted in PSG to obtain a final concentration of 10^5 trypanosomes per ml and inoculated into 4 groups of 6 mice each. Twenty-four hours after inoculation, the mice of three groups were treated with the trypanocidal drugs. The first group was treated intraperitoneally with 1 mg/kg B.W. ISM (Veridium®, Lot No. 173A1), the second and third groups with 10 and 20 mg/kg B.W. DA (Veriben®, Lot No. 500A1) respectively. The control group was injected with distilled water. Mice were monitored twice a week for a period of two months for the presence of trypanosomes by the examination of wet smears of tail blood. An isolate was considered as resistant when more than one out of the 6 treated mice became positive within the observation period. Two reference strains TRT8 and IL1180 sensitive to DA and ISM respectively were added as quality control for the trypanocides used. The TRT8 isolate originates from cattle in the Eastern Province of Zambia and was then cloned visually from a single trypanosome. It shows a *T. congolense* savannah type profile with the 18S-PCR-RFLP and a DA-sensitive profile with Ade2-PCR-RFLP. The IL1180 isolate was described by Peregrine et al. (1997).

2.6. Molecular assays

2.6.1. Field samples

The buffy coats of 411 cattle were spotted on filter paper Whatman 4 (Whatman), dried protected from UV light and stored in plastic bags containing silica gel until further processing.

2.6.2. Samples from mice test

Both treated and untreated mice showing trypanosomes circulating in peripheral blood were bled from the tail and blood was spotted on filter paper Whatman 4 (Whatman), dried protected from UV light and stored in plastic bags containing silica gel until further processing.

2.6.3. Trypanosome species identification (18S-PCR-RFLP)

DNA was extracted using the saponine-PBS method described by de Almeida et al. (1998). For species identification, DNA amplifications were performed using three primers targeting the 18S small ribosomal subunit gene sequence and followed by digestion of the products using *Msp*I enzyme as described by Geysen et al. (2003).

2.6.4. Diminazene resistance test (Ade2-PCR-RFLP)

Primers targeting the gene coding for a P2-type purine transporter and the *Dpn*II restriction enzyme were used as described by Delespaux et al. (2006) and modified by Vitouley et al. (2011) but without the described whole genome amplification step.

2.7. Data analysis

Prevalence and resistance, both binary response variables, were analysed by the logistic regression model introducing intervention as fixed effect and kebele as random effect. When the variation due to kebele is significantly different from zero, the prevalence (of resistance) varies significantly from kebele to kebele. The relapsing time according to drugs and doses was assessed using the Cox regression model with strain as stratum and drug regimen as categorical fixed effect. All tests were done at the 5% global significance level. All analyses were performed using SAS Version 9.2.

Table 1
Summary of *T. congolense* infections based on different test methods.

Kebele	Intervention	Cattle sampled	BCE	18S-PCR-RFLP	Both tests positive
Wedesa	No	38	2	11	1
Shumoro	No	63	3	19	2
Gomsha	No	14	0	0	0
Jatu	No	34	5	4	3
Weira	No	50	0	1	0
Yatu	Yes	75	11	17	3
Keta Bosso	Yes	76	2	8	0
Abelti	Yes	61	1	13	0
Total		411	24 (5.8%)	73 (17.8%)	9 (2.2%)

3. Results

3.1. Prevalence and species identification

Among the 411 samples that were tested, *T. congolense* was diagnosed in 24 (5.8%) and 73 (17.8%) blood samples using BCE and 18S-PCR-RFLP respectively. Nine (2.2%) samples were positive for both tests (Table 1). The 18S-PCR-RFLP confirmed that all the *T. congolense* were savannah type. A few *T. vivax* were observed in single or mixed infection with *T. congolense* but were not further considered in this study.

The prevalences observed in priority and non-priority zones were comparable ($P = 0.83$) with odds ratio equal to 1.21 (95% CI: 0.24–6.02) when using the BCE data. The variance of the random kebele effects was equal to 0.77 (SE = 0.71) and did not differ significantly from zero ($P = 0.31$). Similarly, 18S-PCR-RFLP test showed similar prevalences in priority and non-priority zones ($P = 0.60$) with odds ratio equal to 1.43 (95% CI: 0.40–5.23). The variance of the random kebele effects was equal to 0.59 (SE = 0.49) and did not differ significantly from zero ($P = 0.27$). Therefore, in both test methods, we cannot conclude that there was over-dispersion rather the prevalence was uniform over kebeles.

