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Research Article
Trypanocidal Drugs Resistance and Their Utilization Practices in Benishangul-Gumuz Region, Ethiopia

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Abstract

Background and Objective: Trypanocidal drug resistance is the major threat of cattle production and documentation of trypanocidal drug utilization practices is scanty in Benishangul-Gumuz Regional State. This study conducted to evaluate trypanocidal drug utilization practices and to assess trypanocidal drug resistance in naturally infected Sheko and Boran cattle. **Materials and Methods:** A cross-sectional survey conducted and about 86 farmers were involved. A total of 32 trypanosome-positive cattle randomly assigned to two treatment groups. Treated with a curative dosage of DIM (3.5 mg kg^{-1}) and ISMM (0.5 mg kg^{-1}). An abbreviated 28 days field protocol was applied. Descriptive statistics used to summarized the data. **Results:** About 93% of the respondents use trypanocidal drugs. Diminazene aceturate (DIM) and isometamidium chloride (ISMM) were commonly used drugs. About 31.2 and 45.7% of respondents from Assosa and Bambasi, respectively treated an animal >9 times in a year. The prevention methods practiced are avoiding flies through smoking 32/86 (37.2%), management practices to improve immunity 17/86 (19.8%) and use tsetse repellant chemicals 40/86 (46.5%). Relapse occurred in 12 animals in the first treatment and breed has a significant effect ($\chi^2 = 4.8$, $p<0.05$). But there was no significant ($\chi^2 = 2.1$, $p>0.05$) difference among drugs used. Drugs were switched in treatment failure cases. Treating first with DIM and then with ISMM was better. **Conclusion:** This study indicated the presence of drug resistant trypanosomes. The dependency of farmers on a limited number of drugs aggravate the issue. Strict supervision on drug usage, sanative pair treatment and, further experimental work required using molecular techniques.

Key words: Bovine trypanosomosis, resistance, trypanocidal drugs, utilization practices

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

African trypanosomosis is a major protozoal parasitic disease caused by various species of trypanosome, which can infect vertebrates including livestock, wildlife and human in the Africa continent, South of the Sahara desert. It is a devastating and debilitating infectious disease and transmitted to the host through the bite of an infected vector, the tsetse fly[1-3]. Bovine trypanosomosis is an important disease of cattle in many parts of Ethiopia and it is the major constraint on livestock production[4,5]. Currently, it is found to be one of the factors hampering livestock production and productivity in most settlement areas of Western Ethiopia[6]. One of the most significant problems faced in controlling the spread of trypanosomosis is that there is little economic incentive to conduct research in these impoverished parts of the world that are endemic to the disease[3].

Control and prevention of trypanosomosis is difficult since there is no effective vaccine against the disease. In this situation, control is relayed upon the use of a limited number of trypanocidal drugs and various methods which can reduce vector densities[4]. Formulation of new therapeutic agents in different laboratories through investigating pharmacokinetic properties of the trypanocides, medicinal plants and even combination therapy have not successful in preventing the threat the disease[3]. Vector control is costly and laborious and may involve negative environmental impacts, especially in insecticide applications. So, control of this disease is done mainly through chemotherapy and chemoprophylaxis using trypanocidal drugs. Diminazene aceturate (DIM) and isometamidium chloride (ISMM) have been mostly used in cattle since they are easily available and relatively low toxic. They were introduced and have been in use for more than 60 years. The repeated use of chemicals as chemotherapeutic and prophylactic agents leads to the development of resistance in the target organisms and now a days these drugs are associated with parasitic resistance. Their effectiveness is being eroded by the emergence of drug resistant trypanosomes and drug resistance is increasingly reported[2-4].

The first reports of acquired resistance for trypanocidal drugs were published during the 1960s. Trypanocide resistance remains a huge challenge in the management of animal African trypanosomosis and it is the major draw-back to agricultural development in Africa[2,7]. The rise of drug-resistant trypanosomes is a major challenge in Ethiopia's cattle production. So, detailed experimental investigation is needed in the field to monitor drug-resistant trypanosomes in Ethiopia[4]. The abbreviated 28 days experimental protocol is effective in areas where trypanosomosis risk is high and when their prevalence is greater than ten percent[2,8]. Documentation on utilization practices of trypanocidal drugs is also scanty in Ethiopia[9]. Constant monitoring of drug use and resistance development is crucial for correct trypanocide use and to improve recommendations for first use and for back up drugs to be utilized in a certain area when resistance has been confirmed[10]. Questionnaire survey along with further exploration of trypanocidal efficacy tests are essential for trypanocidal drug utilization practice and drug resistance study[11]. Therefore, a questionnaire survey along with 28 days experimental protocol were used for this particular study with the objectives of:

