Epidemiology and Detection of Multiple Drug-Resistant Trypanosoma congolense in Zebu Cattle in Gurage Zone, Southwest Ethiopia

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Abstract

Background Trypanosomosis in animal is a major threat for the development of livestock sector in many Africa countries as a result it contributes for the prevailing severe hunger and poverty in most sub-Saharan African countries. The aim of this study is to determine the prevalence and drug susceptibility pattern of Trypanosoma congolense in selected districts in Gurage zone, Ethiopia.

Methods A cross-sectional study was carried out in 2000 selected indigenous cattle and eighty (n=80) cattle were selected out of 2000 to carry out drug susceptibility test using longitudinal study. Animals were grouped equally into two (n=40) treatment groups and treated with isometamidium chloride (ISMM) and diminazene aceturate (DIM) according to the manufacturer's instruction. Sample was collected and the microhaematocrit centrifugation technique was used to determine PCV while trypanosomes were detected using dark-ground phase-microscopy.

Results The overall prevalence of trypanosomosis was 4.50% (95% CI=4.28, 4.72) and significantly different among districts (P<0.05) where the highest prevalence (7.1%) was recorded in Abeshege district. The mean PCV for cattle in the longitudinal study (n=80) was 23.2 (95%CI=22.3, 24) with no significance (P>0.05) among the study villages. An overall 37.5% (30/80) drug failure rate was recorded at day 14 post treatment. The failure rate accounted 27.5% (11/40) and 47.5% (19/40) for ISMM and DIM at 14 days post-treatment respectively. Those persistently infected cattle at day 14 were re-treated by exchanging the drug and revealed a failure rate of 26.3% (5/19) and 54.5% (5/11) for ISMM and DA respectively. The mean PCV was variable (P<0.05) before applying treatment (day 0=23.2) and day 28 (PCV=25.09).

Conclusions The study revealed that T. congolense developed resistance to ISM and DIM at the given dosage regimen. Therefore, an integrated control approach against the parasite and vectors should be implemented in the area.

Introduction

In sub-Saharan Africa (SSA), tsetse fly-transmitted African animal trypanosomosis (AAT) is the cause for multibillions of dollars loss annually [1]. Hunger and poverty in SSA is exacerbated due to AAT where it represents a serious impediment to sustainable agricultural rural development [2]. For instance, ATA threaten the survival of draught animals as a result 80% of arable land is tilled by the hand of the farmers which is not efficient to obtain the required productivity to maintain food and nutrition security in the SSA.

In Ethiopia, trypanosomosis is a major bottleneck to the agricultural development in general and livestock production and productivity in particular. An estimated area of 220,000Km^2 is potentially infected by tsetse flies due to high infestation by tsetse flies to previously free areas, [3]. *T. conglense* and *T. vivax* are the most prevalent trypanosma species in the country. Various researchers revealed that the prevalence

of trypanosomosis in tsetse infested areas range from 11.85-37% [4-8] However, in non-tsetse infested areas of Northwest Ethiopia the prevalence of trypanosomosis was in the range of 2-9% [9-11].

Trypanosmiasis is controlled using a combination of different approaches such as chemotherapy, vector control and typanotorolant animals. Chemotherapy is and will continue to be preferred in the control of trypanosomiasis [12]. Currently, a small group of chemo-prophylactic and chemo-therapeutic compounds are in use with new anti-trypanosomal compounds [13]. Despite the use of various drugs to treat trypanosmosis, the rapid development of resistance to the available drugs presents a serious threat that will potentially render them ineffective in trypanosomosis control [14]. Moreover, the benefits of increased trypanocide treatment frequency diminish rapidly with increasing resistance [15]. The dependence of smallholder livestock farming on drugs against trypanosomsis attributed to aggravating the development drug resistance and spread of the trypanosome [16-17].

