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Detection of Bovine Trypanosomosis and Efficacy of Selected Trypanocidal Drugs in Konso District, Southern Ethiopia

Gewado Ayledo Gellebo[1], Sisay Weldegebriel Zeweld[2]*

[1]Konso district, Karat town, Southern Nations Nationalities and People's Regional State SNNPRS), Southern Ethiopia, E-mail: gag_smart@yahoo.com, Tel. +251-0910576419

[2] (*corresponding author): Mekelle University College of Veterinary Medicine, Tel. +251 914745722 E-mail: eversis2985@yahoo.com Fax: +251 344 401595

Abstract

Field and experimental investigations on bovine trypanosomosis and efficacy of selected trypanocidal drugs were undertaken in Konso district, southern Ethiopia, from September 2012 to June 2013, with major views to determine the prevalence and socioeconomic impacts of bovine trypanosomosis and assess the efficacies of selected trypanocidal drugs. Questionnaire interviews were used to collect relevant information. Blood microscopy and packed cell volume (PCV) estimation, estimation of apparent tsetse density, trypanocidal drug sensitivity testing, cattle treatment with experimental drugs, isolation and inoculation of trypanosome isolates and treatment and monitoring approaches were used. The study results revealed trypanosomosis to be a major threat to livestock production with contrasting arrays of socioeconomic impacts. The study on bovine trypanosomosis indicated an overall parasitological prevalence of 17.0 % and 13.4 % during rainy and dry season, respectively, reflecting its significant temporal and spatial variation ($p < 0.001$); and *T. congolense* to be a dominant trypanosome species hampering livestock production in Konso district. Regression analyses on haematological findings disclosed a significant reduction ($p < 0.05$) in the herd mean PCV with an increase in the prevalence of trypanosomosis. Drug sensitivity testing revealed the presence of *T. congolense* populations exhibiting resistance to Diminazene aceturate. In conclusion, the absence of improved veterinary service and indiscriminate use of poor-quality trypanocidal drugs have proven to boost the risk of drug resistance. Therefore, it is recommended that integrated disease control approaches be adopted with chemotherapy restricted to clinically sick animals, and legislations be devised and harmonized to ensure the quality of trypanocidal drugs.

Keywords: *Bovine trypanosomosis, PCV, Prevalence, T. congolense, Trypanocidal drugs*

1. Introduction

Sub-Saharan Africa has been reputed to hold the greatest opportunity for expansion of continental ruminant biodiversity, which plays pivotal roles in the development of sustainable livelihoods mainly for rural communities. However, rampant livestock diseases in general, and especially tsetse-transmitted African animal trypanosomoses have been incriminated as the predominant elements in the extreme deterioration of livestock resource across the continent (FAOSTATA, 2005). Until recently, the deleterious effects of African animal trypanosomosis continue to curtail sustainable livestock development across much of sub-Saharan Africa (Shaw, 2004).

As a key component to improve the productive opportunities of rural communities in tsetse-infested areas, the control methods against animal trypanosomosis have been aimed principally at using suitable trypanocidal drugs as the most important tactics in destroying trypanosomes. However, these drugs are limited in number and have been under extensive administration for over 40 years with little/no regular monitoring (Holmes *et al*., 2004). Consequently, recent case surveys conducted in some sub-Saharan countries, including Ethiopia, have revealed that almost all of the commercially available trypanocidal drugs are gradually losing their efficacy due to the development of multiple resistances by trypanosomes (McDermott *et al*., 2003). Therefore, resistance to trypanocidal drugs is increasingly recognized as a major constraint to sustainable livestock production. Furthermore, the spread of trypanocidal drug resistant to the point where therapeutic and prophylactic failure may occur over large areas is probably the greatest risk to the future use of the currently existing few trypanocidal drugs in tsetse-infested areas of sub-Saharan Africa (OIE, 2004).

In Ethiopia, trypanosomosis is the most prevalent and the biggest constraint to livestock production, where about 220,000 km^2 of fertile land in south and southwestern parts of the country are infested with various *Glossina* species (ILRI, 2002). Socio-economic and ecological constraints involved in initiating and maintaining vector control strategies have compelled the Ethiopian livestock sub-sector to primarily rely on the use of the salts of just three trypanocidal compounds, namely, Diminazene, Isometamidium and Homidium (McDermot *et al*., 2003). Meanwhile, few experimental studies conducted in different tsetse-infested zones of the country, using tests both in ruminants and mice, have revealed the occurrence of varying degrees of resistance in trypanosomes to the commonly applied trypanocides. Here, most of the experimental studies conducted to assess the efficacy of trypanocidal

drugs have involved experimentally infected mice where it was possible to demonstrate the general status of resistance to the drugs used in cattle (Yeshitila *et al*., 2006; Miruk *et al*., 2008).

Given the fact that neither the single-dose nor the multiple-dose tests used in mice are able to accurately predict the curative and prophylactic doses of trypanocidal drugs for cattle infected with a particular trypanosome isolate, it is necessary to ascertain whether or not treatment with a manufacturer's recommended dosage is likely to be successful in cattle infected with this isolate. Nevertheless, most of the currently available information on trypanocidal drug resistance is derived from small number of case reports and does not give any indication of the exact situation of the problem across tsetse-infested zones of Ethiopia. On the other hand, in spite of the long-term supply and indiscriminate application of trypanocides of doubtful quality, mainly by unskilled persons over decades, adequately quantified information is not available for most areas in the southern region of Ethiopia, and particularly for Konso district, about the success of treatment with these drugs. As a foreground step it was, therefore, essential to undertake cross-sectional study in efforts to identify areas with high infection pressure and high suspicion of drug resistance. Furthermore, i*n-vivo* investigations are explicitly required to ascertain the success of treatment with trypanocidal drugs in cattle infected with trypanosome isolates, as a valuable step to generate baseline data as an integral part to the rapid assessments of the true prevalence and probable impacts of trypanocidal drug resistance across tsetse-infested areas of Ethiopia. Therefore, this study was conducted with due emphasis to determine the prevalence of bovine trypanosomosis; to assess the therapeutic and prophylactic activities of selected trypanocidal drugs in experimentally infected village cattle with field isolates of *T. congolense*; and to apply contingent valuation technique to assess the propensity of Konso community, as an aid to define appropriate integrated strategies to control drug resistance in the field.

