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Assessment of Diminazine Aceturate and Isometamidium Chloride Efficacy in Assosa and Bambasi Districts of Benishangul Gumuz Region, Western Ethiopia

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Abstract: Field trial was carried out from December 2018 to February 2019 with the objective to estimate the efficacy of diminazine aceturate and isometamidium chloride against most prevailing trypanosome spp in Assosa and Bambasi districts of Benishangul Gumuz region. An abbreviated 28-day field protocol based on treatment of naturally infected cattle was used to assess the efficacy of trypanocidal drugs. Accordingly, treatment failure was detected in 19/30(63.3%) of cattle treated with 3.5mg/kg body weight diminazene aceturate 14 days post treatment. *Trypanosoma congolense* accounted for 17/19(89.5%) of DIM treatment failure while *T. vivax* and *T. brucie* together accounted for only 2/19 (10.5%). Re-treatment of *T. congolense* positive cattle that had failed DIM treatment at 3.5mg/kg body weight with double dose (7mg/kg) had still resulted in a treatment failure of 6/17(35.3%), however, re-treatment of both *T. vivax* and *T. brucie* positive cattle with double dose of DIM cleared the parasites. Similarly treatment failure was observed in 8/30(26.7%) and 8/22(36.4%) of cattle treated with isometamidium chloride at dose of 0.5mg/kg body weight 14 and 28 days post treatment resulting in cumulative treatment failure of 16/30(53.3%). *Trypanosoma congolense* accounted foremost treatment failure for the two drugs. Assosa district (Megele 38) had higher treatment failure when compared to Bambasi district (Nebar keshimando) for the two trypanocidal drugs; however, no statistically significant variation was observed (P>0.05). Failure of trypanocidal drug efficacy was found to be a constraining factor to control the disease using curative and prophylactic drugs. Hence, vector control using different approach has to be given due attention to mitigate the problem.

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1. Introduction

Ethiopia is one of the richest countries in livestock population. Central statistical Authority report of (2016/17) showed that the country has about 59.49 million heads of cattle, 60.90 million shoats, 11.01 million equines, 1.23 million camels and 59.50 million poultry which are the highest in Africa.

Although the country is the first in livestock population in Africa, productivity of these animals is very low due to a number of factors such as qualitative and quantitative deficiencies of feed, poor performance of animals, lack of knowledge on the dynamics of farming system existing in the country and the presence of livestock diseases throughout the country of which animal trypanosomsis is among the most important (Abebe, 2005).

In Ethiopia, the most important trypanosome spp affecting livestock include *T. congolense*, *T. vivax* and *T. brucei* in cattle, sheep and goats; *T. evansi* in camels and *T. equiperdium* in horses (Abebe, 2005; Alemayehu *et al*., 2012). Western and southern river basins of Ethiopia are the most severely affected areas by trypanosomosis in the country. In the area specifically in the western part, a wide diversity of *Glossina* and trypanosome spp and strains co-exist (Abebe, 2005).

Benishangul Gumuz is one of the five regions of Ethiopia infested with more than one spp of *Glossina* and three of tsetse transmitted trypanosome spp namely *T. congolense*; *T. vivax* and *T. brucei* are found in the region (NTTICC, 2004; Worku *et al*., 2017).

Trypanosomosis is controlled either by controlling the vector or the parasites or by controlling both and for many years, a large number of vector control tools have been developed and implemented (Bauer

et al., 2012). However, control of animal trypanosomosis in poor rural communities has mainly been relied on the use of trypanocidal drugs. But only a small group of chemoprophylactic and chemotherapeutic trypanocidal compounds are currently in use and new compounds are unlikely to become fabricated (De Koning *et al*., 2012). The inevitable outcome of continued use of the same compounds for decades has resulted in drug resistance that has been largely responsible for frequently observed chemotherapeutic failures (Yohannes *et al*., 2012). Of the total doses of veterinary trypanocidal drugs that have been administered each year, Isometamidium chlorides (ISMM), Diminazene aceturate (DIM) and Ethidium bromide (EtBr) represent 40%, 33% and 26%, respectively by market value (Sones, 2015). Isometamidium chloride is mainly used as prophylactic drug and provides on average three months protection against trypanosomal infection whereas DIM has only therapeutic properties and EtBr has limited prophylactic properties and mainly used as a therapeutic agent (Van den bossche *et al*., 2011; Peregrine *et al*., 2015).

