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Chemotherapy Correction of Haematological Changes Induced by *T. evansi* in Nubian Goats

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Abstract: The combined action of Cymelarsan[R] and/or Oxytetracycline (OTC) in goats experimentally infected with *T. evansi* was investigated. Cymelarsan[R] and Cymelarsan[R] OTC combination cleared the parasite from peripheral blood, while OTC alone delayed the death as compared to the untreated group. Haematological indices declined post infection, but returned to normal post treatment except in OTC treated group. The Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and white blood cell and neutraphils increased but, basophiles and eosenophile disappeared post-infection. Monocytes appeared on day 7 and lyomphocytes decreased post-infection. Treatment with Cymelarsan[R] and/or OTC restored the haematological indices to normal in 2 weeks.

Key words: Chemotherapy, haematological changes, Nubian goats, MCHC, MCH, OTC, MCV

INTRODUCTION

Trypanosma evansi caused many changes observed haematologically. El-Malik (1983) and Damayanti *et al.* (1994) studies observed decrease in Haemoglobine (Hb) values. Leukocytes counts dropped post-infection (Naylor, 1971) although in some stuties they were similar in post-infection reading (Elamin, 1980; Elmalik, 1983; Damayanti *et al.*, 1994). Monocytes levels showed little change post-infection (Naylor, 1971; DeVilla *et al.*, 1991) Erythcocyte count were observed to be reduced post-infection (Naylor, 1971; Elmalik, 1983; DeVilla *et al.*, 1991). Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobine (MCH) increased post-infection in Elamin, (1980) study who observed increase in post-infection values in goats infected with *T. evansi*. Mean Corpuscular Haemoglobin Concentration (MCHC) in infected *T. evansi* buffaloes remained normal while, Damayanti (1994) and Elamin (1980) noticed an increase in infected *T. evansi* goats. Packed Cell Volume (PCV) was reduced post-infection (Emalik, 1983; Elamin, 1980).

The commercially available drugs for animal trypanosomosis are limited. Treatment dependents on 2 drugs suramin and quinapyramine sulphate (Bujon, 1990). However, suramin is less effective (Gad-el-Mula and Fayed, 1979) and quina-pyramine sulphate is no larger available from the original manufacture, only samples of dubious origins are now being available Isometamedium chloride (Samorin) only removes parasites from blood steam for 21 h followed by relapse. Moreover it caused some serious adverse effects (Ali and Hassan, 1986) Diminazine aceturate (Berenil) was found tobe toxic at 10 or 20 mg kg^{-1} in camels (Leach and Roberts, 1981).

Trypanosomosis, sometimes associated with other infections such as internal parasites and bacterial infection. Of the commonly used antibiotics, tetracycline group is a broad spectrum, intoxic bacteriostatic (Giovani and Warren, 1983) and has high concentration in the kidney, spleen, liver and lung (Bywater *et al.*, 1991).

In recommend dose Cymelarsan[R] had been showed to be well tolerated and the combination Cymelarsan[R] OTC gave the best result (Fairouz, 2000). Cymelarsan[R] (Rhône-mèrieux-France) was successfully used in the treatment of camel trypanosomosis in Africa.

The main objective of this study is to investigate the effect of administration of Cymelarsan[R] and Oxytetracycline (OTC) simultaneously to animals experimentally infected with *T. evansi*. The study is planned to study the therapeutic effect of the 2 drugs singly or combined by recording the haematological changes compared to control groups.

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MATERIALS AND METHODS

Experimental animals: Animals used in the study were 25 healthy Nubian goats of both sexes, 8-12 months old.

Adaptation period: All animals were stabled in insect prove pens at the Department of Preventive Medicine and Public Health at the Faculty of Veterinary Medicine. They were fed on lucerne and millets and water was given *ad libitum* for 2 weeks.

The parasite: *T. evansi* was isolated from an infected camel at Elmewelh market. It was brought originally from Elgadarif- Eastern Sudan, which is confirmed as non-tsetse zone. The *T. evansi* isolate so obtained was designated as Gad tryp (1).

Drugs: Two drugs were used in this study:

- Cymelarsan^{R}(Rhône-mèrieux-France).
- Oxytetracycline (EMBAcycline*5) (Rhône-mèrieux-France).

