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RESEARCH ARTICLE

FIELD TRIAL OF TRYPANOCIDAL DRUG RESISTANCE USING NATURALLY INFECTED CATTLE IN KONSO DISTRICT, SOUTHERN NATIONS NATIONALITIES AND PEOPLES REGION, ETHIOPIA

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ABSTRACT

The field trial was conducted to assess the problem of trypanocidal drug resistance in Konso District using Diminazeneaceturate (Veriben®) and Isomethamedium chloride (Veridium®). A total of 250 naturally infected animals were screened and divided in to two groups of 125 animals. Group I was treated with Diminazeneaceturate at a dose of 3.5 mg/kg/ bodyweight (b.w.) and group II was treated with Isomethamedium chloride at a dose of 0.5 mg/kg/b.w. and monitored for relapses at 15 days interval for 90 days. The relapsing cases were treated with double dose of the drugs and monitored at the same interval for the same period.It was found that the proportion of relapsing cases was 51.2% and 32.81% in group I and 41.60% and 30.77% in group II after single and double doses of the drugs respectively. The relapses were due to *T.congolense (*75%) followed by *T. vivax* (20.31%) and *T. brucei (*4.65%); and *T. congolense*(86%) followed by *T. vivax* (9%) and *T. brucei*(5%) after single and double doses in group I respectively. similarly the relapses observed in group II were higher for *T. congolense* (71%) followed by *T. vivax* (27%) and *T. brucei*(2%) and *T. congolense*(56%) followed by *T. vivax* (38%) and *T. brucei*(6%) after single and double doses respectively. However, no statistically significant difference has been observed among different sexes (P>0.05) showing their equal susceptibility. The overall mean PCV of the infected animals was 24.34±0.27 with standard deviation (s.d) of 4.34 at 95% CI (23.80-24.88).Therefore, relapses were observed in both single and double doses of both drugs requiring integrated sustainable vector control, enhanced community awareness in the appropriate and strategic use of the drugs, and further research on multiple drug resistance to minimize further development of the problem.

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INTRODUCTION

Trypanosomosis, locally called "Gendi," is one of the major diseases of livestock in Konso District. The disease is widely distributed in the lowland areas of the District and more serious in cattle and equines than other domestic animals. Although losses due to the disease have not been quantified, it was consistently reported to be significantly high and accounted for about 50% of all cattle deaths in the District (Barry and Ejigu Jonfa, 2006).Three economically important *Trypanosoma* species namely, *T. congolense*, *T. vivax* and *T. brucei* were responsible for the disease with some cases of mixed infection. The infected animals show symptoms of loss of body condition, reduced meat and milk production, infertility, reduced draught power and death of properly untreated animals (Getachew, A. 2005; Tewelde *et al.,* 2001; Yohannes *et al.,* 1998). Because of the seriousness and extensive prevalence of the disease livestock keepers have managed to continuously use chemotheraputic and chemoprophylactic Trypanocides to raise their animals. The most commonly used drugs in the District were Isomethamedium chloride (Veridium®) and Diminazene aceturate (Veriben®**)** both manufactured by Ceva Santé Animale**,** Liuborne-France (Morton, 2002). Apart from government sources, 60% of the drugs have been obtained from illegal sources with improper handling, storage and administration by uncertified persons (Morton, 2002). Despite such an intense use of trypanocidal drugs, it is not unusual to hear farmers complaining about the lack of efficacy on the drugs (Barry and Ejigu Jonfa, 2006; Tewelde *et al.,* 2001). Although several authors (Shimelis *et al.,* 2008a) reported the occurrence of drug resistance in Ethiopia, there is no sufficient research evidence in Konso context that explains the problem from either direction. Therefore, the present field trial is aimed at assessing the presence and prevalence of trypanocidal drug resistance to Isomethamedium chloride and Diminazene aceturate in the selected villages of Konso District.

