Haryana Vet. (December, 2023) **62(2),** 93-97 ISSN 0033-4359 (P) **Research Article**

HAEMATOLOGICAL AND CHEMOTHERAPEUTIC EVALUATION IN BUBALINE TRYPANOSOMOSIS

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ABSTRACT

Blood samples from twenty-two buffaloes, naturally infected with *Trypanosoma evansi* were equally divided into two groups and were investigated for haematological alterations before and after treatment. The Group I and II Buffaloes were treated with diminazine aceturate @ 7 mg/kg body wt. i/m and with quinapyramine sulphate and chloride @ 7.4 mg/kg body wt. s/c, respectively along with supportive treatment with meloxicam, dextrose saline, irons dextran, haematinic mixtures, B. complex and liver tonics at recommended doses.

The improvement in the haematological values was more predominant with comparatively higher margins in animals of group II than those of group I. However, the recovery was comparatively faster in Group II animals than group I animals as the parasitaemia was cleared earlier (on day 2) in group II. The combined treatment of quinapyramine sulphate and chloride along with supportive therapy in group II animals showed encouraging results than in group I animals in terms of clearance of parasitaemia, clinical recovery and improvement in haematological values.

Keywords: Buffaloes, Haematogical indices, *Trypanosoma evansi*

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Trypanosoma evansi popularly known as '*Surra*' in the Indian subcontinent, is somewhat unpredictable in large ruminants, particularly in buffaloes ranging from most common chronic to subclinical form and occasional acute form revealing nervous signs and even death. Due to subclinical infection, the incidence of trypanosomosis in cattle and buffaloes has been unnoticed in India and buffaloes may act as reservoirs (Jaiswal *et al.*, 2015; Migri *et al.,* 2016). Buffaloes act as a potent source of *T. evansi* infection to other animals of the same or different species of the region. Although veterinarians in field conditions are not habituated to an investigative approach. Now a days the trend is gradually changing. Haematological and biochemical analysis of complicated cases is being increasingly utilized compared to the past. There is an urgent need to characterize haematological profiles of the disease, particularly in those species of animals which present difficulty in specific diagnosis. The haematological findings thus can offer an important clue to the circumstantial evidence of the disease in the field conditions. Moreover, it can serve as an important biomarker of the disease, particularly in the absence of affordable, user-friendly and reliable diagnostic protocols. Keeping this fact in mind, the present study was undertaken to determine the efficacy of trypanocidal drugs in naturally infected buffaloes. The drugs were also compared for the rate of parasite clearance and the overall changes in haematological values after the treatment.

MATERIALS AND METHODS

Blood samples from 382 buffaloes with pyrexia were randomly collected and examined for trypanosomosis from different parts of Mumbai region of Maharashtra from May 2016 to April 2017. Blood samples from 22 buffaloes positive after confirmation for trypanosomosis by stained blood smear examination were equally divided into two groups i.e., group I and group II and were investigated for haematological alterations before and after treatment. About 3 ml of blood was collected from the jugular vein of each animal in EDTA (anticoagulant) vials for haematological analysis. The haematology of the whole blood was done with a fully automated haematology analyser (Abacus) as per the instructions of the manufacturer. Whole blood collected in EDTA vials for haematological evaluation, was used for the estimation of haemoglobin (Hb) total erythrocyte count (TEC), total leukocyte count (TLC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and differential leukocyte count (DLC).

Chemotherapeutic evaluation of bubaline trypanosomosis

A total of 22 buffaloes naturally infected with *T. evansi* with varying degrees of parasitaemia were divided equally into two treatment groups (group I and group II). The animals of group I and group II were treated with single dose of diminazene aceturate @ 7 mg/kg b. wt. i/m and quinapyramine sulphate and chloride @ 7.4 mg/kg body weight s/c, respectively. The study animals received common supportive therapy (meloxicam, dextrose saline, irons dextran, haematinic mixtures, B. complex and liver tonics) at recommended doses to stimulate the bone

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marrow and proper utilisation of iron (Shahzad *et al.*, 2012). Blood smears of the infected buffaloes were examined microscopically to check parasitaemia on day 0 (pre-treatment), day 1 to 7 and day 15 post-treatment. The efficacy of the trypanocidal drugs was determined based on clearance of parasitaemia and improvement in haematological values post post-treatment. Further, the data were subjected to statistical analysis using paired t-test (WASP 2.0, HTTP: //www.ccari.res.in/wasp2.0/index .php.) and Snedecor and Cochron (1994).