In many parts of Africa most of these trypanocidal drugs are gradually losing their efficacy due to resistance, but trypanosomes are usually not resistant to two of the drugs (DIM and ISMM) at the same time. Thus, these two compounds have been termed as sanative pair since in instances of resistance to one drug, application of the other will control the disease (Machila *et al*., 2015). However, experimental studies conducted by Kazibwe *et al*. (2009) in Uganda, Sow *et al*. (2012) in Burkina Faso and Dagnachew *et al*. (2015) in Ethiopia showed the occurrence of resistance in trypanosomes for both DIM and ISMM which suggested that the concept of sanative pairs might no longer always be valid.

In Benishangul Gumuz region, particularly in Assosa and Bambasi districts, there are reports on frequent treatment of animals with the existing trypanocidal drugs (DIM and ISMM) in which case, farmers cost more and more to treat their animals making them to complain on the efficacy of trypanocidal drugs used (BGRBoA, 2017). However, no study has been made so far on trypanocidal drug efficacy in Assosa and Bambasi districts of the region; as a result information pertaining to drug efficacy is scarce and not well documented in the study areas. Hence, the objective of this research paper was to estimate the efficacy of DIM and ISMM against most prevailing trypanosome spp in the study districts.

2. Materials and methods

2.1 Description of the Study Areas

Benishangul Gumuz region is one of the nine regional states established in 1994 by the new constitution of Ethiopia that created a federal system of governance. The region is located in the western end of the country bounded by the Sudan Republic in the west, the Amhara region in the north and northeast, Oromia region in the east and southeast and Gambella region in the south. According to the current administrative structure, the region is divided in to 3 administrative zones, 20 districts and 482 *rural kebeles* with a total area of approximately 50,380 km^2 (BGRBoC, 2017). The region is found in the northwest part of the country at latitude of 9-11°N and longitude of 34-35°E and altitude ranges generally between 580-2731 m.a.s.l., the highest peak being at Belaya mountain (2731 m.a.s.l) and the lowest (580 m.a.s.l) in the extreme west lowlands near the Ethio-Sudan boundary. It has livestock population of 777,915 cattle, 100,013 sheep, 431,216 goats, 82,080 equines and 1,249,578 poultry (CSA, 2016/17).

The present study was conducted in Assosa and Bambasi districts of Benishangul Gumuz region of Assosa zone, which is located at a distance of about 687 km away from Addis Ababa. Rainfall is uni-modal and occurs for 6 or 7 months between April and October. The mean annual rainfall amount is 1275 mm. The highest rainfall period is between May and September, the highest being in July or August (NMSA, 2015).

Assosa district is located at 9.60^0-10.45^0 N latitude and 34.20^0- 34.58^0E longitude with an altitude that ranges from 580 to 1544 m.a.s.l. The district has a rainfall that ranges from 850 to 1200 mm with uni-modal type that occurs between April and October. Its mean annual temperature ranges between 16.75^0c and 37.9^0c (NMSA, 2015). The total area of the districts is 2317 Km^2 and its livestock population is 27,850 cattle, 25,943 goats, 5,689 sheep, 5,420 donkeys and 53,185 poultry (ADOoA, 2017) whereas Bambasi district is located at 9.45 - 9.75^0N latitude and 34.35-34.88^0 E longitude, with a minimum and maximum altitude of 1350 and 1770 m.a.s.l., respectively. The total area of the district is 2100 km^2 and has an average minimum and maximum annual rainfall of 900 mm and 1200 mm respectively; while the average minimum and maximum temperature is 23^0c and 32^0c, respectively (NMSA, 2015). The total livestock population of the district is 38,964 cattle, 11,990 goats, 3,452 sheep, 1,995 donkeys and 38,442 poultry (BDOoA, 2017) and in both districts, the

livelihood of the society largely depends on mixed livestock and crop production.