3. Materials and methods

3.1. Description of the study area

The present study was conducted in Konso district of southern Ethiopia. Konso is one of the 77 districts in the Southern Nations, Nationalities, and Peoples Region (SNNPR) of Ethiopia. Konso district is a location about 600 Kilometers (Kms) distant southwards from the Addis Ababa, the capital of Ethiopia. The area has an altitude of 550-2300 meters above sea level (m.a.s.l.), with a pronounced rainy season occurring between February and May, and the average rainfall is about 750mm per

annum. The district has two agro-ecologic categories, lowland (560-890 m.a.s.l.) and Middle altitude (1760-1990 m.a.s.l). The annual range of temperature is between 20^0c and 34^0c. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), this district has a total population of 235,087 with an area of 2,273.79 square kilometers. The people reside in subsistence livelihood through mixed agricultural farming practices, as elsewhere in Ethiopia. There are above 150,000 heads of small east African zebu cattle and more than 500,000 heads of small ruminants (sheep and goats) maintained under traditional village management system with multiple ownership raised mainly in the low lying areas for agricultural activities, home consumption and as a source of additional monetary incomes through sales of live animals. Significant numbers of other livestock exist in the area (CSA, 2007).

3.2. Study design

A cross-sectional study was conducted from September 2012 to June 2013 in order to include individual cattle for blood sample collection and packed cell volume /PCV/ estimation and determine the monthly parasitological prevalence of bovine trypanosomosis in the study cattle population. Experimental design was established a month prior to the commencement of the experimental work and experimental animals were moved to a fly-proof accommodation and treated with experimental drugs at recommended dose rates.

3.3 **Sample size determination**

A total of 7 herds, 3 herds from *Jarso* and 4 from *Gumaide* peasant associations (PAs), comprising of 482 cattle heads were sampled during dry and rainy seasons in the PAs to determine the monthly prevalence of bovine trypanosomosis (Table 1).

Table 1: Structure of cattle herds and herd sizes included in study

Agro-ecologic categories	**Study PAs**	**Herd size per PA**
Lowland (560-890 m.a.s.l.)	*Jarso*	230
Middle altitude (1760-1990 m.a.s.l)	*Gumaide*	252
	Total	**482x2 seasons = 964**

The sample size required was determined based on simple random sampling technique, with a previous prevalence of 19.5%; a precision level of 5% and a confidence interval of 95% and calculated using the

formula by Thrusfield (2007). Therefore, a total of 964 cattle head were selected and sampled during the entire visiting period. The selected animals were then ear-tagged for ease of identification during subsequent monthly visits.

3.4 Study methods

3.4.1 Questionnaire survey

Field investigations were conducted in selected sites of the study area, with a view to identify areas with high trypanosomosis risk, and those sites highly suspected with drug resistant trypanosome populations. To this effect, two agro-ecologically distinct study sites, namely, *Jarso* and *Gumaide* were selected for subsequent administration of questionnaire interviews. These sites were the mainstreams where more than three-fourth of the livestock resource was maintained, and a contrasting veterinary service delivery system was experienced. *Jarso* was a livestock-rearing site that was relatively far from vet clinic and, therefore, had poor animal health delivery system so that cattle owners often purchased drugs from drug smugglers and open markets. On the other hand, *Gumaide site* was in close proximity to the local veterinary service, hence with relatively better animal health service delivery. However, previous experience suggested that some CAHWs in this area often provide subnormal treatment doses. Meanwhile, a structured questionnaire was designed and applied to a randomly selected sample of 100 households, and 4 purposely selected focus groups of 36 key informants (8-10 farmers per group). Therefore, a sample comprising 136 farmers (50 % from each site) was organized so as to acquire baseline information on herd composition, socioeconomic activities, the major livestock health problems and their management strategies; source and usage strategies of trypanocidal drugs; and suspected failure of trypanocidal activities.

3.4.2 Blood microscopy and PCV estimation

Individual cattle were selected and parameters like age, sex, breed, body weight; packed cell volume (PCV), parasitaemia, history of previous treatment, etc. were recorded in a monthly data-recording sheet. Blood samples were collected monthly from randomly selected cattle with heparinized capillary tubes from marginal ear-veins, and one end of each capillary tube sealed with a crystal-seal. The collected blood samples were then examined through a combination of micro-haematocrit centrifugation at 12,000 r.p.m for 5 minutes and a Dark-ground/Buffy Coat microscopic study technique, under 40 times objective lens and PCV values of each blood sample were estimated using a micro-haematocrit reader. Animals with lower PCV readings (≤ 24 %) were weighed and treated with

Diminazene aceturate at the dose rate of 3.5mg/kg body weight. For blood samples in which trypanosomal parasites were detected during microscopic examination, thin smears were prepared, stained with Giemsa solution and examined under oil immersion (100x) for species identification of trypanosomes (Uilenberg, 1998).

3.4.3 Estimation of apparent tsetse density

In order to determine the current apparent density of tsetse flies and other vectors, and assess their relative importance in bovine trypanosomosis in the study areas, biconical traps odour-baited with cow-urine were deployed at an interval of about 100 meters during both the rainy and dry season in the two PAs. A total of 80 biconical traps, 40 traps at *Jarso* and 40 at *Gumaide* Pas, were deployed in grazing lands, along river banks, wet and dense areas. Cow-urine, as an odour attractant, was regularly brought and placed in locally available containers, which were permanently kept under each trap and regularly topped up with urine on weekly basis. All the traps were coated with grease in order to prevent the trapped flies from being preyed out by ants and other predators. Identification of the flies into their species level was carried out on the basis of morphological features (Leak, 1999), such as specific colorations of their abdominal and tarsal segments on their front legs. To facilitate this technique, a magnifier hand lens was used. Distinguishing tsetse flies from other mechanical vectors was based mainly on their external appearances and microscopic structures like the hatchet cell on their wings, a structure unique for tsetse. Sexing of tsetse flies was based on the genital structure of respective sexes. The traps were emptied and the number of fly trapped was properly recorded at every 24 hours interval.