Experimental design: Animals were divided into 5 groups, 5 animals in each as follows:

Group (C) Uninfected-untreated.
Group (R) Infected-treated with Cymelarsan^{R}.
Group (O) Infected-treated with OTC.
Group (Z) Infected-treated with Cymelarsan^{R} and OTC combination.
Group (A) Infected-untreated group.
Group (C2) Uninfected-treated with the combination of the two used drugs.

Experimental inoculation: Each goat was inoculated intravenously with 0.75 mL blood of rat infected it contained (5×10^{5} organisms).

Blood values determination: Blood samples for haemogram were withdrawn from the jugular veins of all goats before and after infection and after treatment using a vacutainer system (Becton-Dickinson France) with an anticoagulant (Ethylene Diamine Tetra Acetic Acid (EDTA). The parameter under investigation includes: Hb, PCV, blood cells counts, differential WBC counts and indices were calculated.

Parasitological methods: The examination of wet blood film, thin film, thick film and Buffy Coats Technique (BCT) was done to determine the presence of trypanosomes in goats.

Statistical analysis: All data was computerized using MSTAT-C program (Michgan State University), for the analysis of variance and for means separation.

RESULTS

Parasitological findings

Pre-patent period: Incubation period ranged between 4-9 days, seven out of twenty animals showed parasitaemina in 4 days and the rest become patent within 9 days. This was summarized in Table 1.

Course of infection: Death was frequently preceded by appearance of trypanosomes in the peripheral blood. In group (A) death began by the 2nd week, all animal died by day 20. The treated groups (group (R) and (Z) were found negative within 26 h of drug inoculation. All animals of group (O) started to die on day 47 and all animals died by day 54. This was summarized in Table 1.

Haematological changes: Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC) showed significant decreased post-infection and they were within the normal level post-treatment. This was summarized in Table 2.

Mean Corpuscular Haemoglobin Concentration (MCHC) values increased post-infection, post-treatment with OTC and it was normal in the rest treated groups.

Mean Corpuscular Haemoglobin (MCH) values increased slightly post-infection and increased post-treatment with OTC alone or with combination and it was normal in Cymelarsan treated group.

Mean Corpuscular Volume (MCV) values decreased post-infection and it began to normal at the end except when animal treated with the combination. This was summarized in Table 3.

The leukocytes counts (WBC) increased slightly post-infection, it declined to normal levels post-treatment.

The lymphocyte counts decreased post-infection and increased post-treatment. The neutrophile counts increased post-infection and post-treatment.

No basophiles and eosenophiles cells appeared post infection, but they appeared post treatment. Monocytes counts appeared on day 7 in goats of group (C), (R), (Z) and after injection the combination in goats of group (C2) it ranged between 1-3%. This was summarized in Table 4.

Table 1: The Parasitaemia in the different groups

			Days											
Groups	Animal No	Base -line	4	7	10	13	16	19	26	33	40	47	54	61
C	1													
	2													
	3						-ve							
	4													
	5													
R	6		+	+	++	++++								
	7				+	++								
	8				+	+++					-ve			
	9				+	++								
	10				++	+++								
O	11		+	++	++	+++	+++	+++	+++	+++	++++	Died	Died	Died
	12			++	+	+	+	++	++	++	++	+++	Did	
	13	-ve		+	++	++	++	+	+	+	++	++	++	
	14			+	++	++	+	++	++	++	+++	+++	++++	
	15			+	++	+	++	++	++	++	++	++	+++	
Z	16		+	++	+++	++++	++++							
	17			+	+	+	+							
	18			++	++	++	++				-ve			
	19			+	+	++	+++							
	20			++	++	++	++							
A	21		++	+++										
	22		++	+										
	23		++	++					Died					
	24		++	+										
	25		+	++										
C2	26													
	27													
	28						-ve							
	29													
	30													

Parasitaemia grade: 1-3 = +, 3-5 = ++, 5-10 = +++ and above 10 = ++++

Table 2: The Mean±SE of Packed Cell Volume (PCV), Haemoglobin (Hb) and Red Blood Cell (RBC) in Nubian goats infected with *T. evansi*