- To evaluate trypanocidal drug utilization practices
- To gain information on trypanocidal drug resistance in cattle

MATERIALS AND METHODS

Description of study area: The study was conducted from October 01 to December 31, 2020 in Assosa and Bambasiworedas of Benishangul-Gumuz Regional State, Ethiopia. The region is located in the West and Northwest part of Ethiopia between latitude 10° and 04°N and longitude 34° and 31°E. It consists of three administrative zones namely Assosa, Kamashi and Metekel divided into 18 woredas and two special woredas (Mao-komo and Pawe). The total area of the region is 50,380 km^2. The total farmers association is 487 and about 86% are rural dwellers which depend on agriculture and related activities for livelihood.

The altitude of Assosaworeda ranges from 580 to 1,544 m above sea level. The total area of Assosaworeda is 2,317 km^2 characterized by low land plane agro-ecology with average annual rainfall of 1,316 mm with unimodal type of rainfall that occurs between April and October. It has a livestock population of 36,916 cattle, 23,500 goats, 14,325 sheep, 5,890 equine and 35,125 poultry. Bambasiworeda is located Southwestern part of Assosa zone with elevation of 1,668 meters above sea level. The total area of the woreda is 2,210 km^2. It has the livestock population of 38,964 cattle, 11,990 goats, 3,739 sheep, 4,467 equine and 41,438 poultry (BGRBOA, 2018). Traditional housing and grazing of natural pasture are the predominant husbandry practices. The study woredas selected based on easily accessibility and high reports of bovine try panosomosis from woreda veterinarians.

Data collection: Questionnaire survey conducted to know trypanocidal drug utilization practices in the study areas. The study woredas and kebelles were selected purposively

where bovine trypanosomosis is highly prevalent, having high number of cattle and easily accessible. The respondents were 86 small holder farmers. Those who have cattle and experience of bovine trypanosomosis were included for interview and they were selected by kebelle animal health personnel.

Field trypanocidal drug resistance trial conducted at Assosa Agricultural Research Center on naturally infected Sheko and Boran cattle breeds. The detection of parasites in body fluids is currently the most widely practiced method for the diagnosis of animal trypanosomosis in endemic areas[12]. Blood samples were collected from the ear vein of each animal using capillary tube. Two capillary tubes were then filled (75% of its size) and sealed with sealant and it was centrifuged at 1500 rpm for 5 min in a microhematocrit centrifuge. All animals showing clinical signs were screened for trypanosomes using the buffy-coat technique (BCT) and the value of their PCV were recorded. Animals that were parasitaemic, had a packed cell volume (PCV) below 24% and with clinical signs of trypanosomosis were purposively selected for this study. Based on these parameters 32 infected animals were included for this study.

Trypanosome-positive Sheko and Boran cattle breeds were randomly allocated into two treatment groups with eight animals assigned to each group. Equal number of study animals per group were assigned to detect the presence of at least one resistant isolate, using an infinite population size and an expected proportion of trypanosome-resistant isolates of thirty percent. The body weights estimated using heart girth to determine the dose of the drugs to be administered[2,13].

They were treated with curative dosage of ISMM and DIM. The animals in the first group were treated with ISMM at a dose rate of 0.5 mg kg^{-1} body weight. Curative dosage (1% solution) of ISMM prepared, 1 g powder in a sachet dissolved in 100 mL distilled water. The other group treated with DIM at a dose rate of 3.5 mg kg^{-1} body weight. The solution prepared by dissolving a bag of 2.36 g in 15 mL distilled water for injection as described by Mungube *et al.*[14]. Drugs administered with deep intramuscular injections in gluteal muscle. The initial treatment day was considered as day zero. Treated cattle were monitored for trypanosomes and packed cell volume (PCV) levels at day 14 (for DIM and ISMM) and 28 (for ISMM) post-treatment as described in Degneh *et al.*[15] in 28 days experimental protocol. For animals that were persistently positive for trypanosomes, trypanocidal drugs were switched to expose the trypanosome previously treated with DIM to ISMM and vice versa and to detect if there was resistance to only one drug or to both drugs.

A treatment failure rate of 25% of the cattle in the ISMM treated group on days 14 and 28 were indicative of resistance[16]. But DIM response was considered only 14 days post-treatment due to its short prophylactic activity.