3.4.4 Trypanocidal drug sensitivity testing

In order to assess the therapeutic and prophylactic efficacy of the commonly used trypanocidal drugs (Diminazene aceturate and Isometamidium chloride), field isolates of *T. congolense* were randomly collected from parasitaemic cattle at *Jarso* study site. A treatment group of ten calves and a control group of five calves with about 5 to 6 months of age were selected from small east African zebu cattle. All the calves meant for the experimental purpose were obtained from, and kept at, Durro experimental site (2268 m.a.s.l.) until the termination of the experiment. Then, *in-vivo* experimental infection with the trypanosome isolates, one stabilate per five calves, was made by intravenous injection. The main features of the experimental calves at the time of inoculation are summarized in Table 2.

Table 2: Major characteristics of experimental animals at the time of inoculation with *T. congolense* isolates

Animal categories	ID No.	Sex	Age in days	Body weight (kg)	PCV value (%)	*T. congolense* isolate inoculated
Treatment group	K0101	M	159	88.5	26	ET/07/Konso 59
	K0102	M	176	98.0	28	ET/07/Konso 59
	K0103	F	158	88.0	27	ET/07/Konso 59
	K0104	M	167	92.9	25	ET/07/Konso 59
	K0105	F	181	100.7	28	ET/07/Konso 59
	K0106	F	163	90.7	24	ET/07/Konso 114
	K0107	M	171	96.8	27	ET/07/Konso 114
	K0108	M	178	99.3	26	ET/07/Konso 114
	K0109	F	175	98.5	27	ET/07/Konso 114
	K0110	M	180	100.1	26	ET/07/Konso 114
Control group	K0111	M	174	97.0	28	_
	K0112	M	170	96.5	25	_
	K0113	F	169	94.2	27	_
	K0114	M	179	100.1	29	_
	K0115	M	171	95.8	26	_

3.4.5 Treatment with experimental drugs

A month prior to the commencement of the experimental work and experimental animals were moved to a fly-proof accommodation and treated with long-acting oxytetracyline, Albendazole 2500mg, ivermectin, and Diminazene aceturate at recommended dose rates. After two weeks, the animals were examined for presence of trypanosomes in their blood. Starting from this day and continuing until the end of the experiment, PCV and parasitaemia were monitored three times per week by examination of peripheral blood from marginal ear-veins through Dark ground/Buffy coat microscopic technique. Moreover, the calves were also examined, on daily basis, for clinical conditions throughout the study period (Eisler *et al*., 2001).

3.4.6 Isolation and inoculation of trypanosome isolates

Parasitological examinations of blood samples, by a combination of micro-haematocrit centrifugation and Buffy coat methods, were conducted in the field at Jarso site in order to identify cattle naturally

infected with heavy scores of trypanosomal parasitaemia. Thin blood smears stained with Giemsa solution were prepared and examined for species identification of trypanosomes. Two adult cattle, ID Number K59 and K114 of Table 2, were randomly selected from the cattle having heavy scores of parasitaemia (*T. congolense strain*). The isolates were named as ET/07/Konso 59 and ET/07/Konso 114, according to (FAO, 2003). Jugular vein blood samples were collected into EDTA-treated vacutainer tubes, placed in liquid nitrogen and carried to the experiment site called Durro. After confirming the viability of the trypanosome stabilates microscopically, the isolates were injected into the jugular veins of the experimental calves that were found negative for trypanosomal parasites upon previous examination.

3.4.7 Treatment and monitoring

The experimental calves inoculated with the trypanosome isolates were regularly monitored for clinical and parasitological parameters. When the first peak of parasitaemia was detected, they were weighed and treated, on the same day, intramuscularly with Diminazene aceturate at a dose rate of 3.5 mg/kg body weight. For calves in which relapse/breakthrough infections were detected after the treatment with Diminazene aceturate, clinical and parasitological monitoring was carried out every three other day for 45 days in order to obtain basic information on the pathogenecity of drug resistant trypanosomes. Based on the consideration of parasitological and clinical examination, the relapsed calves whose PCV values had revealed a fall by one-fifth of their value at the time of relapse, and those with significantly deteriorated clinical manifestations were intramuscularly treated, on day 60, with Isometamidium chloride at a dose rate of 0.5 mg/kg body weight. All experimental animals were monitored and followed up for 100 consecutive days, from 15 December 2012 to 24 March 2013, in which they were maintained on natural grass supplementation and water *ad libitum*.

3.5 Data management and statistical analysis

Both qualitative and quantitative data, collected through a combination of questionnaire interviews, cross-sectional as well as experimental study designs were handled properly in MS Excel spread sheets and analyzed. A 95 % confidence level was employed for most of the quantitative data, in order to extrapolate sample results to the target population at large. All the data used for description as well as inference purposes were analyzed using the Statistical Package for Social Sciences (SPSS) Version 20 software. Linear regression analysis model was employed to assess the relationship between parasitological prevalence of trypanosomal infections and herd average PCV. Fly population in

respective study sites was estimated in terms of the relative fly density which was calculated as fly number per trap per 24 hours. One-way ANOVA test was applied to determine the significant variations in the mean daily catches of the respective fly vectors. The prevalence of relapse/breakthrough infections in experimental calves was calculated as the number of animals with relapse/breakthrough infections on the day of monitoring divided by the total number of animals examined at that particular day. Hence, interpretation of the results on this entity was made based on the standardized experimental protocols described for drug sensitivity testing in cattle (Eisler *et al*., 2001).