2.2 Study animals

For this field trial, indigenous zebu cattle kept under traditional smallholder farming system were used.

2.3 Study design

Field trial was carried out from December 2018 to February 2019 to establish trypanocidal drug efficacy in the study districts.

2.4 Trypanocidal drug efficacy test

To conduct trypanocidal drug efficacy test, an abbreviated 28-day field protocol based on treatment of naturally infected cattle was used. To carry out this, two study sites, one from each district was selected by simple random method and 60 positive cattle (35 from Assosa and 25 from Bambasi) were identified and grouped into two and one group from each site were treated with ISMM (Veridium ®, Ceva Sante Animale, France) at a dose rate of 0.5mg/kg b.w of 2% solution while the other group were treated with DIM (YZ-Diminazene; YZPC, Hebei Y unzheng, Pharmaceutical Co., LTD) at a dose of 3.5 mg/kg b.w through a deep intramuscular injection after estimating the body weight of each animal using weight measuring tape for cattle and by identifying each animal by its respective name from the owner since farmers were not interested to use ear tag.

After treatment, Smash /deltametrin/ 1% pour on ready to use formulation was poured on the back of animals starting from the base of the ear to hip of the tail at a dose rate of 1ml/10 kg b.w (NTTICC, 2014) to minimize the risk of re-infestation and all farmers were told to bring their animals at day 14 post treatment. The initial treatment day was considered as day zero. Treated animals were monitored for trypanosomes and PCV on days 14 and 28 post treatment. Trypanosome positive cattle in DIM treated group at days 14 were re-treated with 7mg/kg body weight DIM.

2.5 Data management and analysis

Raw data collected were entered into a Microsoft Excel spreadsheet. The data were summarized and presented in tables and analyzed using STATA version 13.0 for Windows (Stata Corp. College Station, TX). Fisher's exact test was used to compare treatment failure between study sites and paired t-test was used to compare mean PCV differences observed at the beginning and end of DIM and ISMM intervention. Persisting trypanosomes in DIM or ISMM treated animals were indicative of resistance to the respective drug. Throughout the analysis, the test result was considered as significant when the calculated P-value was ≤ 0.05 at 95% confidence interval and 5% absolute precision (Thrusfield, 2005).

3. Results and discussion

3.1 DIM treatment response

In the present study, relapse of parasitaemia was detected in cattle treated with DIM in the two districts. From animals treated with this drug at 3.5 mg/kg b.w 14 days later, relapse was observed in 19/30 (63.3%). Megele 38 *rural kebele* of Assosa district had higher DIM treatment failure 14/19(73.7%) when compared to Nebar keshimando *rural kebele* of Bambasi district which had DIM treatment failure of 5/11(45.5%) with no significant difference (P>0.05). *Trypanosoma congolense* accounted for 17/19(89.5%) of DIM treatment failure while *T. vivax* and *T. brucie* together accounted for only 2/19 (10.5%) as shown in Table 1.

This finding was much higher than research results reported by John *et al*. (2003) who reported 14.7% DIM treatment failure at a dose rate of 3.5mg/kg in their study on field studies of drug-resistant cattle trypanosomes in Ke´ne´dougou province, Burkina Faso as well as 30.6% treatment failure reported by Mungube *et al*. (2012) in their study on detection of multiple drug-resistant *T. congolense* populations in village cattle of southeast Mali; similarly, the present finding was higher than research result registered by W/yohannes *et al*. (2010) in their study on assessment of drug resistance on *T. vivax* in Tselemti woreda of Tigray region whose finding was (50%) relapse at DIM 3.5mg/kg b.w.