The existence of multiple drug resistance trypanosome species were reported from different corners African countries where Trypanosma is endemic [18]. At present, 21 African countries has been reported the existence of resistance against trypanocidal drug such as diminazene aceturate (DA), isometamedium (ISM) and homodium has been reported in trypanosome populations in ten African countries [19,20]

Drug Incubation Glossian Infective Test (DIGIT) proved to be extremely sensitive in detecting drug-resistant *T. congolense* populations, but requires availability of tsetse flies. Genetic markers can be used to characterize trypanocidal drug resistance [21, 22]. The practical application of genetic markers is still limited in many sub-Saharan African countries (SSA) due to the lack of appropriately equipped laboratories and skilled personnel. Some researcher recommends longitudinal study is reliable though it takes long (up to 90 days) to generate results [23, 24].

Isometamidium chloride and diminazene aceturate has been used for more than 40 years in Ethiopia to treat cattle against *T.congolense* and *T.vivax* infection [25]. There are some studies on trypanocidal resistance particularly against *T.congolense* infection as reported in the Ghibe valley and other parts of infested areas with trypanosmosis. However, still there are limited information on occurrence of drug resistant trypanosome across Ethiopia is not well known. Therefore, this study was undertaken to detect multidrug resistance *T. Congolese* against commonly used trpanocidal drugs (ISMM and DIM) in south west Ethiopia.

Materials And Methods

Study Area Description

The study was carried out in selected districts in Gurage zone, south-west Ethiopia which is located between7° 76'and8° 45'N latitude and 37° 46'and38° 71'E longitude, and at altitude of 1500-1850 meter above sea level (Fig 1). The annual temperature and rainfall ranges are 11-22□C and 800-1200 mm with peaks in July and August, respectively. The society based on mixed farming system which include both

crops and livestock production. The livestock population in the district accounted 40.204 bovine, 4.217 equine and 7.624 caprine, 1.825 ovine and 35.680 poultry.

Study animals

The study was carried out in freely grazing indigenous zebu cattle of both sexes in the selected study areas. Cattle aged 12 months and above were sampled and examined in the study districts as recommended by [25]. Animals for the drug sensitivity analysis were known to be infected with *T. congulense*.

Study design

A cross-sectional study was carried out to determine the prevalence of *T. congulense* in the study areas. Accordingly, five (Fig 1) out of 40 villages were selected depending on the prevalence of trypanosomes. Blood samples were collected from 2000 cattle in five villages and screened for *T. congulense* using Woo test techniques in order to determine the prevalence of Trypanosome in the study village [26].

Study on Drug sensitivity

Longitudinal study was conducted from November-December 2013 on 80 known *T. congulense* positive cattle which were randomly allocated into two equal treatment groups. An abbreviated 28-day field protocol was used to estimate resistance to 3.5 mg/kg and 7.0 mg/kg bw DIM and to 0.5 mg/kg bw ISMM in trypanosome-positive cattle [3, 27]. Animals were grouped, ear-tagged and their weights estimated using a weighing band and conversion tables developed for local zebu cattle [28]. Group one was treated with Isometamidium chloride (TRYPAMIDIUM-SAMORIN®, Merial, France) at the dose of 0.5mg/kg /bw (2% solution) and group 2 was treated with diminazene aceturate (DIM) (Veriben®, Ceva Animal Health Inc., France) at 3.5 mg/kg bw (7% solution) against *T. congulense*. The preparations were injected deep intramuscular (i.m). Animals were monitored for trypanosomes and Paced Cell Volume (PCV) on day 14 and 28 post-treatment for ISMM and DIM. Those cattle failed to respond for each treatment were re- treated by shifting the drugs and were monitored for trypanosomes at day 14 and 28 post-treatment.

Sample collection and laboratory analysis

A jugular vein blood sample was extracted from every cattle into vacutainer tubes coated with di-sodium salt of ethylene diamine tetra-acetate (EDTA) using 18 gage needle and Hawksley micro haematocrit reader (Hawksley, Lancing, United Kingdom) was performed on blood in a capillary tube to determine the PCV whereas trypanosomes were detected using the dark-ground phasemicroscopy.