MATERIALS AND METHODS

Study area: The study was conducted between October and January 2010 in five randomly selected villages. The District has a total area of 2273.9 square kms located in the Southern Rift Valley of Ethiopia at about 595 km South of Addis Ababa and 369 kms South of Hawassa (CSA, 2007).. The District is found at 37^0 02' 35" and 37^0 58' 23"E Longitude and 5^0 21' 14" and 5^0 61' 27"N Latitude sharing the same border with Derashe District on the North, Ale District on the North west, Burji and Amaro Districts on the East, Oromiya region on the South and South omo Zone on the West (Fig 1). The District is located at an altitude ranging from 500 to 2300 meters above the sea

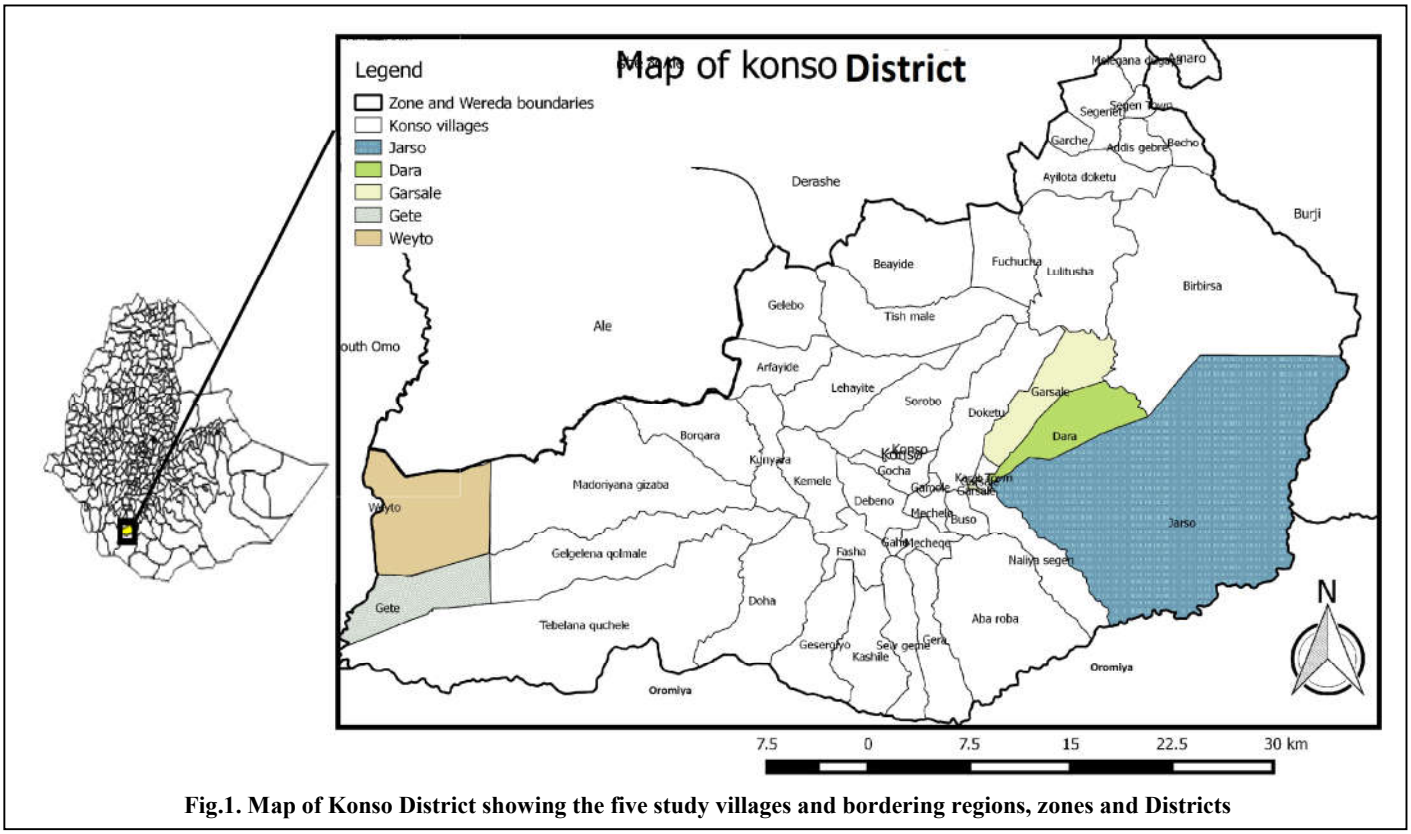

Fig.1. Map of Konso District showing the five study villages and bordering regions, zones and Districts

level (m.a.s.l) with annual average rainfall of 750 mm and temperature ranging from 12.2 ^{0}C- 34^{0}C. The livestock population of the district was estimated to 146,316 cattle, 6,492 equines, 146,070 sheep, 267,938 goat, 146,034 poultry and 29,518 beehives (CSA, 2007).