Re-treatment of *T. congolense* positive cattle that had failed DIM treatment at 3.5mg/kg b.w with double dose (7mg/kg) had still resulted in a treatment failure of 6/17(35.3%), however, re-treatment of both *T. vivax* and *T. brucie* positive cattle with double dose of DIM cleared the parasites. Assosa district (Megele 38) still had higher DIM treatment failure 5/12(41.7%) when compared to Bambasi district (Nebar keshimando) which had treatment failure of 1/5(20%) as indicated in Table 1 and the association was not statistically significant (P > 0.05).

Although the current finding was slightly higher, it was comparable with the finding of (Mungube *et al*., 2012) where 26.3% *T. congolense* failed to respond to re-treatment of DIM at 7mg/kg b.w. It

was in line with the finding of (W/yohannes *et al*., 2010) who reported no (zero) relapse of *T. vivax* at a dose rate of 7mg/kg b.w. However, in contrast to the current finding, Codjia *et al*. (1993) reported that, 12 trypanosome isolates collected from cattle in Ghibe, Ethiopia were found to be resistant to 7mg/kg b.w DIM and Mulugeta *et al*. (1997) reported in Ghibe, Ethiopia that all 10 calves which were infected with *T. congolense* and treated with DIM at a dose rate of 7mg/kg failed to respond to treatment within 5-16 days. In other studies higher dose like 17.5 mg/kg b.w still failed to clear *T. congolense* resistant to 3.5mg/kg DIM (Clausen *et al*., 1992).

Table 1: Cattle with failed diminazene aceturate treatment over total treated in Assosa and Bambasi districts

RK	**Response at day 14 post treatment with 3.5 mg/kg b.w DIM (%)**				**Re-treatment response at day 14 PT with 7 mg/kg b.w DIM (%)**
	T. c	**T. v**	**T. b**	**Total**	**T. c**
M- 38	12/18(66.7)	1/5(20)	1/1(100)	14/19(73.7)	5/12(41.7)
N/K	5/10(50)	0/2(0)	0	5/11(45.5)	1/5(20)
Total	**17/28(60.7)**	**1/7(14.28)**	**1/1(100)**	**19/30(63.3)**	**6/17(35.3)**

Single infection (T.c =23, T. v= 1), mixed infection (T.c & T.v =5, Tb & T.v =1), DIM = Diminazene aceturate, T.c = *T. congolense*, T.v = *T. vivax*, T.b = *T. brucei,* M-38=Megele 38, N/K= Nebar Keshimando, RK= *Rural kebele,* PT= post treatment

3.2 **ISMM treatment response**

At day 14 post treatment, response of trypanosomes to 0.5 mg/kg b.w ISMM prophylactic preparation varied between the study districts (Table 2). Of the total 30 trypanosome-positive cattle treated with ISMM at this dose 14 days later, 8/30(26.7%) had persistent infection. Slightly higher ISMM treatment failure was registered in Megele 38 *rural kebele* 5/16(33.3%) when compared to Nebar Keshimando which had 3/14(23.1%) treatment failure even though the difference was not significant (P>0.05). *Trypanosoma congolense* accounted for all 8/8(100%) treatment failure with ISMM at day 14 post treatment in the two districts. *Trypanosoma vivax* positive cattle that received ISMM treatment were all cleared of trypanosome at 14 day post treatment. Among the 22 cattle found aparasitaemic 14 days later, 8/22(36.4%) had persistent infection at day 28 post treatment. Megele 38 still had higher treatment failure 5/12(41.7%) than Nebar Keshimando which had treatment failure of 3/10(30%) although the difference was not significant (P>0.05). *Trypanosoma congolense* accounted for 7/8(87.5%) of the failed treatment while *T. vivax* accounted for only 1/8 (12.5%) of the treatment failure. The cumulative treatment failure rate when summing days 14 and 28 was 16/30(53.3%).