Data analysis

All data were coded and entered into excel spread sheet. Descriptive, Pearson chi square ($\chi 2$) and student's t-test analyses were carried out using SPSS (IBM SPSS Statistics for Windows, Version 20.0.

IBM Corp, Armonk, NY.). Fisher exact test compared treatment failure between villages while Student's t-test was used to deter-mine differences in PCV values. Pearsonchi square ($\chi 2$) used to test differences in trypanosome prevalence between villages. The differences were considered statistically significant at $P < 0.05$.

Results

Overall prevalence of trypanosomosis

The current study revealed that an overall prevalence of trypanosomosis was 4.50% (95% CI=4.28, 4.72) in the studied areas. The prevalence of the disease was significantly different among districts (P<0.05 and the highest prevalence (7.1%) was recorded in Abeshege district. However, the difference was not significant (P>0.05) for sex, age and body condition (Table 1)

Table 1: Comparison of trypanosomosis prevalence among different variables

Variable	No. sampled	No. negative	No. positive	Prevalence	95% CI	$\chi 2$	P-value
Sex							
Female	815	772	43	5.30%	5.08,5.52	1.93	0.16
Male	1185	1138	47	4.00%	3.14,4.85		
Total	2000	1910	90	4.50%	4.28,4.72		
Age							
<1year	113	108	5	3.80%	3.56,4.01	0.69	0.708
1-3Year	448	431	17	4.70%	4.48,4.92		
>3year	1439	1371	68	4.80%	4.58,5.02		
Total	2000	1910	90	4.50%	4.28,4.72		
Body condition							
Fat	1155	1100	55	4.80%	4.58,5.02	1.076	0.584
Medium	167	162	5	3.00%	2.25,3.75		
Lean	678	648	30	4.40%	4.18,4.62		
Total	2000	1910	90	4.50%	4.28,4.72		
Woreda							
Abeshge	800	743	57	7.10%	6.90,7.30	25.1	0.001
Enomor	549	538	11	2.00%	1.39,2.61		
Checha	451	432	19	4.20%	3.90,4.41		
Ameya	200	197	3	1.50%	1.34,1.66		
Total	2000	1910	90	4.50%	4.28,4.72		

Evaluation paced cell volume for normal and infected cattle

Table 2 below showed that 35.5% and 64.5% animals had PCV <24 and PCV>24 respectively. The level of PCV was significantly different (P<0.05) among infection with different species of trypanosome. The

mean PCV for cattle selected for follow-up study (n=80) was 23.2 (95%CI=22.3, 24) with no significance (P>0.05) among the study villages (Table 3)

Table 2: Overall packed cell volume for all animals (n=2000)

Status	No. sampled	PCV< 24 (anemic) (%)	PCV >24 (Normal) (%)	$\chi 2$	P-value
Negative	1910	658 (34.5)	1252(66.5)	30.1	0.001
T.congulonse	55	33(60)	22(40)		
T. vivax	16	13(81.2)	3(18.8)		
T.theileri	10	3(30.3))	7(66.7)		
Mixed	9	3(33.3)	6(66.7)		
Total	**2000**	**710(35.5)**	**1290 (64.5)**		

Table 3: Descriptive and one way ANOVA analysis for PCV in cattle selected for follow- up study (n=80)

Villages	№	Minimum	Maximum	Mean (95% CI)	F test	P-value
Misreta	16	16	32	21.9(19.7,24.0)	1.862	0.126
Wolayita	16	20	30	24.1(22.3,25.8)		
Wuha Limat	16	18	30	24.1(22.2,26.1)		
Borer-4	16	15	32	24.1(21.7,26.4)		
Borer-5	16	20	26	21.9(20.9,22.8)		
Total	80	15	32	23.2(22.4,24.0)		