Study animals: The study was conducted on local breeds of cattle (*Bos indicus*) maintained on traditional communal management system. The animals have made part of the field trial by using trypanosome positive animals.

Study design: Five villages namely, Garsale, Jarso, Dara, Weyto and Gete were randomly selected from 15 villages purposely identified based on high trypanosomosis prevalence, broad access to illegal drug smugglers, limited access to public veterinary service, and history of low trypanocidal drug efficacy. The villages were marked with numbers 1-15 and five villages were randomly selected using simple lottery system. A total of 50 households (10 per village) were selected for an interview using expected frequency of 3.4% at 5% confidence limits and a confidence interval of 95% with Epi Info 7. The households were purposely selected to include individuals owning livestock to obtain full information on the required data. A total of 250 positive animals (cattle) were selected for the trial using expected frequency of 20.5% with precision level of 5% and a confidence interval of 95% with Epi Info 7. The total sample was equally divided for five villages (50 samples per village) which were further subdivided in to two groups of 25 animals for the field trial. The first group of 25 animals was identified with yellow ear tag and treated with Diminazene aceturate (Veriben®) at a dose of 3.5 mg/kg/b.w. and the second group was identified with red ear tags and treated with Isomethamedium chloride (Veridium®) at a dose of 0.5 mg/kg/b.w. The relapsing cases in both groups were monitored for 3 months at 15 days interval and treated with double dose of the drugs. Despite the length of gaps of monitoring days, no single animal has been lost from either group during the study period.

Questionnaire survey: A semi-structured questionnaire was developed to collect information on the farming system, trypanosomosis problem, and veterinary service provision including sources and usages of trypanocidal drugs and the presence of drug resistance (ineffectiveness) in the villages.

A preparatory school complete student was selected from each village and training with rehearsal session was organized for one day at Karat town to create better understanding on the questionnaire. The number of interviewees was limited to 10 per village to reduce the burden of work on the limited number of enumerators and enable them to complete the questionnaire correctly and effectively.

Blood sample collection and examination: A total of 187, 253, 316, 325 and 389 animals were randomly selected from Garsale, Jarso, Dara, Weyto and Gete villages, respectively, to screen fifty naturally infected animals from each village. Blood samples were collected from ear vein of the animals using heparinised capillary tubes and examined according to the procedures described by Uilenberge (1998) as follows. Two capillary tubes were used for each animal and filled with blood until 3/4th of their length. One end of the capillary tubes were sealed with crystal seal and symmetrically loaded on the Microhematocrit centrifuge (Hettich®, Germany) with the sealed end pointing out ward and centrifuged at 12,000 revolutions per minute (rpm) for 5 minutes and packed cell volume (PCV) was measured using heamatocrit reader.

The capillary tubes were broken 1 mm below the buffy-coat to include red blood cell layers and the content was expressed on a microscope slide, mixed with the edge of other slide and covered with 22x22 mm cover slip. The samples were examined under 40x objective using dark ground buffy-coat technique to detect the presence of trypanosomes with their characteristic movements. Thin blood smears were prepared from positive samples, fixed with 95% methanol for 5 minutes and stained with Giemsa stain solution for 30 minutes. Then the samples were examined under 100 x objectives to identify the trypanosomes at species level. The PCV values were recorded and the reading below 24% was considered as anaemia (Marcotty *et al.,* 2008).

Data analysis: The data collected from the villages were entered in to Microsoft excel spread sheets-2007. Epi Info version 7 and SPSS version 20 were used for statistical analysis. Descriptive statistics like frequency proportion and mean values were used and the presence or absence of significant difference between groups was detected at $p< 0.05$ using T - test.

RESULTS

Questionnaire survey: All respondents (100%) indicated that trypanosomosis was the most important disease in the villages and treated with Trypanocidal drugs supplied in sachets containing brown or yellow powders. Some farmers have shown empty sachets of Isomethamidium chloride and Diminazene aceturate supplied in different trademarks consisting of Samorin®, Trypamedium® and Veridium® for Isometamedium chloride and Berenil®, Fatribanil® and Veriben® for Diminazene aceturate. According to the majority of respondents (78%) the drugs were obtained mainly from illegal sources. Sixty six percent of the respondents indicated that the drugs were administered by livestock owners (Table 1). The respondents also mentioned that Homidium salts consisting of Homidium bromide (Ethidium®) and Homidium chloride (Novidium®) have been used for decades before losing acceptance by the farmers due to their ineffectiveness. According to the study 38% of respondents used a sachet of 2.36 g Diminazene aceturate per animal while 62% used for 2-3 animals. Likewise, 40% of the respondents used a sachet of 1 g of Isomethamedium chloride for 10 animals while 60% of the respondents used for 15-20 animals. The respondents indicated that the effectiveness of the drugs was declining from time to time requiring weekly (5%), biweekly (23.5%), every fortnight (15%), and monthly (41.2%) and bimonthly (15.5%) treatments. The farmers said that they are losing confidence on the drugs since they have to treat their animals about 8 times a year.