4. Results

4.1 Questionnaire survey

Structured questionnaire were administered to a total of 136 households and key informants in order to acquire baseline information on socioeconomic activities and other essential issues pertaining to livestock health and resistance to trypanocidal drugs, as perceived by farmers. Response rate of the questionnaire interviews was 100 %. The majority of members of the households (97.8 %) were within the productive age category (25-35 years), with the mean household size of 6.83. However, the household heads involved in this study were relatively older in *Gumaide* PA than those in *Jarso*. Respondents indicated that cattle were often tended in communal herds and allowed for free grazing mainly on natural grasslands, though supplementation with crop residues (aftermath) following harvesting times was also common. Livestock owners wholly (100 %) disclosed that there have long been multi-factorial livestock health problems in their respective localities, with animal diseases and recurrent drought being the commonest ones among several others. Among the several diseases, respondents incriminated trypanosomosis, contagious bovine and caprine pleuropneumonia (CCPP & CBPP), various parasites as the most important livestock health problems posing severe annual losses to livestock sub-sector. Most of the annual livestock losses were attributed to animal trypanosomosis, a serious disease affecting several livestock species. Animal trypanosomosis was an endemic livestock problem occurring during rainy months of the year (April-October) and the disease was characterized, among several other signs, by depression, rough hair coat, reduced appetite, emaciation, weakness and, thus, a reduction in the working power of their draft oxen. The great majority of livestock owners attributed the transmission mode of trypanosomosis to flies, but none of them were able to distinguish tsetse from biting flies.

Sixty seven percent of the households witnessed that there has been a decreasing tendency in the occurrence and impacts of animal trypanosomosis, at least, since the last 8 years. Nevertheless, particularly in *Jarso* site where highly promising irrigation-based agricultural projects have currently been under implementation as setouts to improve food security, most of the respondents claimed that there had never been any improvement in the occurrence and impacts of trypanosomosis in their localities. For this reason, they were desperate that if such a problem remained unsolved, their livelihoods would be highly hampered. On the other hand, the results revealed that the successful tsetse control over the past few years resulted in significant reduction in annual livestock production losses and improved access to pasture and water resources.

Respondents all in all (100 %) reported Diminazene aceturate and Isometamidium chloride to be the most common trypanocidal drugs used for the treatment of cattle against trypanosomosis. The Konso community most frequently used curative trypanocidal drugs (69.8 %) than prophylactic drugs (26.5 %). Seventy-nine percent of the interviewed households witnessed that they have been using these drugs at least for the last 15 years, and this phenomenon of drug usage showed an increasing tendency for the last 10 years. On the other hand, most of the farmers (98 %) claimed that they had quitted the use of Homidium tablets over the past 8 years because of its lower trypanocidal effects and abortion problems associated with its use in pregnant animals. According to the testimony of the households, majority of the farmers (86.9 %) often treated only sick cattle, focusing mainly on matured cows and oxen. Moreover, nearly 70% of the farmers acquired trypanocidal drugs from open markets, community animal health workers (CAHWs) and drug smugglers, non-professional persons. However, respondents claimed that more than 40 % of the animals treated with trypanocidal drugs did not recover from the disease, despite repeated treatments. In addition, results of the questionnaire survey on trypanocidal dosage regime revealed that statistically significant proportions of the total trypanocidal drugs were applied below the manufacturers' recommended doses.

4.2 Detection of trypanosome

Blood samples from a total of 964 village cattle were examined with combination of micro-haematocrit centrifugation and Buffy coat microscopic procedure monthly during the rainy and dry seasons. Blood samples of 230 and 252 heads of cattle of *Jarso* and *Gumaide* peasant associations (PAs), respectively, were examined during the rainy months (September-October) and trypanosome parasites were detected

in 45 (19.6%) and 43 (17.1%) of the animals, respectively, and 36 (15.6%) and 33 (13.1%) of animals during the dry season, respectively. The highest mean parasitological prevalence (20.4 %) was recorded in *Jarso* and the lowest (12.6 %) in *Gumaide* peasant association. Among the positive animals detected at both the study PAs, 133 (84.47 %), 19 (12.10 %) and 5 (3.43 %) cases were due to *T. congolense*, *T. vivax* and mixed infections of *T. congolense* and *T. vivax*, respectively. The monthly prevalence of *T. congolense* infection was significantly higher ($p < 0.001$) than the infections due to *T. vivax* and mixed species. Comparing trypanosomal infection on spatial basis, the mean monthly prevalence was significantly higher ($p < 0.001$) in *Jarso* than those in *Gumaide* during rainy months. Temporally, however, the mean monthly prevalence during dry season did not show statistically significant differences between the two PAs ($p > 0.01$). Nevertheless, the reduction in mean parasitological prevalence of trypanosomal infection from rainy season to dry season at both PAs revealed statistically significant changes ($p < 0.05$).

4.3 Haematological examination

The average PCV readings and distribution patterns of positive and negative animals or of both parasitaemic and aparasitaemic animals in *Gumaide* area were slightly higher than the average values in *Jarso* PA (Table 3). Thus, the differences in PCV values for parasitaemic and aparasitaemic animal categories were statistically significant ($p < 0.05$) and the overall patterns of mean PCV readings for both parasitaemic and aparasitaemic animals are depicted in Figure 1 and 2.

Table 3: Haematological results of cattle herds at two study PAs of Konso district

Sampling PAs	Sample size per season		Mean PCV (± 1 S.E)	
	Rainy season	**Dry season**	**Parasitaemic animals**	**Aparasitaemic animals**
Jarso	230	230	25.7 ± 0.3	27.5 ± 0.5
Gumaide	252	252	27.3 ± 0.2	28.9 ± 0.6

The average PCV values of aparasitaemic animal were significantly higher than the corresponding average values of parasitaemic animals ($p < 0.001$). However, despite the presence of trypanosomal parasites in their blood, about 15 % of the parasitologically positive animals had PCV values of 28 % or more (Figure 1). On the other hand, about 10 % of the parasitologically negative animals had PCV scores below 26 % (Figure 2).