Parameter/Groups	PCV	Hb	RBC
Group(C1)	28.06±0.061[a]	7.614±0.015[a]	13.79±0.076[a]
Group(R)	25.44±0.012[a]	6.924±0.012[a]	12.434±0.012[a]
Group(O)	15.543±0.013[b]	4.843±0.014[b]	7.242±0.015[b]
Group(Z)	26.44±0.015[a]	7.465±0.010[a]	10.08±0.014[a]
Group(A)	15.23±0.091[b]	4.677±0.012[b]	8.34±0.06[b]
Group(C2)	28.200±0.011[a]	7.645±0.011[a]	13.809±0.020[a]

The different letter in one column showed the significant changes p = 0.05

Table 3: The Mean±SE of the Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Handmean Corpuscular Haemoglobin Concentration (HCHC) in Nubian goats infected with *T. evansi*

Parameter/Groups	MCV	MCH	MCHC
Group(C1)	20.34±0.100[a]	55.21±0.111[a]	27.13±0.152[a]
Group(R)	20.4±0.120[a]	55.68±0.153[a]	27.21±0.147[a]
Group(O)	21.4±0.201[a]	66.87±0.141[ab]	31.15±0.121[a]
Group(Z)	26.23±0.130[a]	74.05±0.121[b]	28.23±0.1232[a]
Group(A)	18.26±0.141[a]	56.07±0.132[a]	30.70±0.147[a]
Group(C2)	20.42±0.151[a]	55.36±0.112[a]	27.109±0.123[a]

The different letter in one column showed the significant changes p = 0.05

Table 4: The Mean ±SE of White Blood Cell (WBC), neutrophile, lymphocyte, eosenophile, and monocyte count in Nubian goats infected with *T. evansi*

Parameter/ Groups	WBC	Neutrophile	Lymphocyte	Basophile	Eosenophile	Monocyte
Group(C1)	11.47±0.121[a]	20.397±0.214[a]	50.53±0.153[a]	0.000±[a]	0.000±[a]	1.000±[a]
Group(R)	12.075±0.215[a]	17.98±0.125[a]	54.00±0.169[a]	1.000±[b]	2.000±[b]	1.000±[b]
Group(O)	12.377±0.148[a]	18.06±0.121[a]	66.77±0.168[a]	1.500±[b]	1.000±[b]	0.000±[a]
Group(Z)	13.673±0.123[a]	16.82±0.123[a]	75.71±0.136[b]	2.000±[b]	2.000±[b]	2.000±[b]
Group(A)	12.89±0.125[a]	15.52±0.014[b]	30.42±0.124[c]	0.000±[a]	0.000±[a]	3.000±[b]
Group(C2)	11.68±0.125[a]	33.02±0.012[b]	70.82±0.121[b]	1.000±[b]	2.000±[b]	3.000±[b]

The different letter in one column showed the significant changes p = 0.05

DISCUSSION

Haemoglobin (Hb) values were reduced significantly post-infection this agrees with several workers (Naylor, 1971; Elamin, 1980 and Damaganti *et al*., 1994) treatment with Cymelarsan[R] however reversed the decline of Hb significantly. The increase being more pronounced on the combination of Oxytetracycline (OTC) with Cymelarsan[R] treatment. However, OTC alone slightly reduced Hb in the infect goats. These suggest that the high percentage decrease of Hb levels may be ascribed to an enhanced destruction of the Red Blood Cells (RBC) due to the *T. evansi* or to precipitation of defective infection. A further documented possibility is an adverse effect of *T. evansi* infection on the animals (Kimeto, 1980).