3.2. Ade2-PCR-RFLP resistance study

All 73 samples positive for *T. congolense* savannah type using the 18S-PCR-RFLP were tested for DA susceptibility using the Ade2-PCR-RFLP (Table 2). Among the 73 samples, 37 (50.7%) gave amplification products. Among the 37 positive, 35 (94.6%) showed resistant profiles, 1 (2.7%) sensitive and 1 (2.7%) mixed profile. There was almost no variation in resistance over the different kebeles, as almost all the strains were found to be resistant.

3.3. T. congolense isolation

Among the 24 fresh blood samples that were diagnosed as *T. congolense* by BCE and injected into mice, 12 (50%) were successfully isolated and preserved in liquid nitrogen.

3.4. Resistance tests in mice

The 12 *T. congolense* isolates were found resistant to DA at 10 and 20 mg/kg B.W. The median prepatent period was 9 and 12 days for 10 and 20 mg/kg B.W. DA treatment groups respectively. The same isolates were found resistant to ISM at the dose of 1 mg/kg B.W. and the median prepatent period was 8.8 days (Table 3).

The hazard ratio of getting parasitaemic after treatment with DA at 20 mg/kg B.W. compared to the control group was 0.38 (95%, CI: 0.26–0.56) which differs significantly from one ($P < 0.001$). Relapsing time after treatment with DA 10 mg/kg B.W. or ISM 1 mg/kg B.W. was also significantly longer than the appearance of parasites in the control group. As expected, the sensitive strain TRT8 did not relapse in mice after treatment as confirmed by negative microscopical examination and 18S-PCR-RFLP during the two months of observation.

3.5. 18S and Ade2-PCR-RFLP on samples from mice

All 12 isolates were confirmed to be *T. congolense* savannah type and resistant to DA using Ade2-PCR-RFLP with blood spotted on filter papers from mice. The samples collected in the field and spotted on filter paper corresponding to those 12 isolates were also checked with the two PCR-RFLPs. Only six (50%) of those field samples gave PCR amplification products. All were confirmed to have a DA-resistant profile.

3.6. Mice isolation versus BCE and 18S-PCR-RFLP on DNA from filter papers

For the 24 samples that were diagnosed in the field as *T. congolense* by BCE and inoculated in mice, the similarities and discrepancies between the BCE and molecular identification techniques are summarized in Table 4.

From the 24 samples, 9 were diagnosed correctly by both tests, 12 were negative with the 18S-PCR-RFLP, 1 was characterized as *T. theileri* by 18S-PCR-RFLP, 1 mixed infection *T. congolense–T. vivax* was found to be *T. theileri* by 18S-PCR-RFLP, another mixed infection *T. congolense–T. vivax* was diagnosed as *T. vivax* by 18S-PCR-RFLP. More importantly, the 18S-PCR-RFLP detected three times more positive than the microscopic examination.

4. Discussion and conclusions

The aims of this survey were to evaluate the current situation of trypanocidal drug resistance in the Ghibe valley using fast and easy molecular methods and validating those results by tests in mice. For achieving this, the molecular tests were performed on 411 samples from which a

Table 2
Diminazene resistance results of *T. congolense* using Ade2-PCR-RFLP according to study sites.

Kebele	Intervention	18S-PCR-RFLP		Ade2-PCR-RFLP		
		Positive	Amplified	Resistant	Sensitive	Mixed
Wedesa	No	11	8	8	0	0
Shumoro	No	19	8	8	0	0
Gomsha	No	0	0	0	0	0
Jatu	No	4	2	2	0	0
Weira	No	1	0	0	0	0
Yatu	Yes	17	8	7	0	1
Keta Bosso	Yes	8	5	4	1	0
Abelti	Yes	13	6	6	0	0
Total		73 (17.8%)	39 (53.4%)[a]	35 (89.7%)[b]	1 (2.6%)[b]	1 (2.6%)[b]

[a] % of the 18S-PCR-RFLP positive.
[b] % of the 18S-PCR-RFLP positive that also amplified using Ade2-PCR-RFLP.