Data analysis: Data collected during questionnaire survey were coded and entered into Microsoft Excel Spread Sheet and analyzed with descriptive statistics of SPSS version 20 software package. GLM and descriptive statistics were used to calculate frequencies, percentages, 95% confidence interval and others for different parameters. The $p<0.05$ at 95% confidence level was considered significant. Packed cell value would be categorized as anemic if it was <24% and normal if it was $\geq$24%.

RESULTS AND DISCUSSION

Trypanocidal drug utilization practice towards bovine trypanosomosis were evaluated with questionnaire survey. About 86 small holder farmers were involved for the interview. The number of cattle owned by each respondent was variable (0-30 in number). Communal grazing are predominant in the study areas and all practices extensive production system. Point of watering is from river (90.7%) and the rest 9.3% is both from river and at home. The risk factors considered for bovine trypanosomosis were presented at Table 1.

All farmers from both woredas considered bovine trypanosomosis as the major threat in the area. It was in line with the report of Dagnachew *et al.*[17], trypanosomosis was a significant animal health constraint for 100% of the farmers questioned in tsetse infested areas of Northwest Ethiopia. But, only 95.7% of the respondents from Tselemti woreda, Tigray regional state replied that it is a major challenge[18]. This might be due to the agro-ecology and tsetse infestation rate difference.

The respondents' knowledge about bovine trypanosomosis is essential for control and prevention of the disease. All of the respondents in this study were able to describe bovine trypanosomosis. The symptoms they observed suggestive of bovine trypanosomosis were indicated in Table 2.

Responses of respondents in trypanocidal drug utilization practices indicated that risk factors are common for the occurrence of trypanosomosis in Northwest Ethiopia[17]. And, in this study age of cattle are one of the risk factor for bovine trypanosomosis and 52.3% (45/86) of them respond that cattle more affected at old age, and38.4% of them said that all age groups of cattle were affected. About 70.9% of them respond

Table 1: Risk factors for bovine trypanosomosis

Some risk factors	Woredas		Total
	Assosa	Bambasi	
Grazing area			
Forest	4 (25)	16 (22.9)	20 (23.3)
Grass land/savanna, bush	10 (62.5)	30 (42.9)	40 (46.5)
Around river	1 (6.3)	17 (24.3)	18 (20.9)
Forest and around river	1 (6.3)	7 (10)	8 (9.3)
Point of watering			
River	14 (87.5)	64 (91.4)	78 (90.7)
River and at home	2 (12.5)	6 (8.6)	8 (9.3)
Home only	0	0	0
Housing practices			
Housed free from flies' together human	1 (6.3)	3 (4.3)	4 (4.7)
Housed free from flies separated from human	11 (68.7)	36 (51.4)	47 (54.7)
Barn without shade	4 (25)	31 (44.3)	35 (40.7)

Table 2: Symptoms suggestive of bovine trypanosomosis by respondents

Symptoms suggestive of trypanosomosis	Frequency (N = 86)	Percentage
Rough hair coat	34	39.5
Weakness and constipation	1	1.2
Rough hair coat and emaciation	7	8.1
Rough hair coat, weakness and constipation	3	3.5
Lymph node enlargement and low appetite	2	2.3
All above signs were observed	39	45.3

N: Number of respondents

Table 3: Respondents reason for preference of trypanocidal drugs

Reason of preference	Percentage
Quick response of DIM	10 (11.6)
Adaptation of DIM but not ISMM	1 (1.2)
Relapse of infection is rare if treated with ISMM	10 (11.6)
They eat well after ISMM treatment	2 (2.4)

that sex of cattle have no significant difference for trypanosomosis infection but 18.6% and 8.1% of them respond that male and female cattle are more affected, respectively.

When their animals diseased with trypanosomosis, 93% of the respondents use trypanocidal drugs and 3.5% use traditional medicine to treat their animals but 3.5% of them sell their animals. From those who use drugs, 77.9% of them treat their animal at government clinics and 22.1% at both government and private clinics. In this study drugs from government clinic were more effective (87.2%) than private sources, which is in agreement with the report of Tesfaye *et al.*[11], drugs obtained from private drug stores were less effective compared to those obtained from governmental veterinary clinics. ISMM and DIM were commonly used trypanocidal drugs in the study area. About 60.5% and 29.1% of respondents prefer to use ISMM and DIM drugs respectively. But, 9.3% of respondents prefer to use both drugs, first they treat their animal with DIM and then at 14th day of treatment they treat with ISMM. Reason for preference of drugs were indicated in Table 3. In this study the respondents prefer ISMM than DIM in contrast to the report of Tesfaye *et al.*[11] and Tekle *et al.*[9], even though DIM preferred by farmers, it shows high treatment failure.