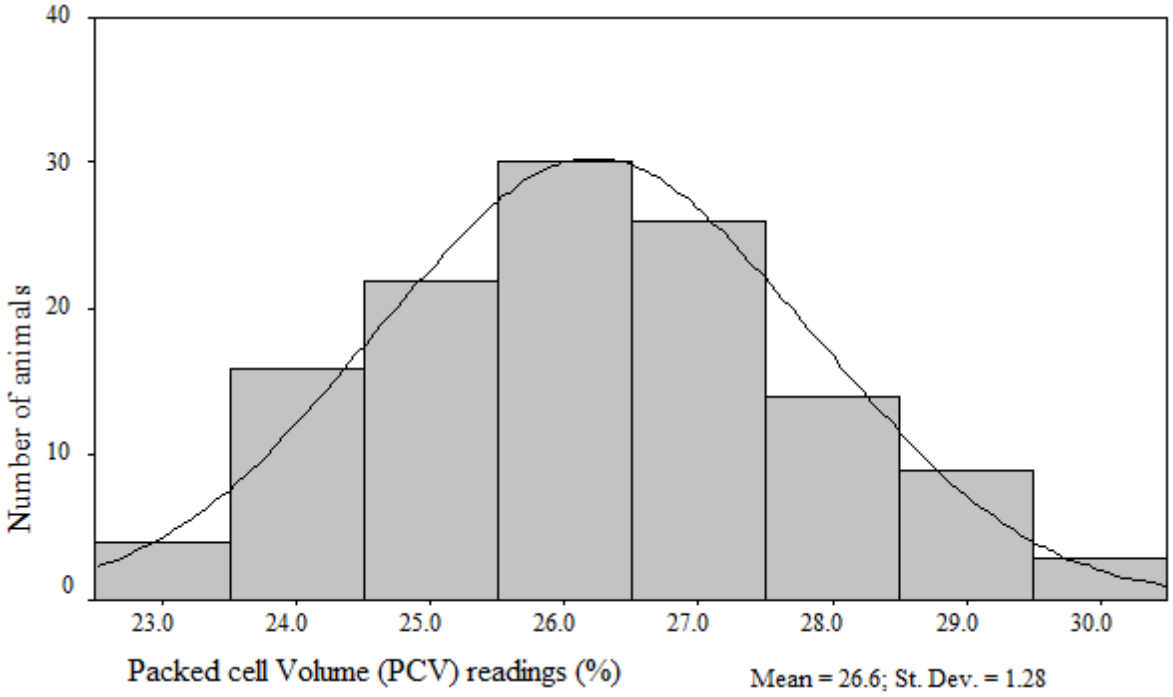

Figure 1: Patterns in the distribution of PCV readings of parasitologically positive animals

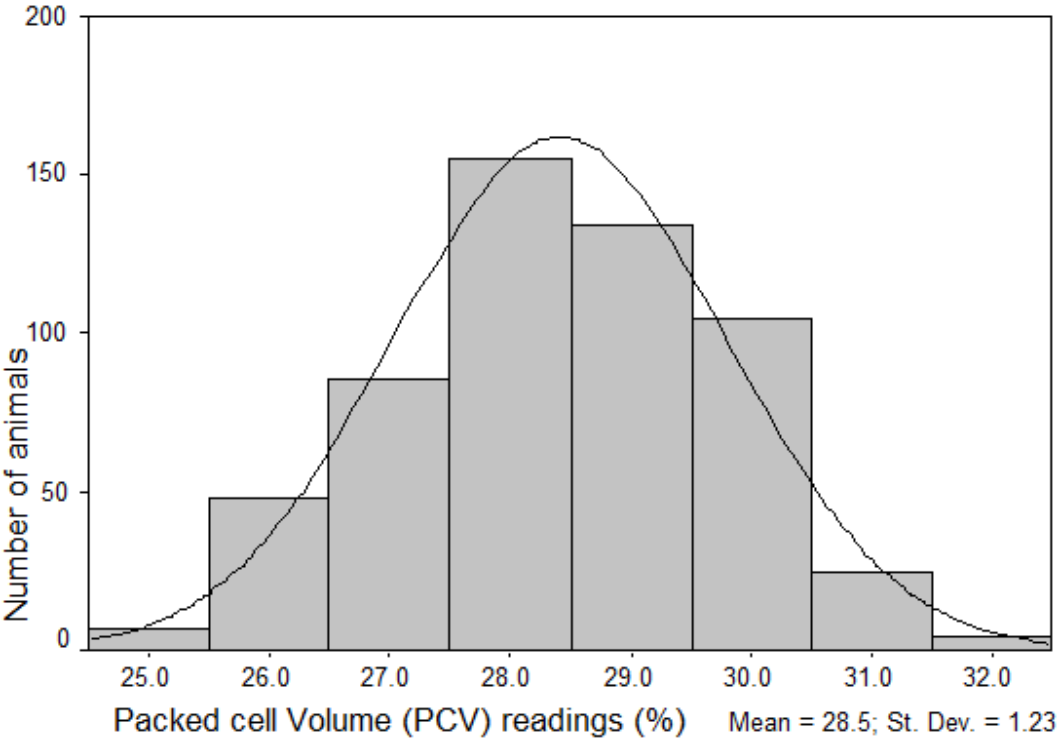

Figure 2: Patterns of PCV readings of cattle negative for bovine trypanosomosis

In relation to seasonal variation, the average PCV values of the herds were relatively higher during rainy months as compared to those values for dry months, the variation being statistically significant ($p < 0.05$). Across the herds at the two sampling PAs, a negative association was seen between the herd average PCV readings and parasitological prevalence of trypanosomal infections. Especially with abrupt rises in the parasitological prevalence of trypanosomal infection in the herds (above 18 %), the reduction in the herd mean PCV values seems to be higher (Figure 3).