RESULTS AND DISCUSSION

Haematological findings

The effect of treatment on haematological parameters in both groups was studied and the improvement in the haematological values was more predominant with comparatively higher margins in animals of group II than those of group I as depicted in Tables 1 and 2. It was observed that haematological parameters like Hb, PCV, and TEC were lower in infected animals of both treatment groups than normal range. These haematological values were found to be improved in both treatment groups (Group I and Group II) treated with diminazene aceturate and quinapyramine sulphate and chloride respectively. However, Various workers *viz*., Aulakh *et al.* (2005), Puri (2009), Sulaiman and Adeyemi (2010), Kumar *et al.* (2012) and Rakesh Kumar *et al.* (2015) in general reported low levels of Hb, PCV, and TEC in infected animals and improvement was observed after treatment with either of the trypanocidal drugs. Omoja *et al.* (2012) reported increased PCV and RBC counts in rats infected with *T. brucei brucei* after treatment with diminazene aceturate @ 7 mg/kg body weight. However, when Joshi and Singh (2000), Birajdar (2007), Ramakrishna and Yoganand (2008) and Shrinivasulu (2011) specifically compared the efficacy of diminazene aceturate with quinapyramine sulphate and chloride and found comparatively more improvement in haematological values of the group treated with the latter. The observations of these authors support the findings of the present study. Lower means of Hb, PCV and TEC in infected buffaloes before the treatment during the present study might be either due to increased inhibition of erythropoiesis or increased rate of erythrocytes due to endotoxins released by trypanosomes. The decreased erythrocytic count indicates a hypochromic trend in *T. evansi* infected buffaloes, which may be due to immune-mediated destruction of red blood cells and it also indicates that there is a trypanosome-induced reduction in haemoglobin (Mishra *et al.*, 2017).

A significant ($P<0.01$) high level of MCV and low level of MCHC indicates that the anaemia is macrocytic hypochromic. These observations are in close agreement with the report of Hilali *et al.* (2006) who also observed macrocytic hypochromic anaemia in infected buffaloes with *T. evansi* while Birajdar (2007) reported all types of anaemia in naturally infected buffaloes of the Mumbai region with *T. evansi*. Furthermore, lower means of PCV, TEC and TLC levels in *T. evansi* infected buffaloes are in close agreement with the observations of Sulaiman and Adeyemi (2010), Sivajothi *et al.* (2014) and Hussain *et al.* (2016). The reduction in leucocytes in trypanosomosis has been reported to be due to ineffective or depressed granulopoiesis in the bone marrow (Anosa *et al.*, 1997). Moreover, the differential leucocyte count in the present study revealed leucocytopenia associated with lymphocytopenia, neutrophilia, eosinophilia and monocytosis in infected animals before the treatment. Similar findings were reported by other researchers *viz*., Chaudhary and Iqbal (2000); Rakesh Kumar *et al.* (2015), Hussain *et al.* (2016) and Mishra *et al.* (2017). The eosinophilia indices also showed the same pattern of increase and decrease in various groups before and after treatment. Eosinophilia and lymphocytopenia is produced due to stress in infected animals (Benjamin, 1985). Sivajothi *et al.* (2014) also reported that eosinophilia is the main feature of parasitic infection and is associated with immediate type of hypersensitivity reactions.