In almost all animal cases, animal health practitioners administer drugs, only 4.7% of respondents administer themselves in acute cases and in absence of health practitioner at the clinic. It was comparable with the study of trypanocidal drug utilization practices in Northwestern part of Ethiopia, treatment administration is mainly by animal health personnel[17].

Cattle living in tsetse infested areas are frequently infected by bovine trypanosomosis. Frequency of an animal treated with trypanocidal drugs in the two study woredas were assessed and presented in Table 4. About 45.7% of respondents from Bambasi treat an animal more than nine times in one year, whereas 50% of respondents from Assosa treat an animal 1-3 times in a year.

About 54.7% (47/86) of respondents in this study indicated that an animal receives more than six treatments per a year in contrast to the report of Tekle *et al.*[9], 85% of respondents from South-Western Ethiopia treat an animal more than six treatments per a year.

According to the respondents in this study risk factors play a great role for trypanocidal drug resistance development. Treatment failures are also frequently seen almost in every respondents. It was comparable with report of

Table 4: Frequency of an animal treated with trypanocidal drugs in a year

	Frequency of an animal treated per a year				
Woredas	1-3	4-6	7-9	>9	χ^2 (p-value)
Assosa	8 (50)	3 (18.8)	0 (0)	5 (31.2)	5.955 (0.114)
Bambasi	17 (24.3)	11 (15.7)	10 (14.3)	32 (45.7)	
Total	25 (29.1)	14 (16.3)	10 (11.6)	37 (43.0)	

Table 5: Response of infected animals treated with trypanocidal drugs

	Woredas		
Treatment responses	Assosa	Bambasi	Percentage
Cured	8 (50)	27 (38.6)	35 (40.7)
Relapsing after Rx	5 (31.3)	34 (48.6)	39 (45.3)
Death	0 (0)	7 (10)	7 (8.1)

Table 6: Trypanosomosis prevention methods practiced by small householders

	Woredas		
Prevention mechanisms	Assosa	Bambasi	Total
Tsetse control methods using chemicals	2 (12.5)	12 (17.1)	14 (16.3)
Avoid flies through smoking	0	6 (8.6)	6 (7)
Prevent animals grazing in forest and savannah areas	0	4 (5.7)	4 (4.7)
Take animals to grazing and watering areas during low fly burden	0	1 (1.4)	1 (1.2)
Tsetse control methods using chemicals and avoid flies through smoking	4 (25)	22 (31.4)	26 (30.2)
Good management practice, feeding and barn cleaning	8 (50)	9 (12.9)	17 (19.8)
Prevent animals grazing in forest and savannah areas and take animals to grazing and watering areas during low fly burden	0 (0)	5 (7.1)	5 (5.8)

Dagnachew *et al.*[17]. Response of infected animals treated with trypanocidal drugs were presented in Table 5.

Trypanocidal drugs remain the principal method of animal trypanosomosis control in the country[19]. However, in addition to a proper use of the few already licensed trypanocidal drugs, trypanosomosis control requires an integrated approach that includes both vector control and appropriate livestock management[10]. Prevention methods practiced in the study area were described in Table 6.

Field trypanocidal drug resistance trial was conducted on naturally infected Sheko and Boran cattle breeds available at Assosa Agricultural Research Center livestock farm. Those animals showing clinical signs and positive for trypanosomosis were purposively selected. And, 32 infected animals were included for this study.

The overall mean PCV of parasitaemic Sheko and Boran cattle breeds at day zero in this study is better than the study conducted in Northwest Ethiopia, 20±2.3 SD for parasitaemic animals (Table 7)[17]. In the present study treatment failure was higher for DIM than ISMM and it was in agreement with the report of Mulandane *et al.*[2].

Out of eight trypanosome positive Sheko and Boran cattle breeds treated with ISMM, 12.5 and 37.5%, respectively had persistent Trypanosome infections on day 28 post-treatment. Similarly, 25 and 75% of DIM treated Sheko and Boran cattle breeds, respectively had persistent trypanosomes on day 14 post treatment. Treatment failure were higher in Boran compared to Sheko cattle breeds in this study. At day 14 for DIM and 28 for ISMM treated animals relapse of trypanosomosis occurred in 12 animals (3 Sheko and 9Boran cattle breeds). Breed has a significant effect ($p<0.05$) = 0.033 for relapsing of bovine trypanosomosis. Proportion of drug resistance after treatment was indicated in Table 8. Case relapse was also higher in DIM treated (8/16) than ISMM (4/16) treated animals. However, there is no significant difference ($p>0.05$) = 0.144 among drugs used (Table 9).