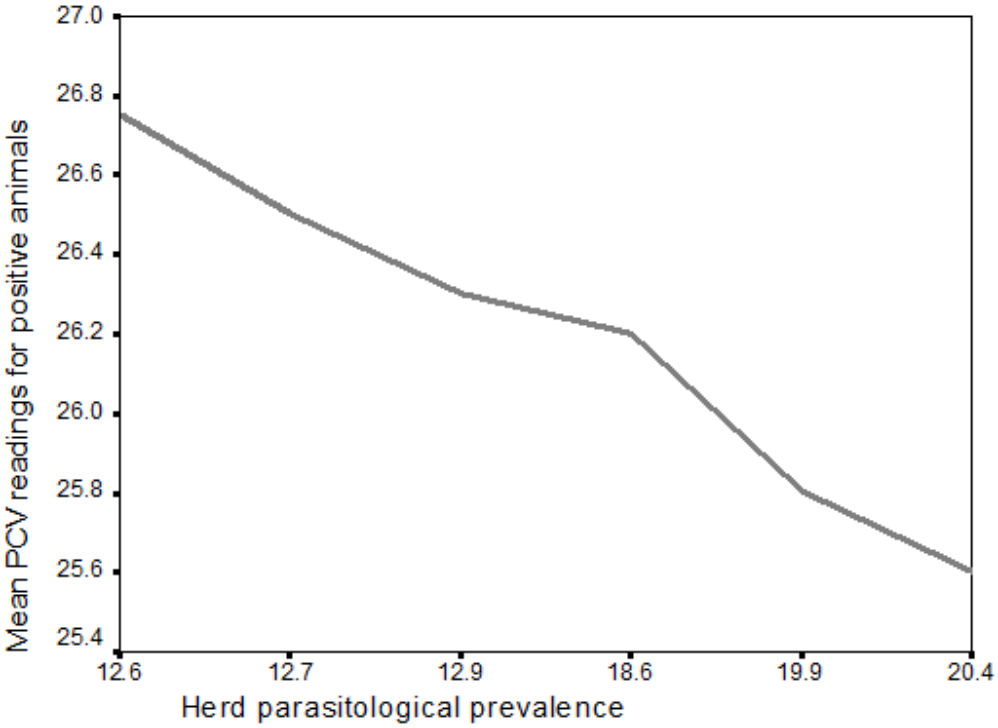

Figure 3: The relationship between herds means PCV and prevalence of trypanosomal infections

In addition, linear regression analyses of herds mean PCV readings on the prevalence of trypanosomal infection revealed that the PCV value of parasitologically positive animals significantly decreased ($p < 0.001$) with high prevalence of trypanosomal infections (Table 4).

Table 41: Linear regression of herd means PCV on the prevalence of trypanosomal infection in the PAs during the rainy and dry seasons

Sampling PAs	Sampling season	No. herds sampled	Herd size sampled	Regression coefficient and its significance*			
				α (± 1 S.E)	β (± 1 S.E)	r	P-value

Jarso	Rainy	3	230	28.6 ± 0.8	-0.21 ± 0.09	0.73	0.0004
	Dry	3	230	26.2 ± 0.5	-0.43 ± 0.12	0.69	0.0001
Gumaide	Rainy	4	252	29.9 ± 0.7	-0.37 ± 0.05	0.82	0.0001
	Dry	4	252	27.4 ± 0.9	-0.28 ± 0.17	0.63	0.0002

* : α, intercept; β, regression coefficient; r, correlation coefficient

4.4 Estimation of tsetse density

A total of 287 vector flies representing four families were captured in 80 biconical traps deployed in selected sites of the study areas. The mean catches of major flies were expressed as the mean number of flies caught per trap per day, in each season of respective sites (Table 5).

Table 52: Summary of the major fly vectors trapped at seven sampling sites

		Composition and mean catches (fly/trap/24hours) of main vectors*							
		Glossina		*Tabanus*		*Stomoxys*		*Hippobosca*	
PAs	Seasons	Total	Mean	Total	Mean	Total	Mean	Total	Mean
Jarso	Rainy	67	2.95	26	1.31	21	1.05	17	0.85
	Dry	36	1.80	8	0.42	5	0.25	3	0.15
Gumaide	Rainy	39	1.94	16	0.80	13	0.65	9	0.45
	Dry	15	0.75	7	0.35	3	0.15	2	0.10
	Total	157	1.86	57	0.71	42	0.53	31	0.38

* Significantly higher mean catches recorded for *Glossina* compared to other vectors in both seasons ($p < 0.05$)

Glossina species formed the predominant proportion of the fly catches (54.47%), followed by *Tabanus* (19.86 %) , *Stomoxys* (14.63 %) and *Hippobosca* (10.80 %) (Table 5). Thus, the results of the analysis of one way-ANOVA for mean catches of the flies indicated that the mean daily catches of *Glossina* species were in statistically significant excess ($p < 0.05$) than the corresponding means of other vectors both during the rainy and during the dry seasons in all the sampling sites. For all the flies, the proportion of female population revealed a higher preponderance (65.8 %) over the male population (34.2 %). Across the sampling sites, the overall fly catches were relatively higher in the rainy months than in the dry season. All the *Glossina* species captured at each sampling site were belonged to *G. pallidipes* sub-group.

4.5 Drug sensitivity test

Trypanocidal activities of Diminazene aceturate and Isometamidium chloride were assessed in ten calves experimentally infected with field isolates of *T. congolense* collected from randomly selected cattle at *Jarso*. The first peaks of parasitaemia were detected between 13-15 days following intravenous inoculation of the trypanosome isolates. The infected calves manifested typical clinical signs of trypanosomosis such as: depression, fever, inappetance, swelling of pre-scapular and pre-femoral lymph nodes, rough hair coat, and overall reduction in PCV. After 9 days of intramuscular treatment with Diminazene aceturate, neither of the calves revealed relapse or breakthrough infections. However, relapse infections were detected after 12 days (calf ID No. K0101) and 15 days (calf ID No. K0104) following treatment. Then, these calves were treated with Isometamidium chloride on day 60. On the other hand, no relapses of trypanosomal infections were detected in any of the remaining eight calves until the termination of the experiment (Table 6).

Table 6: Patterns of relapse infections of *T. congolense* isolates in experimentally infected calves after treatment with Diminazene aceturate (days = 15).

Animal ID	Days to the first detection and persistence of relapse/breakthrough infection*					
	18^{th}	21^{st}	24^{th}	27^{th}	30^{th}	60^{th}
K0101	b	b	b	a	a	a
K0102	b	b	b	b	b	b
K0103	b	b	b	b	b	b
K0104	b	b	b	b	a	a
K0105	b	b	b	b	b	b
K0106	b	b	b	b	b	b
K0107	b	b	b	b	b	b
K0108	b	b	b	b	b	b
K0109	b	b	b	b	b	b
K0110	b	b	b	b	b	b

*: a, Relapse detected or persisted on the specified day; b, relapse neither detected nor persisted on the specified day.

Linear regression analysis of the effects of persistent trypanosomal infections on mean PCV and body weight gain in the relapsed calves revealed that the reduction in these parameters was not statistically significant ($p > 0.05$) for the experimental period (Table 7).