PCV values were reduced post-infection and this is suggested by reports of Naylor (1971) Elmalik (1983) and Katunguka *et al.* (1997). PCV levels increased post-treatment in Cymelarsan[R] and the combination treated group (R) and (Z), it remained as it was in OTC treated group. The reduction in PCV is due to infection with *T. evansi,* which is known to produce anaemia and reduction in PCV values (Anosa, 1988) through destruction of erythrocytes. It is known that stimulation of Central Nervous System (CNS) particularly hypothalamic area, leads to increased erthythropoiesis and hence increased PCV levels (Schalm *et al*., 1975). It is likely that Cymelarsan[R] might have stimulated under this circumstance relevant parts of the CNS thereby raising the levels of PCV. Red Blood Cell (RBC) levels decreased post-infection, this is supported by Moulton and Sollod (1976) and Ostile *et al.* (1991). RBC increased post-treatment except in group (O) and control group after injection with the combination. RBC destruction during trypanosome infection due to splenomegaly and over-activity of the Mononuclear Phagocyte System (MPS) was thought to be the main factor responsible for erythrolysis during chronic crisis (steady-state) when the parasitaemia disappears but anaemia persists (Anosa, 1988). Basophils and eosinophils disappeared post-infection (Naylor, 1971; De Villa *et al*., 1991). Monocytes were slightly changed from pre-infection counts. They appeared on day 7 post treatment (Naylor, 1971) and De Villa *et al.* (1991) and after injection with the combination it ranged between 1-3%. Lymphocytes counts were reduced post-infection (Naylor, 1971) it increased post-treatment with Cymelarsan[R] treated group (R) returned to normal levels while, group (C2) showed increase after injection with the combination. Neutrophils level increased post-infection (DeVilla *et al*., 1991) it decreased post-treatment, group (C2) showed increase in neutrophils level after injection with the combination and groups (Rand Z) became normal post-treatment with cymelarson[R]. One of the probable major factors of eosinopenia, particularly during early infection, is the narrow granulocyte hypoplasia, the second one is the splenic sequestration (Anosa, 1988). Since hypersplensim, which is thought to be associated with the splenomegaly present in trypanosomosis usually results in neutropenia. Monocytosis in most infection indicates that the depression of myeloid colony formation reported had no appreciable effects on the monocytes precursors suggesting that the inhibitor acted at point acted the developmental divergence of granulocyte and monocyte and monocyte cell lines. Monocytosis was matched by a proliferation of macrophage in several tissues in trypanosome-infected animals. These macrophages are activated and epithelied cells and giant cells are also formed, these change stimulated by increased demand to remove particulate matter including trypanosomes, RBC, leukocytes and dead tissue cells, this was clear after injection of the combination in the control group (group C). Anosa (1988) showed that a lymphopenia which invariably developed in an affected animals was associated with a marked lymphoid hypoplasia and disappearance of germinal centers in the spleen, lymph nodes and haemolymph nodes. The etiology of early lymphocytopenia is not clear but may involve redistribution of lymphocytes to other sites, such as lymphoid organs (Anosa and Isoun, 1976). Leukocytes levels increased slightly post-infection (Elamin, 1980). This is an indication of tolerance, which previously, was clearly shown in similar breeds of goats indigenous to this country (Elmalik and Mahmoud, 1978).

MCV levels decreased post infection, it increased post-treatment with combination. Anosa (1988) reported that the increase of MCV occurred during the early acute phase of infection and became normal in the animal that developed chronic disease. In most animals polychromatophilic RBC i.e., reticulocytes, were essentially normocytic. There was a highly significant correlation between MCV values and reticulocytes counts. In the present study measurement of reticulocytes response was not done, reticulocytes were seldom seen in sheep and goats with severe anaemia (Anosa, 1988).

Nevertheless, our finding are consistent with those reported by Valli and Mills (1980) who demonstrated a macrocytic normochronic anaemia in cattle with *T. congolense.* OTC stopped the MCV level decline similarly Cymelarsan[R] treatment resulted in a normal level of MCV.

The MCHC levels increase post-infection (Elamin, 1980) and post-treatment with combination this is a reflection of changes in MCV and RBC counts discussed above.

During regenerative anaemia, increased erythrogensis characteristically leads to the release in to

the circulation of reticulocytes (decreased MCV level), macrocytic immature red cell (increased MCV level) (Schalm *et al*., 1975).

MCH levels revealed a slight increase post treatment with OTC or the combination, it remained, as it was post infection, this is disagreed with Elamin (1980) Naylor (1971) and in agreed with (Damayanti *et al*., 1994).

The upward shift in MCH of all infected animals indicates an increase in Hb concentration with the increase of RBC size. However, recorded MCH of Cymelarsan^{R} treated group (group R) was found to be higher than the mean value for combination treated group (group Z). This may imply that group (Z) animals had a relatively more impaired erythropoiesis and/or more rapid haemolysis of young RBC (Anosa, 1988) compared to group (R). Therefore, it is tempting to assume that group (R) probably had suffered a more rapid and severe RBC breakdown despite possession of a superior capacity (compared to the combination treated, group (Z) of injecting RBC, into circulation. However, the combination protected the trypanosomosis infected goats from the haemolysis whereas it didn't affect the MCH level in the control group.

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