Hematological findings: The overall mean PCV of the infected animals was 24.34±0.27 with standard deviation (s.d). of 4.34 at 95% CI (23.80-24.88). The highest PCV was recorded from Gete village (34%) and the lowest was from Jarso village (14%). Among the total number of positive animals the majority (69.6%) had mean PCV below 24% (Table 2).

Parasitological findings: The prevalence of trypanosomosis in Garsale, Jarso, Dara, Weyto and Gete was 26.74%, 19.76%, and 15.82%, 15.38% and 12.85%, respectively, with the overall prevalence of 17%. According to the study T. congolense (61.02%) was the most dominant trypanosome species in the District (Table 3). When the proportion of sex of infected animals was considered, females were more infected (52%) than the males (48.4%), although the difference was not statistically significant (P>0.05) (Fig.2).

Relapsing cases following trypanocidal drug treatment: Among the animals in group I 51.2% relapses were recorded after treatment with 3.5 mg/kg/b.w. of Diminazene aceturate and 32.81% relapses from animals treated with double dose (7 mg/kg/bw) of the drug. Similarly, 41.6% relapses were recorded from animals treated with 0.5mg/kg/bw of Isomethamedium chloride and 30.70% relapses after doubling the dose (Table 4.

Trypanosome parasites detected in the relapsing cases: The study showed that the relapsing cases were mainly attributed to

Table 1. Sources and use of trypanocidal drugs

Villages	No of farmers interviewed	Source of trypanocidal drugs		Treatment given by		
		Illegal	Government and illegal	Farmers	Veterinary personnel	Vet and farmers
Garsale	10	9	1	5	0	5
Jarso	10	7	3	5	0	5
Dara	10	8	2	6	0	4
Weyto	10	8	2	8	0	2
Gete	10	7	3	9	0	1
Total	50	39 (78%)	11(22%)	33(66%)	0	17(34%)

Table 2. Haematological findings of parasitemic animals

Village	Animals sampled	positive animals	PCV ≤20%	PCV 21-24%	PCV 25-29%	PCV ≥30%	Mean ±SEM	S.d	95% C.I.	Sig (2 tailed)
Garsale	187	50	13	23	13	1	24.13±.59	4.16	22.95-25.31	.000
Jarso	253	50	16	20	14	0	23.57±.64	4.52	22.29-24.85	.000
Dara	316	50	12	23	12	3	25.11±.65	4.63	23.79-26.43	.000
Weyto	325	50	12	23	15	0	24.29±.57	4.01	23.15-25.43	.000
Gete	389	50	10	22	17	1	24.59±.62	4.36	23.35-25.83	.000
Total	1470	250	63 (25.2%)	111 (44.4%)	71 (28.4%)	5 (2%)	24.34±.27	4.34	23.80-24.88	.000

Table 3 Prevalence of trypanosomosis in the selected villages

Village	Animals sampled	Positive animals	Trypanosomes sp diagnosed				Prevalence (%)
			T. congolense	*T. vivax*	*T. brucei*	Mixed (*T.c and T.v*)	
Garsale	187	50	30	12	5	3	26.74
Jarso	253	50	32	7	9	2	19.76
Dara	316	50	29	14	2	5	15.82
Weyto	325	50	31	13	4	2	15.38
Gete	389	50	32	11	3	4	12.85
Total	1470	250	154 (61.02%)	57 (22.8%)	23 (9.2%)	16 (6.4%)	17

Table 4. Relapses after treatment with normal and double doses of Isometamedium chloride and Diminazene aceturate in five villages of konso District