Table 7: Linear regression of mean PCV and body weight gain of experimental calves on the persistence of *T. congolense* isolates

Major health parameters	Regression and correlation coefficients and significance *			
	α (± 1 S.E)	β (± 1 S.E)	r	P-value
Mean PCV (%)	27.2 ± 1.5	-0.01 ± 0.02	0.35	0.062
Mean body weight gain (gm)	248 ± 1.3	-0.03 ± 0.04	0.28	0.078

*: α, intercept; β, regression coefficient; r, correlation coefficient

Despite the deterioration of the clinical and haematological conditions during the first few weeks post inoculation of the trypanosome isolates, there was progressive improvement in parameters following treatment with Diminazene aceturate and Isometamidium chloride. On the basis of deterioration in both the clinical and hematological conditions described above, the relapsed calves were treated, 45 days later (on day = 60), with Isometamidium chloride at the dose rate of 0.5mg/ Kg body weight. Relapse infections were not detected on microscopy in either of the calves from day 63, until the termination of the experiment (Table 8).

Table 8: Patterns of relapse infections of *T. congolense* isolates in experimental calves after treatment with Isometamidium chloride in Konso district, southern Ethiopia.

Animal ID	Detection and persistence of relapse /break through infection *										
	63rd	66th	69th	72nd	75th	78th	84th	90th	93rd	97th	100th
K0101	n	n	n	n	n	n	n	n	n	n	n
K0104	n	n	n	n	n	n	n	n	n	n	n

*: n, Relapse infection neither detected nor persisted on the specified day.

5. Discussion

Tsetse-transmitted African Animal Trypanosomosis (AAT) is a disease complex with profound social economic consequences on African scene. Understanding the perceptions and knowledge of livestock owners about the impacts of trypanosomosis are valuable steps in the formulation of assumptions about livestock productivity. The aspect of primary importance in the present study is the evidence that animal trypanosomosis was the most important threat to livestock production in Konso district of Southern Ethiopia. The perception by livestock owners that trypanosomosis primarily constrains livestock production has also been reported in other areas of Ethiopia. Similarly, reports of

socioeconomic surveys and temporal comparison of livestock herds under varying risk levels across sub-Saharan Africa revealed that most of the annual livestock losses are attributed to trypanosomosis (Swallow *et al*., 2000). Majority of the respondents in the peasant associations reported a declining tendency in the occurrence and impacts of animal trypanosomosis over the last few years. This indicates the dramatic effects associated with tsetse control operation through deployment of traps/targets and application of 'pour-on' formulation on the back of cattle. On the other hand, the contention by many livestock owners that the disease problem remained apparent till the present could be explained by the fact that in dry months livestock are confined to graze around the main rivers where tsetse flies concentrate posing great challenges and the relatively higher insufficiency of livestock feed in the study areas exacerbate the intolerance of livestock to trypanosomosis during this period. The current survey has revealed significant increases in the percentage of livestock owners using trypanocides. A similar situation was observed in Northern Cote d'Ivoire, with more farmers in tsetse controlled areas using trypanocidal drugs than those in non tsetse-controlled areas (Pokou *et al*., 1998).

Delivery of poor-quality drugs and administration of over-diluted quantities mostly by unskilled persons were identified as the common treatment strategies. The majority of livestock owners witnessed a more inclination to the use of curative trypanocidal drugs than prophylactic ones over the last decade, and this trend indicated inadequate knowledge on appropriate drug usage. In a similar manner, surveys in the Zambia have shown that farmers administer most of the trypanocide treatments, with a strong tendency to use curative drugs than prophylactic ones (Van den Bossche *et al*., 2000). Generally, it has been revealed that most livestock owners do not have adequate knowledge on the diagnosis and appropriate drug usage. Moreover, the choice between the use of therapeutic and prophylactic drugs is made on the basis of cost per dose, without understanding the advantages of prophylactic drugs under essential circumstances (Holmes *et al*., 2004). Therefore, the similar situation expressed by livestock owners in the present study area adds to the complex circumstances, which could booster drug resistance in sub-Saharan Africa (FAO, 2003). The evidence by the respondents that most treatments are confined to sick animals, with major emphasis on mature cows and oxen, reflects the preference of livestock owners to treat the most productive animals as a priority. Similarly, there are evidences from other parts of sub-Saharan Africa indicating that, farmers treat not all their animals

for economic reasons and that, irrespective of the drugs used, the most productive animals receive the majority of treatments (Sinyangwe *et al*., 2004).

The present study indicated that *Glossina* species constitutes a predominant fly population in the spatial and temporal distribution over other vectors, with female sex preponderating over their male counterparts. The dominance of *Glossina* could be due to the presence of suitable savannah dominating the vegetative physiognomy in this area. In addition, the preponderance of female flies over the male population indicates the impeding challenge of tsetse and other vectors, and the corresponding risk of animal trypanosomosis. Indeed, a significantly higher reduction in the apparent fly density was recorded in the present survey period, compared to the corresponding values in the past 10 years. This scenario reflects the achievements attained following successive vector control. Furthermore, human settlement and mixed crop-livestock farming have been more intensively expanded in the lowland areas particularly over the last seven years, with concomitant bush clearing, which might have resulted in the destruction of fly habitats. Obviously, the adverse effects of human settlement, indiscriminate deforestation and expansion of mixed agriculture on the habitat and population dynamics of tsetse have been explicitly described in sub-Saharan Africa (Vale and Torr, 2004). All the above findings consistently add to other entomological studies about the seasonal and ecological dynamics in the population of tsetse flies and mechanical transmitters; the higher confinement of G*lossina morsitans* group into habitats with savannah vegetation and the significant impacts of female fly population on the vector challenges and associated risk of animal trypanosomosis (Rogers and Robinson, 2004). The recorded evidence that no G*lossina* species other than *G. pallidipes* were recorded in all the sampling areas, during both seasons, further strengthens previous entomological findings in the same area and the STEP-target areas of the southern rift valley (Gemechu *et al*, 1998).