Multi Drug Resistance

ISM treatment: Of the 40 trypanosome-positive cattle treated with ISMM (0.5 mg/kg/bw), 27.5% (11/40) had persistent infection at day 14 post-treatment (Table 4). Resistance to ISMM treatment were recorded in all 5 villages though Misreta, wolaita and Borer-4 had the highest treatment failure rate of 27.3% (3/11) and Wuha-limat and Borer-5 had the lowest treatment failure rate of 9.1% (1/11) at day 14 post treatment However, the failure rate was not significantly different among villages (χ^2 =3.413, P > 0.05). Cattle showing treatment failure (n=11) at day 14 post treatment for isometamedium chloride were re-treated with diminazene aceturate (3.5 mg/kg/bw) and the failure rate was 54.5% (6/11) at 28th days of post treatment (Table 5). The highest and lowest treatment failure was recorded in Borer-5 50% (3/6)) and Misreta, Wolaita and Borer-4 of 16.7%1(1/6)) respectively.

Table 4: Proportion of infection at day 14 post treatment with ISMM (0.5 mg/kg/bw)

Infection status	№. animals	Percent
Positive	11	27.5
Negative	29	72.5
Total	**40**	**100**

Table 5: Response to ISMM and DIM treatment at 14th and 28th day respectively

Villages	**ISMM (0.5 mg/Kg/bw) 14th day (n=40)**		**ISM**(0.5 mg/kg/bw) **28th day (n=19)**	
	No. Positive (%)	No. Negative (%)	No. Positive (%)	No. Negative (%)
Misreta	3 (27.3)	5(17.2)	1(20)	2(14.3)
Wolayita	3(27.3)	5(17.2)	1(20)	3(15.8)
Wuha Limat	1(9.1)	7(24.1)	1(20)	3(15.8)
Borer-4	3(27.3)	5(17.2)	1(20)	3(15.8)
Borer-5	1(9.1)	7(24.1)	1(20)	3(15.8)
Total	**11(100)**	**29(100)**	**5(100)**	**14(100)**

DIM treatment: Out of 40 *T.congulonse* cattle treated with diminazene aceturate (3.5 mg/kg/bw), 47.5% (19/40) had persistant trypanosma infection 14 days post treatment (Table 6). Four out of five villages (Wolaita, wuha-limat, borer-4 and Borer-5) had the highest treatment failure rate 21% (4/19) and the lowest treatment failure 15.8% (3/19) was observed in Misreta village (Table 5) though the failure rate was not significantly different among the villages (χ^2=1.067,P=0.90). Cattle which were found positive 14 days post treatment with 3.5mg/kg/bw diminazene aceturate were re-treated with isometamedium chloride (0.5mg/kg/bw) and 27.8% (5/18) were showed treatment failure at day 28 post treatment. All the villages had the same treatment failure rate 5.3% (1/19) (Table 7) and there was no significant difference amongst villages on treatment failure (P> 0.05).

Table 6: Proportion of infection of animals treated with DIM (3.5mg/kg/bw)

Infection Type	Frequency	Percent
Positive	19	47.5
Negative	21	52.5
Total	**40**	**100**

Table 7: Response to DIM and ISM treatment at 14th and 28th day respectively

Villages	DIM (3.5 mg/Kg/bw) 14th day (n=40)		DIM(3.5 gm/Kg/bw) 28th day(n=11)	
	No. Positive	No. Negative	No. Positive	No. Negative
Misreta	3(15.8)	5(23.8)	1(16.7)	1(20)
Wolayita	4(21.1)	4(19.0)	1(16.7)	1(20)
Wuha Limat	4(21.1)	4(19.0)	0(0.0)	0(0.0)
Borer-4	4(21.1)	4(19.0)	1(16.7)	1(20)
Borer-5	4(21.1)	4(19.0)	3(50)	2(40)
Total	**19(100)**	**21(100)**	**6(100)**	**5(100)**

Table 8 below showed the mean PCV value at day 0, 14 and 28 was 23.2±0.41, 23.7±0.37, 25.1±0.41 respectively. The mean PCV was variable before applying treatment (day 0) and day 28 post treatment (t=-4.096, P<0.05); between day 14 and 28 post treatment (t=-59.6, P<0.05). However, the mean PCV was no significantly different between day zero and 14 (t=-1.461, P>0.05).