Trypanocidal resistance is increasing over time[20]. Trypanosome species which persist in trypanocidal drugs treated animals were an indication of resistance to the specific drug type used. The DIM treatment failure were high (75%) in Boran cattle of this study.

The second treatment were administered by shifting the type of drug for relapsed cases as presented at Table 8. From ISMM treated animals, only 33.3% (1/3) of Boran cattle breed was cured with DIM treatment. However, from DIM treated animals, 50% (3/6) of Boran and all (2/2) of Sheko cattle breeds were cured with ISMM. Therefore, treatment of diseased animals first with DIM and after two weeks with ISMM is better to minimize relapse of infection. This finding is comparable with the report of Desalegn *et al.*[18], use of sanative pair of DIM and ISMM confirmed by the occurrence of no relapses after first treatment with DIM and then ISMM. The use of sanative

Table 7: Mean PCV (%) of animals included for this study

Treatment days	Breeds	Drug types	Treatment response	Number of animals	Mean PCV (%)	95% confidence interval		SD	p-value
						Lower	Upper		
Day 0	Sheko	-	-	16	22.3	21.67	23.0	1.25	0.021
	Boran	-	-	16	21.2	20.44	21.9	1.4	
Day 14/28	Sheko	DIM	Positive	2 (25)	22.7	18.9	26.5	0.42	0.003
			Negative	6 (75)	24.9	24.3	25.5	0.6	
		ISMM	Positive	1 (12.5)	21	-	-	-	0.000
			Negative	7 (87.5)	25.9	25.4	26.3	0.48	
	Boran	DIM	Positive	6 (75)	21.9	20.9	22.8	0.91	0.008
			Negative	2 (25)	24.6	29.5	29.7	0.57	
		ISMM	Positive	3 (37.5)	21.57	16.8	26.3	1.9	0.013
			Negative	5 (62.5)	24.56	24	25.1	0.46	
Day 42	Sheko	DIM	Positive	1 (100)	20	-	-	-	-
			Negative	0 (0)	-	-	-	-	
		ISMM	Positive	0 (0)	-	-	-	-	-
			Negative	2 (100)	25.9	17	34.8	0.99	
	Boran	DIM	Positive	2 (66.7)	19.6	14.5	24.7	0.57	0.069
			Negative	1 (33.3)	26	-	-	-	
		ISMM	Positive	3 (50)	23	21.9	24.2	0.45	0.09
			Negative	3 (50)	25.1	21.3	28.8	1.5	

SD: Standard deviation

Table 8: Sheko and Boran cattle breeds treatment response and proportion of drug resistance

Cattle breed	Drug types	Number of animals	Relapsing cases	Relapse (%)	χ^2 (p-value)
Sheko	DIM	8	2	25	4.8 (0.033)
	ISMM	8	1	12.5	
Boran	DIM	8	6	75	
	ISMM	8	3	37.5	
Treatment 2 after relapse					
Sheko	DIM	1	1	100	2.13 (0.166)
	ISMM	2	0 (no relapse)	0	
Boran	DIM	3	2	66.7	
	ISMM	6	3	50	

Table 9: Treatment response following treatment with curative dose of DIM and ISMM

Drug treatments	Treatment response one		χ^2 (p-value)
	Negative	Positive	
DIM	8	8	2.1333 (0.144)
ISMM	12	4	
Treatment response two			
DIM	1	3	1.5 (0.225)
ISMM	5	3	

pairs is essential, although ineffective when multiple-drug resistant trypanosomes are present[10]. On day 42, 31.25% (5/16) Boran and 6.25% (1/16) Sheko animals remained positive after drug swap.

CONCLUSION

The present study indicated that the existence of drug resistant strains of trypanosomes in the therapeutic dose of diminazene aceturate (3.5 mg kg^{-1}) and isometamidium chloride (0.5 mg kg^{-1}). Even though the respondents involved in this study reported the relapse of infection after trypanocidal drugs treatment, most of them use those drugs to treat their animals. Therefore, strict supervision on the usage of trypanocidal drugs should be implemented. Sanative pair treatment should be considered to minimize relapse of infection. Single and multi-drug resistance should be considered during choosing control options. Further experimental work required in the field to monitor the development of drug resistance using advanced molecular techniques.

SIGNIFICANCE STATEMENT

Bovine trypanosomosis is an important disease of cattle. Currently, it is found to be one of the factors hampering livestock production and productivity in most settlement areas of Western Ethiopia. It is prevalent and there is complaint of treatment failure in Benishangul-Gumuz region. Trypanocidal drugs utilization practices are not documented and occurrence of trypanocidal drug resistance are not studied.

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