Table 3
Median prepatent periods according to doses and treatments of *T. congolense* infections in mice.

Group	Median PP[a] (days)	Maximum PP[a] (days)	Minimum PP[a] (days)
Control	8	13	6
ISM 1 mg	8.75	60	6
DA 10 mg	9	25.5	6
DA 20 mg	12	20	8.5

[a] Prepatent period.

subset (24 samples that were microscopically trypanosome positive in BCE) was also tested in an *in vivo* mouse test for drug resistance. When examining the discrepancies between the BCE and 18S-PCR-RFLP (Table 4), it might seem intriguing that positive samples by microscopical examination were found negative by PCR-RFLP. This is probably due to the low concentration in parasites and the random effect created by the low volume of blood (50 μl – the content of a capillary tube) examined by both methods. A parasitaemia of 20 trypanosomes/ml blood corresponds to 1 single trypanosome in 50 μl. The probability of getting a 50 μl sample with no parasite is thus not negligible. Furthermore, the buffy coats that are examined with the microscope or that are submitted to PCR-RFLP diagnosis are two different individual samples. It is thus expected to find some discrepancies between the two methods. An option to avoid this problem could be to replace the sampling on filter paper by whole blood (500 μl) mixed to a protective buffers such as AS1 (Qiagen®) or a 6 M guanidine hydrochloride / 0.2 M EDTA buffer (Avila et al., 1990).

Table 4
Summary of discrepancies between 18S-PCR-RFLP and BCE for 24 mice injected samples.

Isolates Ref. No.	Kebele	BCE	18S-PCR-RFLP	Ade2-PCR-RFLP	Isolated
F042	Shumoro	T.c.	N	N	No
F072	Shumoro	T.c.—T.v.	T.c.	N	No
F127	Jatu	T.c.	N	N	No
F140	Jatu	T.c.	T.c.	N	No
F153	Yatu	T.c.+T.v.	N	N	No
F159	Yatu	T.c.+T.v.	T.v.	N	No
F165	Yatu	T.c.	N	N	No
F171	Yatu	T.c.	N	N	No
F178	Yatu	T.c.	N	N	No
F189	Yatu	T.c.+T.v.	T.th.	N	No
F244	Keta Bosso	T.c.	T.th.	N	No
F246	Keta Bosso	T.c.+T.v.	N	N	No
F015	Wedessa	T.c.	T.c.	R	Yes
F018	Wedessa	T.c.	N	N	Yes
F058	Shumoro	T.c.	T.c.	R	Yes
F119	Jatu	T.c.	N	N	Yes
F125	Jatu	T.c.	T.c.	R	Yes
F148	Jatu	T.c.	T.c.	R	Yes
F164	Yatu	T.c.+T.v.	N	N	Yes
F166	Yatu	T.c.	U	N	Yes
F172	Yatu	T.c.+T.v.	T.c.	N	Yes
F187	Yatu	T.c.+T.v.	T.c.	R	Yes
F203	Yatu	T.c.	T.c.	R	Yes
F393	Abelti	T.c.	N	N	Yes

With T.c. as *T. congolense*; T.v. as *T. vivax*; T.th. as *T. theileri*; N as negative; U as unidentified and R as resistant.

This alternative presents a double advantage: (i) the sampling volume is larger, increasing the probability of getting at least one trypanosome in the collected blood and (ii) it decreases the risk of contamination between samples. This is usually not a big issue as all samples are stored individually and as the parasitaemia are normally very low but it can constitute a hinder in cases of high parasitaemias as it is observed in some spots (Moti et al., unpublished data). The 18S-PCR-RFLP was as expected, more sensitive than BCE with three times more positive samples. Our results (17.8%) with the 18S-PCR-RFLP for the prevalence of trypanosomosis are in concordance with previous studies (13.3%) (Rowlands et al., 2001).