Ser No	Days after treatment	Relapses after treatment with Diminazene aceturate				Relapse after treatment with Isomethamedium chloride			
		3.5mg/kg/b.w.		7mg/kgb.w.		0.5mg/kg/b.w.		1mg/kg/b.w.	
1	15	12/125	9.6%	5/64	7.81%	15/125	12%	6/52	11.54%
2	30	20/125	16%	6/64	9.38%	14/125	11.2%	2/52	3.92%
3	60	15/125	12%	6/64	9.38%	11/125	8.8%	3/52	5.77%
4	90	17/125	13.6%	4/64	6.25%	12/125	9.6%	5/52	9.62%
Total	90	64/125	51.2%	21/64	32.81%	52/125	41.60%	16/52	30.77%

T. concolense followed by *T. vivax* and *T. brucei* in both types of drugs (Table 5).

Table 5. Ttrypanosome parasites detected during the first and second relapses of both drugs

Trypanosome parasites	Diminazene aceturate				Isomethamediumchloride			
	First relapses		Second relapses		First relapses		Second relapses	
	No	%	No	%	No	%	No	%
T.congolense	48	75	19	86	35	71	9	56
T.vivax	13	20	2	9	13	27	6	38
T.brucei	3	5	1	5	1	2	1	6
Total relapses	64	100	22	100	49	100	16	100

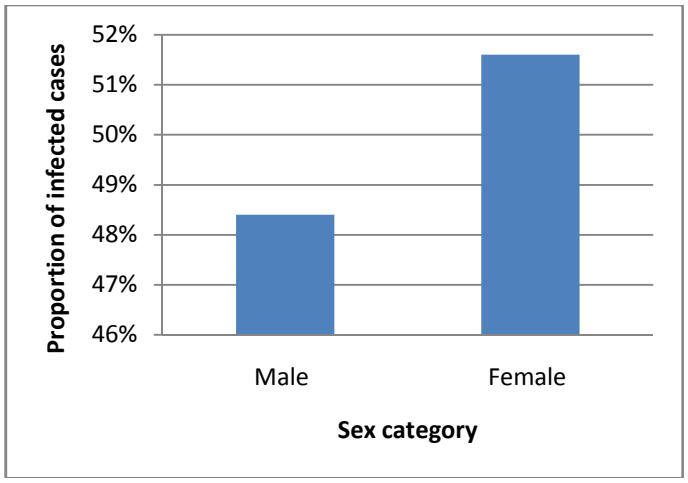

Fig. 2.Proportion of infected animals of different sexes

DISCUSSION

The questionnaire survey as well as field trial on naturally infected cattle indicated that trypanosomosis existed in the study area for several years and left most of the Konso farmers with few unproductive cattle. Limited government drug supply coupled with inadequate knowledge of livestock owners on appropriate use of drugs had contributed to the use of services delivered by illegal or uncertified individuals who administer poor quality and over diluted drugs (Gizaw Woldeyes and Gezehagne Aboset, 1997). According to this study the problem of service delivery by uncertified individuals is more severe (66%) than the reports of Yohannes Afework (1998) and Nega Tewelde (2001) who indicated 43% and 57% in the villages of Metekel District, Northwest Ethiopia and Farming in Tsetse Controlled Areas (FITCA) in Western Ethiopia, respectively. However, Daya (2004) reported 97.5% service delivery by livestock owners and drug smugglers in Boloso Sore District which is higher than the present findings. Gizaw Woldeyes and Gezehagne Aboset (1997) reported similar situation in Kola shara village of Arbaminch Zuria District where all farmers were armed with syringes and needles and treat their own animals with trypanocidal drugs. This clearly describes the weakness of the prevailing animal health service delivery and loose regulatory imposition on drug transaction. The frequency of treatment reported during this field trial is about 8 times a year which is in-agreement with similar findings of Muturi (1999) at Merab Abaya (7-9 times) and higher than the report of Yohannes Afewerk (1998) at Pawe (3.1 times). Uilenberg (1997) described that the frequency of treatment over a year reflects the magnitude of trypanosome challenge and the declining efficiency of the drugs is an indication of drug resistance likely due to poor handling and under-dosing of drugs from either legal or illegal sources and less active ingredient than anticipated in some drugs. Yohannes Afework (1998) and Nega Tewelde (2001) reported more than 40% respondents using trypanocidal drugs below the recommended dose due to inefficient government services contributing to trypanocidal drug misuse and development of drug resistance supporting to the present field trial. The current field trial indicated that about 69.6% of positive animals had PCV < 25% which agrees with similar report of Rowlands *et al.* (2001) in Ghibe, South west Ethiopia. Although various factors are responsible for the occurrence of anaemia (Conner, 1994), low PCV is an indication of anaemia, which is the most indicative sign of trypanosomosis in endemic areas (Leak, 1987; Murray and Dexter, 1988; Trail *et al*.,1991; Yohannes Afewerk, 1998; Muturi, 1999, and Rowlands *et al.,* 2001). The present study showed an overall average PCV of 22.57% which agrees with the report of Vanden Bossche (2001) who found 23% and stated that the average PCV of trypanosomosis positive herds decrease with increasing prevalence of trypanosomosis. The trial has detected an overall prevalence of 17% which is in agreement with the findings of Yohannes Afewek (1998), NegaTewelde (2001) and Muturi (1999) who reported the prevalence of 17.2%, 21%, and 17.5% in Metekel District, upper Dedessa valley and Southern Rift Valley of Ethiopia respectively. It was observed that *T. congolense* is more dominant *(*61.6%) than *T. viviax* (22.8%), *T. burcei* (9.2%) and mixed infection (6.4%) which is in agreement with the findings of Getachew Abebe and Yilma Jobre (1996) (58.5%), Muturi (1999) (66.10%) and Yohannes Afewek (1998) (60,9%).The results reported by Gizaw Woldeys and Gezehagn Aboset (1997) (85.2%) from Arbaminch Zuria District and Rowland *et al.* (1993) (84%) from Ghibe valley are similar but higher than the present findings. The proportion of infected female in this field trial is higher than the males which are similar to the findings of Rowlands *et al*. (1995) who reported higher prevalence in cows than males. However, Yohannes Afewrk (1998); Muturi (1999) and NegaTewelde (2001) reported higher infection proportion in males than female animals, although the difference is not significant in both cases showing equal susceptibility of both sexes.