Parasitaemia and regression of clinical signs post-treatment

In group, I, the average level of parasitaemia was 12.94 tryps/high power field (HPF) before treatment which reduced to an average of 4.83 tryps/HPF and 1.33 tryps/HPF on day 1 and day 2, respectively. However, the parasitaemia was completely cleared in all animals on day 3 post-treatment. Six animals had average parasitaemia < 4.6 tryps/HPF and were completely cleared only in one animal on day 1 while the parasitaemia was cleared from seven animals on day 2 post-treatment and the parasitaemia in the rest of 3 animals was cleared on day 3 after the treatment (Table 3). The animals were active and alert and showed improvement in appetite after 3 days post-treatment and overall recovery was observed on 15th day after the treatment. In group II, the average parasitaemia was 12.54 tryps/HPF before treatment (day 0) with three animals having high parasitaemia of 28.6 to 30 tryps/HPF. This parasitaemia was reduced to an average of 3.94 tryps/HPF on day 1 post-treatment. The parasitaemia was completely cleared from the remaining 11 animals in the group on day 2 post-treatment. On day 1 post-treatment, only three animals had average parasitaemia of 10.4, 10.8 and 11.2/HPF respectively while it was completely cleared from the blood of 8 animals of group II. On day 2 post-

Table 1. Haematological profile of *T. evansi* infected buffaloes (Group I) before and after treatment with diminazene aceturate.

NS-Non-Significant (P>0.05), *Significant (P<0.05), **Highly Significant (P<0.01)

Sr. No.	Parameter	Before treatment Mean ± SE	After treatment Mean ± SE	Normal Range
1.	Hb (gm/dl)	8.99±0.35**	11.11±0.26	11.07–12.83
2.	TEC (10^6/mm^3)	5.09±0.20**	6.81±0.26	6.39 – 7.37
3.	TLC (10^3/mm^3)	6.83±0.32**	8.29±0.36	7.01 – 10.30
4.	PCV (%)	27.33±0.70**	30.45±0.25	29.42 – 32.17
5.	MCV (fl)	55.93±0.63*	48.05±1.51	43.00 – 49.52
6.	MCH (pg)	13.84±0.52**	18.09±0.36	15.90 – 19.53
7	MCHC (gm/dl)	30.61±0.39**	34.28±0.66	35.10 – 41.83
8.	N (%)	33.46±0.65**	29.73±0.42	22 – 32
9.	E (%)	5.91±0.49**	3.00±0.19	0 – 3
10.	L (%)	56.36±1.02**	64.18±0.60	64 – 71
11.	M (%)	3.91±0.21**	2.82±0.18	2 – 6
12	B (%)	$0.36±0.15^{NS}$	0.27±1.14	0 – 1

Table 2. Haematological profile of T. evansi infected buffaloes (Group II) before and after treatment with quinapyramine sulphate and chloride.

Sr. No	Parameters	Mean ± SE Before treatment	Mean ± SE After treatment	Normal Range
1.	Hb (gm/dl)	9.83±0.30**	11.56±0.11	11.07–12.83
2.	TEC (10^6/mm^3)	4.66± 0.30**	6.84 ±0.22	6.39–7.37
3.	TLC (10^3/mm^3)	6.53±0.29**	8.32±0.34	7.01–10.30
4.	PCV (%)	27.46± 0.69**	31.13±0.55	29.42–32.17
5.	MCV (fl)	$55.96±3.66^{NS}$	49.26±1.05	43–49.52
6.	MCH (pg)	15.93±0.33*	16.36±0.31	15.90–19.53
7	MCHC (gm/dl)	33.85±0.30*	35.21±0.59	35.10–41.83
8.	N (%)	33.46.45±0.45 **	29.91±0.39	22–32
9.	E (%)	5.46± 0.34 **	2.82±0.12	0–3
10.	L (%)	57.18±0.88**	64.64±0.38	64 –71
11.	M (%)	3.46±0.28*	2.27±0.19	2–6
12	B (%)	$0.46 ±0.15^{NS}$	0.36±0.15	0–1

NS–Non-Significant (P>0.05), *Significant (P<0.05), ** Highly Significant (P<0.01)

Table 3. Post-treatment parasitaemia and regression of clinical signs

Day	Group I Diminazene aceturate	% Efficacy	Group II Qinapyramine sulphate and chloride	% Efficacy
	Avg. Parasitaemia		Avg. Parasitaemia	
1	12.95		12.55	
2	4.83	62.70	2.95	76.49
3	1.33	89.72	0	100
4	0	100	0	
5	0		0	
7	0		0	
15	0		0	
			0	

treatment, parasitaemia was cleared completely from the remaining animals indicating 100 per cent elimination of trypanosomes in group II animals (Table 3). However, clearance of parasitaemia was only 89.72 per cent in group I which took a day more to clear the parasitaemia to 100 per cent as compared to group II animals as clearance of parasitaemia and regression in clinical symptoms were noticed on day 2nd and overall recovery was observed on day 15th post-treatment.