Table 8: Comparison of PCV before and after treatment using paired sample T test (n=80)

Treatment	№ animals	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
PCV day zero	80	15.0	32.0	23.20	.405	3.62	13.12
PCV day 14	80	16.0	34.0	23.73	.373	3.34	11.18
PCV day 28	80	15.0	30.0	25.09	.412	3.69	13.60

Discussion

The current study revealed that an overall prevalence of 4.50% (95% CI=4.28, 4.72) trypanosomiases in the study areas. The prevalence was significantly different among the study villages (P<0.05). The prevalence of the disease was in agreement with 4.9% reported by Geremew et al. [29], 5.43% Lelisa et al. [30], 6.11% Terefe et al.[31], 6.25% Abera et al. [32] 7.24% Sheferaw et al. [33] and 7.30% Birhanu et al. [34]. However, by far lower than 10.8% by Lelisa et al. [31], 12.14% Tesfaye et al. ([35], 12.41% [336] and 21.33% Ataro et al. [37] in different part of the country. The lower prevalence during this study might be due to difference in season of sampling and/or changes in ecological/ climatic conditions that affect vector fly density/distribution overtime, animal susceptibility, trypanocidal drug use and existence of fly control which affects the epidemiology of the disease [39-40]

Eighty (n=80) known infected cattle were used to investigate the susceptibility *T. congolense* to two trypanocidal drugs namely DIM and ISM at different dose at day 14 and 28 post treatment. Accordingly, the study revealed that there was variability for failure rates at day 14 as compared to s at day 28 post treatment which was in agreement with other findings in different parts of the country. For instance, the

dynamic nature of the epidemiology of drug resistant infection in the Ghibe valley, which was reported to be 6% in 1986 and in1989, is increased to 14% [22]. The current finding showed that the incidence of multi drug resistance was increasing to 51.25% which was almost four fold increment as compared to the incidence in 1989.

Afework et al. [41] detected for the presence of resistance in mice using clones of *T. congolense* against both diminazene and isometamidium. According to Chaka and Abebe [3] *T. congolense* 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. Afewerk et al. [41] also showed the presence of multiple-drug-resistant *T. congolense* in the village cattle of Metekel district, northwest Ethiopia. Codjia et al. [18] reported thatonly one out of 12 trypanosome isolates collected from cattle in Ghibe were sensitive to 0.5 mg/kg b.w. isometamidium chloride, all were found to be resistant to 7.0 mg/kg, 0.5 mg/kg and 1.0 mg/kg body weight of diminazene, isometamidium and homidium chloride, respectively.

An overall failure rate for DIM in this finding was 47.5% (19/40) at 14 days post infection. The observed 3.5 mg/kg/bw DIM resistance is agreement with Afework et al. [41] had been reported the same failure rate in Ethiopia. Moreover, twenty percent (20%) failure rate was recorded to ISM during this study. Since long time ISMM resistance *T.congolense* has been reported in Ethiopia [42-43]. The multiple-drug resistance in the Ghibe valley of Ethiopia was related intensive use of quinapyramine sulphate for treating trypanosomiases [41]. The prolonged use of drugs without replacement, as is true for trypanocides principally encourages the development of resistance in accordance with Waller [44].

Conclusion

In conclusion, despite the low prevalence of trypanosomosis in the study areas, results from the current study revealed that *T.congulonse* developed multiple drug resistance against diminazene aceturate and isometamidium chloride. This is a serious impediment for controlling the disease in the areas. Therefore, the control of trypanosomosis in the study area should be quite rational in utilizing the available drugs and implementing an integrated control approach against the parasite and the vector.