The recorded seasonal variation in the prevalence of trypanosomal infection in the study areas reflects the changes associated with temporal alterations in the apparent density of fly vectors. The present findings on the apparent fly density support this trend, where highly varied mean fly catches were observed in relation to season of sampling. The higher dependence of tsetse-transmitted animal trypanosomosis on the temporal and spatial variations in vector population has been described across several areas of sub-Saharan Africa (Hargrove, 2004). Despite effective tsetse control measures over the last few years, the present study found no significant declines in the overall prevalence of

trypanosomal infections (15.6 %), as compared to the previous reports (19.5 %). The evidence that *T. congolense* is the dominant species in the present study areas and this is in accordance with most of the previously reports in the southern rift valley of Ethiopia (Gemechu *et al*, 1998) and in the Ghibe valley (Rowlands *et al*, 2001). In general, it has been proved that *T. congolense* is the most prevalent and virulent trypanosome species in Eastern Africa, although certain hemorrhagic *T. vivax* strains prevail in this area (Taylor and Authie, 2004).

The lower PCV readings in parasitaemic cattle and the higher corresponding values in aparasitaemic animals reveals an inverse relationship between herd PCV and trypanosomal infection. It also reflects the depressive effects of animal trypanosomosis on normal physiologic aspects of livestock. This relationship is in agreement with the findings of Van den Bossche and Rowlands (2001). Despite the absence of trypanosomal parasitaemia in their blood, the evidence that some aparasitaemic animals had PCV values below 26 % could possibly be due to the compounded effects of concurrent infections by haematophagous helminth parasites. On the other side, the parasitaemia observed in some animals with PCV values above 27 % could be explained either in relation to the presence of very recent infections or to the variation among cattle in the tolerance to trypanosomosis. Similar findings were reported from Ghibe valley of Ethiopia (ILRI, 2002; Rowlands *et al*., 2001). The season of sampling had a profound effect on the herd average PCV and its relation with trypanosomal infection and the severity of anaemia is influenced by plane of nutrition (Taylor and Authie, 2004) and poor pasture and high temperatures pose recurrent nutritional stress. Therefore, the observed seasonal effects on the association between herd PCV and trypanosomal infection are very likely to be due to poor nutrition during the dry season. Secondly, trypanosomosis seems to be less well tolerated during the dry season, which indicate that higher proportion of trypanocidal drug treatments are being administered during the dry season and this pattern is in line with inferences drawn from the report of Van den Bossche *et al*, 2000.

The detection of relapse infections in some of the experimental calves following treatment with Diminazene aceturate at the dose of 3.5 mg/Kg body weight is clearly indicative of the presence of at least sub-populations of trypanosome isolates resistant to this drug. This conclusion steams from the fact that Diminazene aceturate could maintain therapeutic blood levels until 22 days following treatment, unless resistance is present. This finding on the relapse date in resistant strains is comparable with inferences drawn from a previous study at the Ghibe valley where trypanosomes resistant to Diminazene aceturate have relapsed after over 14 days following treatment (Peregrine *et al*., 2000). As

there is an increasing number of case reports from other trypanosomosis-endemic areas of Ethiopia, disclosing a range of prevalence of *T. congolense* resistant to Diminazene aceturate and Isometamidium chloride (Afework, 2004; Tewelde *et al*., 2004), the demonstration of resistance to Diminazene aceturate (20%) manifested by the current *T. congolense* isolates in study district was an expected outcome. Retreatment of the cattle with Isometamidium chloride could have resulted in a complete elimination of the sub-population that revealed resistance to Diminazene aceturate. Improvement in livestock health parameters following treatment of infected animals with trypanocidal drugs at specified times has been observed by Holmes *et al*. (2004). The results on the sensitivity testing of the isolates to Isometamidium chloride are generally not conclusive as to whether or not the isolates were sensitive to this drug at the specified dose. In this procedure, the possible effects of selection biases are of crucial importance, since the trypanosome population against which the second drug was administered might not be a complete representative of the original field population. A possible explanation for this scenario is the fact that by the time of testing against Isometamidium chloride, previous treatment with Diminazene aceturate might have eliminated the sub-population resistant to Isometamidium chloride. Indeed, the possible effects of selection biases in trypanocidal drug sensitivity tests following administration of the first drug have been explicitly described (Eisler *et al*., 2004).

6. Conclusions and Recommendations

Until recently, the majority of control methods against animal trypanosomosis have been aimed at using suitable trypanocidal drugs as the most important tactics in destroying trypanosomes. However, the heavy reliance of the veterinary sector on these drugs, the alarming emergence and vulnerability to resistance together with the low adoption of integrated control approaches have aggregately created a greater dilemma in the management of this disease complex. Therefore, resistance to trypanocidal drugs is increasingly recognized as a major constraint to reliable livestock production. Furthermore, the unlikelihood of new trypanocides appearing in the foreseeable future and the spread of drug resistance to the point where therapeutic failure may occur over large areas is probably the greatest risk to the future use of these trypanocidal drugs. Animal trypanosomosis has proved a major and continuing threat to livestock production in Konso district. Efforts to combat the devastating effects of this menace have faced sustainability problems due to the absence of concerted coordination among different stockholders, and because of little/no direct public participation. Therefore, treatment of sick animals without prior diagnosis has remained to be a standard approach in the management of the disease at the farmers' level. Nevertheless, continuous supply of trypanocidal drugs of doubtful quality by drug

smugglers, indiscriminate use and application of over-diluted quantities of drugs mainly by unskilled persons coupled with poor veterinary services have greatly contributed to the development of resistance to trypanocidal drugs. Therefore, drug resistance is currently identified to be amongst the major determinants curtailing livestock production and productive opportunities of the community in Konso district. Therefore, temporal and spatial surveys should be conducted in order to provide valuable data to the rapid assessments of the true prevalence and probable impacts of drug resistance across trypanosomosis-endemic areas of Ethiopia. The use of sanative pairs of trypanocidal drugs should be intensified, and resistance to the available trypanocidal drugs should be monitored over time on regular basis. The prophylactic application of Isometamidium chloride should be and chemoprophylaxis should be combined with other control measures in an integrated manner and application of trypanocidal drugs should be restricted to the treatment of parasitaemic animals. Establishment and harmonization of the existing legislations on drug delivery and usage is essential in order to ensure drug quality. To this effect, particular attention should be paid to training and recruitment of qualified professionals so as to improve the delivery of veterinary services to rural communities.

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