Effect of chemotherapy on haematological parameters

In animals of both groups, haematological values were improved on day 15 after the treatment. However, substantial improvement in the mean values of Group II animals treated with quinapyramine sulphate and chloride was comparatively at par with higher margins than those of Group I which received therapy of diminazene aceturate. However, animals of Group I recovered clinically with marginally lower values of Hb, TEC and MCHC in 5, 3, and 6 buffaloes on the 15th day post-treatment. The findings of the present study corroborate with the findings of Birajdar (2007) who reported the absence of trypanosomes in buffaloes treated with Berenil (7mg/kg b. wt. i/m) and Antrycide prosalt (7.4 mg/kg b. wt. s/c), showed considerable clinical recovery and improvement in the blood parameters, except slight degree of anaemia in few animals 10 days post-treatment. The utility of diminazene aceturate in *T. evansi* infection in bovines was also studied by Singh and Joshi (1991) who reported 100 per cent efficacy based on clinical recovery and clearance of parasites from the blood smears. In contrast, Soulsby (1982) and Bhatia *et al.* (2006) and Shrinivasullu (2011) reported inadequate efficacy of diminazene against *T. evansi* irrespective of species of hosts.

Bal *et al.* (2014) reported that treatment of infected animals with Triquin (quinapyramine sulphate and chloride) @ 5mg/kg b. wt. showed improvement in general body condition within a period of two weeks and results of PCR were also negative for *T. evansi* in all the blood samples. Further, they reported the highest efficacy in terms of restoration of haematological parameters after one month post-treatment. Similarly, Shrinivasullu (2011) and Suman Kumar *et. al.* (2009) also reported clinical response on the 3rd day and complete recovery by 10th day of post-treatment using quinapyramine sulphate and chloride @ 4.4 mg/kg. b.wt. s/c. along with the same supportive therapy. Various researchers *viz*., Rajguru *et al.* (2000), Sulaiman and Adeyemi (2010), Rajesh *et al.* (2010), Kumar *et al.* (2015) and Ponnudurai *et al.* (2015) reported 100 per cent efficacy in different species of animals suffering from trypanosomosis after treatment with either of the trypanocidal drugs. Bidyasankar *et. al.* (2005) compared the efficacy of Triquin and Berenil in *T. evansi* infected buffaloes and found that the overall recovery rate was earlier in Triquin-treated animals compared to Berenil-treated animals. On the contrary, Ramkrishna and Yoganand (2008) reported that there was no significant difference in the recovery rate among animals treated with Triquin and Berenil, though Triquin had the highest efficacy compared to Berenil which was similar to the observations of the present study. Our research findings corroborate with the findings of Pathak and Singh (2005), Birajdar (2007) and Shrinivasulu (2011) who specifically compared both diminazine aceturate with quinapyramine sulphate and chloride and found that the latter was comparatively 100 per cent efficacious than former for the treatment of bubaline trypanosomosis.

CONCLUSION

The present study revealed that haematological values were improved on day 15 after the treatment in animals of both groups. However, substantial improvement in the mean values of Group II animals treated with quinapyramine sulphate and chloride was comparatively at par with higher margins than those of Group I which received therapy of diminazene aceturate. Moreover, the recovery was comparatively faster in Group II animals than in group I as the parasitaemia was cleared earlier (on day 2) in group II animals than in group I. It was observed that the efficacy of quinapyramine sulphate and chloride and diminazene aceturate was found to be 100 and 89.72 per cent, respectively. The combined treatment of quinapyramine sulphate and chloride along with supportive therapy showed more encouraging results than diminazene aceturate in terms of clearance of parasitaemia, clinical recovery and improvement in haematological values.

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