The relapses encountered after Diminazene aceturate treatment at a dose of 3.5 mg/kg/bw of this field trial (51.2%) is similar to the findings of Rowlands *et al*. (1993) who reported the same figure at the same dose. Likewise relapses encountered at double dose (7 mg/kg/bw) of the drug (32.81%) is not very different from findings of McDermott *et al*. (2003) who reported 36.8% resistance to Diminazene aceturate 7 mg/kg/b.w. but significantly lower than the findings of Codjia *et al*. (1993) (100%) who reported resistance to the maximum recommended dose (7 mg/kg/b.w.) of Diminazene aceturate from 12 isolates of *T. congolense* collected from Ghibe valley. The present field trial detected 41.6% relapses of Isometamedium chloride treatment at a dose of 0.5 mg/kg bw which is lower than the findings of Yohannes Afewerk (1998) (50%) at Pawe, North west Ethiopia and McDermott et al. (2003) (63.8%) at Kenedougou province of Burkina Faso. Eisler *et al*. (2000) indicated that resistance against this drug is strongly suspected when more than 25% of Isometamidium chloride treated cattle become parasitaemic within 8 weeks of exposure. Codjia *et al.* (1993); Leak *et al*. (1993); Rowlands *et al*. (1993) and Yohannes Afewerk (1998) reported that *T. congolense* is the most predominant drug resistant trypanosome which agrees with the findings of the present field trial. The development of drug resistance in the District can be assumed to occur due to the extensive use of low doses of standard as well as substandard drugs. According to Clausen *et al.* (1992) and Geerts and Holmes (1998) a prolonged and frequent use of trypanocides in high challenge areas, even when well applied, is likely to cause drug resistance apart from under dosing. As reported by Barry and Ejigu Jonfa (2006) the vector control efforts made since 1994 in some villages have improved the situation by reducing disease prevalence from 16% to zero and fly apparent density from 3.5 to 0.32 after a year in 1995 but interruption of the program resulted severe damage. The present field trial showed the presence and severity of drug resistance to both drugs which could not be completely cleared by doubling their doses. The infections detected from 60 to 90 days after treatment with Diminazene aceturate could be confused with new infections which could not be true for Isometamedium chloride because of its long acting and prophylactic effect. In conclusion, the present study showed the presence and widespread of trypanocidal drug resistance to both Diminazene aceturate and Isomethamedium chloride in Konso District. Thus improved supply of trypanocidal drugs and government veterinary services with strengthened community awareness and vector control will help minimize further development of the problem and interrupt transmission of drug resistant strains among susceptible animals. This should be complemented with further research on the

mechanism of development and presence of multiple drug resistance and cross resistance using more sensitive molecular techniques.

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