Even though the present finding was slightly lower, it was comparable with prior findings of (McDermott *et al*., 2003; Mungube *et al*., 2012) who reported 37.8% and 31.7% treatment failure at dose 0.5mg/kg b.w ISMM 14 days post treatment, respectively. Although, the result of the present research work was slightly higher, it was comparable with (Mungube *et al*., 2012) who registered treatment failure of (25.6%) 28 days later and cumulative treatment failure of 49.2%. It was also consistent with (Mungube *et al*., 2012) where 90% of failed ISMM treatment was accounted for *T. congolense.*

In contrst, the present finding was much lower than prior reports of Codjia *et al*. (1993) who found that, 12 trypanosome isolates collected from cattle in Ghibe, Ethiopia were found to be resistant to 0.5 mg/kg body weight ISMM, except for one isolate and Mulugeta *et al*. (1997) in Ghibe, Ethiopia, who reported that all 10 calves infected with *T. congolense* were failed to respond to ISMM treatment at a dose rate of 0.5mg/kg within 4-26 days. However, the current finding was higher than earlier research result of (W/yohannes *et al*., 2010) who reported no (zero) relapse of *T. vivax* in animals treated with ISMM at a dose rate of 0.5mg/kg b.w.

In this study, one cattle with *T. vivax* failed to respond to ISMM treatment were detected at day 28 post treatment which might indicate that at high plasma concentration of ISMM, *T. vivax* are effectively suppressed, flourishing as ISMM plasma concentration wanes. Study carried out by (Leak *et al*., 1993) had indicated that *T. vivax* is highly

susceptible to treatment while the problem of drug resistance is higher in *T. congolense* which support the present research work

Table 2: Cattle with isometamidium chloride treatment failure over total treated at a dose of 0.5 mg/kg body weight

RK	Response at day 14 post treatment (%)			Response at day 28 post treatment (%)			
	T.c	T. v	Total	T.c	T. v	Total	Cumulative
M -38	5/15(33.3)	0/3(0)	5/16(33.3)	4/10(40)	1/3(33.3)	5/11(45.5)	10/16(62.5)
N/K	3/13(23.1)	0/1(0)	3/14(23.1)	3/10(30)	0/1(0)	3/11(27.3)	6/14(42.9)
Total	**8/28(28.6)**	**0/4(0)**	**8/30(26.7)**	**7/20(35)**	**1/4(20)**	**8/22(36.4)**	**16/30(53.3)**

Single infection (T.c =26, T.v =2), mixed infection (T.c & T.v =2), T.c = *T. congolense*, T.v = *T. vivax*, T.b = *T. brucei, M-38*=Megele 38, N/K= Nebar Keshimando, RK= *Rural kebele*

3.3 Response of PCV level to treatment

Treatment of trypanosome positive cattle with either DIM or ISMM increased the mean PCV level (%) from 23.38 ± 1.6 SD at day 0 (95% CI =22.97-23.79) to 24.25 ± 1.3 at day 14 (95% CI =23.90-24.60) post treatment with significant variation ($P < 0.05$) and subsequently to 25.18 ±1.5 at day 28 (95% CI=24.79-25.58) post treatment with statistically significant difference ($P < 0.05$) as shown in Table 3.

Table 3: Response of mean PCV level to DIM and ISMM treatment at days 0, 14 and 28

Treatment day	Observation	Mean	SD	95% CI	P-value
Day 0	60	23.38	1.6	22.97-23.79	0.0000
Day 14	60	24.25	1.3	23.90-24.60	0.0000
Day 28	60	25.18	1.5	24.79-25.58	

In conclusion, the application of an abbreviated 28-day field protocol based on treatment of naturally infected cattle provided valuable information on the presence of DIM resistant *T. congolense, T. vivax* and *T. brucei* and ISMM resistant *T. congolense* and *T. vivax* in the study districts. Therefore, further study on trypanocidal drug efficacy under controlled environmental condition using advanced trypanocidal drug efficacy test should be conducted in the region in general and the study districts in particular.

Conflict of interest statement

The authors declare that they have no competing interests and have no any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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