Abbreviations

ISMM: Isometamidium chloride; DIM: Diminazene aceturate; PCV: Packed cell volume;

Declarations

Ethics approval

The Research Ethics Review Committee (RERC) of the College of Veterinary Medicine and Agriculture at Addis Ababa University reviewed and approved this research work with letter Ref. No. VM/ERC/05/07/06/2013

Consent for publication

All authors have consent for publication of this research work

Competing interests

The authors declare that they have no competing interests

Funding source

Not applicable

Authors' contributions

TT carried out this study and generated data and partially prepared the manuscript. AT analyzed and interprets data and completes the manuscript.

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Figures

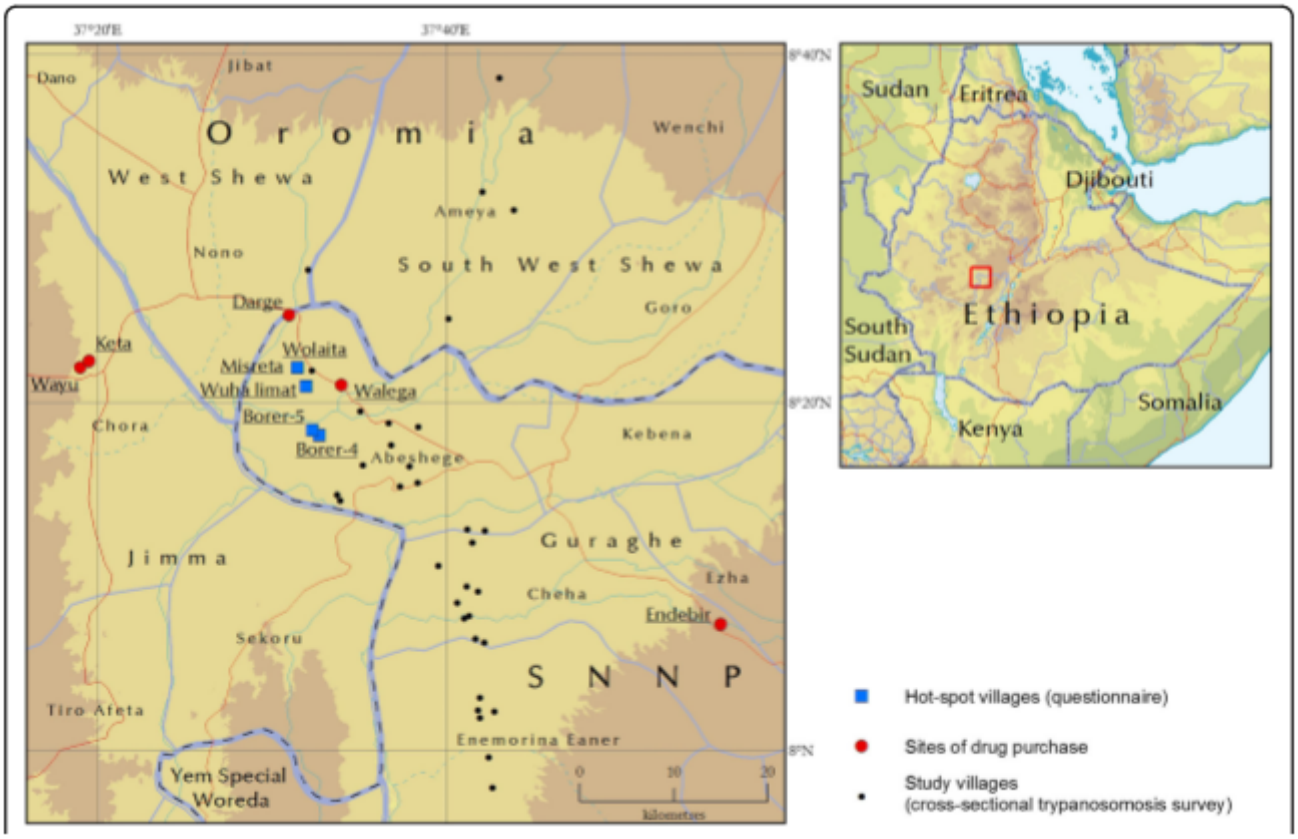

Figure 1

Study villages for cross-sectional and trypanocidal drug resistance evaluation

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