The results of the species determination with *T. congolense* savannah type as predominant species are also comparable with previous studies (Mulugeta et al., 1997). The prevalence of *T. congolense* infections using BCE fluctuated around 5–6% and was not significantly different across the 8 kebeles. Our results indicate that the monthly systematic administration of DA (Veriben®) and deltamethrin pour-on treatment in the priority zones manage to reduce the burden of the disease to a level comparable to the non priority zones where no systematic and organized interventions were found necessary. There, the control relies on the sole administration of trypanocidal drugs based on clinical signs or breeder assumptions. The fact that farmers continue administrating trypanocides even when the presence of drug resistance is confirmed in the region, might be not surprising as it was demonstrated that even for infections with drug resistant trypanosomes the treatment with trypanocides remained beneficial. In an experimental model where cattle were inoculated with ISM-resistant *T. congolense* and treated at the first peak of parasitaemia, the impact of the infection on the PCV was not very pronounced with an average PCV reduction 8–14 weeks after treatment of only 5.9% (95% CI: 4.5–7.3) (Delespaux et al., 2010). Limiting the treatment to anaemic animals would certainly decrease the volume of drugs used as well as the costs for the farmers as recommended by Van den Bossche and Delespaux (2011).

Cases of drug resistance in the Ghibe valley have been described earlier. Rowlands et al. (1991) documented 12/12 isolates resistant to DA and 11/12 isolates to ISM in South-Western Ethiopia. Codjia et al. (1993) collected 12 isolates from cattle bred in the Ghibe valley and reported resistance to the maximum recommended dose of DA (7.0 mg/kg B.W.). Later, in the same region, the bell was rung about the threat of drug resistance for the efficiency of animal production (Mulugeta et al., 1997; Chaka and Abebe, 2003). Tewelde et al. (2004) also reported ISM resistance in cattle sampled from upper Didessa valley of Western Ethiopia by the use of block treatments (Eisler et al., 2000). Our study shows that molecular tools greatly facilitate the diagnosis of DA resistance allowing for more large scale surveys but still has to be complemented by tests in mice or block treatments for the diagnosis of ISM resistance as no trustable molecular tool has been developed for this drug.

The molecular technique for the diagnosis of DA resistance in *T. congolense* proved to be sensitive, fast and reliable even if only half of the samples positive with the 18S-PCR-RFLP gave amplification products with the Ade2-PCR-RFLP. This can be explained by the fact that the target gene of the 18S-PCR-RFLP is multicopy as opposite to the target gene of the Ade2-PCR-RFLP which is a single copy gene. The correlation between the Ade2-PCR-RFLP and the gold standard (test in mice) was 100% as all 12 isolates tested in mice for DA-resistance were also diagnosed as such by the molecular tool allowing for a reliable extrapolation to all the *T. congolense* isolates.

The test in mice complements the Ade2-PCR-RFLP by providing information on ISM resistance. All isolates were resistant to ISM too jeopardizing thus the use of the sanative pair to eliminate drug resistant trypanosomes as proposed by Whiteside (1962). Briefly, the concept of the sanative pair recommends the use of two trypanocides (*e.g.* DA and ISM) which are chemically unrelated and, therefore, are unlikely to induce cross-resistance. The first pair is used until resistant strains of trypanosomes appear and then the second is substituted and used until the resistant strains have vanished from cattle and tsetse (Whiteside, 1962).

The relative control of the disease in the area despite the high level of drug resistance is a relief as a recent resettlement programme launched in the region by the government for farmers from draught struck areas is placing the Ghibe valley under high pressure for food production (Getachew, 2005; Lemecha et al., 2006).

Furthermore, the impact of human activities on the environment will certainly improve the health status of the cattle at the cost of ecological deterioration of the landscape, limiting infested zones to regions not suitable for agriculture or grazing and facilitating thus focalized vector control operations. Forests were occupying nearly 60% of the territory in 1990 for 30% in 2008. In opposition, cultivated and built up areas increased from 20 to 32% and from 13 to 27% respectively (Girma, 2010). This trend will be accelerated by the migratory flux.

Human encroaching on the wildlife territory is creating preserved residual islands (game or forest reserves) where flies are restricted to a sylvatic cycle where trypanosomes gain in virulence (Van den Bossche et al., 2011). This creates a shift from an endemic to an epidemic situation in the vicinity of those wildlife sanctuaries. The option of vector control is there particularly indicated (Van den Bossche and Delespaux, 2011).

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