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Efficacy of Buparvaquone Treatment in Pregnant Cows Infect with *Theileria* Species in Sudan

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Authors' contributions

This work was carried out in collaboration among all authors. Author EI carried out the experiments. Author SBM wrote the manuscript and supervised the project. Authors AEG and DAS conceived and planned the experiments. Author SMH contributed to the final version of the manuscript. Author AMK analyzed the data. All authors read and approved the final manuscript.

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ABSTRACT

A total of 100 pregnant cows from various private livestock farms of Al Nuba town, Al Gezira State, Sudan was selected to assess the therapeutic effect of buparvaquone in bovine theileriosis. Theileriosis was diagnosed before treatment by blood smears and PCR. *Theileria* spp. infected animals (n=96) were divided into three groups based on their gestation period; A (6 months, n=25), B (7 months, n=41) and C (8 months, n=30). Each group was subdivided into two subgroups; treated animals [T (n=68) including; TA (n=16), TB (n=28) and TC (n=24)] and non-treated animals [NT (n=28) including; NTA (n=9), NTB (n=13) and NTC (n=6)]. The subgroups TA, TB and TC were treated with buparvaquone at the dose rate of 2.5 mg/kg body weight intramuscularly. All cows were re-examined clinically and laboratory using blood smears and PCR four and six weeks after treatment. The overall recovered cows showed significant (P≤ 0.05) differences between treated and non-treated animals; the recovery percentages in treated animals (T) were 38.2% and 66.2%

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after 4 and 6 weeks of treatment, respectively, while in the non-treated groups (NT) the percentages were 0% and 39.3% after the same time. A variation in recovery percentage was observed among animals based on the gestation period; the recovery percentages were 18.7%, 32.1% and 58.3% after 4 weeks of treatment and 81.3%, 57.1% and 66.7% after 6 weeks of treatment for group TA, TB, and TC, respectively. The recovery percentage was 0% for NTA, NTB and NTC after 4 weeks and 33.3%, 38.5% and 50% after 6 weeks for the same groups. In conclusion, buparvaquone is quite effective against bovine theileriosis. However, it could be less effective between 6 and 8 months of pregnancy. The highest recovery percentage is found in cows in 8th and 6th months of gestation after 4 and 6 weeks of treatment, respectively.

Keywords: Theileriosis; buparvaquone; pregnant cows; Sudan.

1. INTRODUCTION

Tick-borne diseases (TBDs) are present throughout the world especially in the tropical and subtropical regions and affect approximately 80% of the world's cattle population. They are considered a significant threat to the global food security [1]. Tropical theileriosis is a tick-borne disease caused by *Theileria annulata*, which is an apicomplexan protozoa parasite that exists in a wide zone of Southern Europe, Africa, and a major part of Asia [2]. Globally, around 250 million cattle are at risk of tropical theileriosis [3]. The disease is mainly transmitted by *Hyalomma anatolicum* in Sudan [4]. It is a destructive disease that affects cattle of all ages, breeds and sex and leads to severe losses in production and reproduction [5]. The typical clinical symptoms are fever, lacrimation, nasal discharge, diarrhoea, abortion, anemia and decreased milk and meat production. Death usually occurs within 10–25 days after the onset of clinical signs [6]. The disease can be diagnosed by using blood smears or Polymerase Chain Reaction (PCR) or serological tests [7,8].

In Khartoum State, Sudan, it has been reported that 85% of cattle experienced clinical theileriosis and death rate of 30% and 22% in heifers and young calves, respectively [9]. In addition to that, tropical theileriosis is highly endemic in Al Gezira State, Central Sudan due to the high prevalence of *H. anatolicum*, which is the main vector of this disease [10].

It is well known that livestock farming constitutes a very important component of the agricultural sector for the provision of animal-derived food products as a source of income especially for poor people and farmers [11]. Therefore, supplementation of the diseased animals with treatment is recommended to help those animals to resume their normal productivity early [12]. A number of antibacterial and antiprotozoal drugs, such as parvaquone, buparvaquone and halofuginone, have been applied in treatment of theileriosis [13,14]. Buparvaquone (Butalex®) is a second-generation hydroxynaphthoquinone and given as a single dose of 2.5 mg/kg body weight intramuscularly. The effect of the drug is directed against the parasites and not against the host cells [15]. Although, Butalex® is widely used in treatment of tropical theileriosis, it becomes effective when used at the early stages of the disease [16]. Moreover, resistance against drugs was observed in apicomplexan infections in general, which in turn leads to a decrease in the efficiency of these drugs [13,17]. Limited studies were conducted to evaluate the Butalex® efficacy against theileriosis in the Sudan. Thus, this study aimed to estimate the therapeutic efficacy of Butalex® in the treatment of pregnant cows infected naturally with theileriasis in Al Nuba town, Al Gezira State, Sudan.

2. MATERIALS AND METHODS

2.1 The Study Area

The present study was conducted during the period from March 2015 to December 2016 in AL Nuba town, Central Sudan, which is located between latitudes 15°14'-15°18'N and longitude 32°51' -33°55'E in the north part of Al Gezira State approximately 54 kilometres south Khartoum (Fig. 1) along Blue Nile River. About, 100 dairy cattle farms are located in the town, which supply Khartoum State with milk.

2.2 Collection of Samples

A total of 100 cows at different stages of gestation period (Table 1) from three different dairy farms was selected for this study. Blood samples were collected directly from the Jugular veins into EDTA tubes. Each specimen was labelled indicating farm number, animal number, stage of gestation and date of collection.

Fig. 1. The study area, Al Nuba town, Al Gezira State, Sudan. (https://ar.wikipedia. org/wiki/%D9%85%D9%84%D9%81:Sudan_location_map.svg)

Table 1. Number of cows and their distribution based on the gestational period

Gestation period (Months)	6	7	8	Total
No of cows	27	41	32	100

2.3 Examination of Blood Smears

Firstly, the blood smears were stained with 10% Giemsa's stain and then examined for the presence of *Theileria* species piroplasms under 100× oil immersion objective using a light microscope. At least 25 microscopic fields per slide were observed.

2.4 Molecular Detection

2.4.1 DNA extraction

DNA was extracted from the whole blood samples using phenol-chloroform extraction method following the protocol described by Sambrook [18], stored at -20oC until used for PCR test. For quality assessment, 5 µl of extracted DNA were analyzed on 1% agarose gel.

2.4.2 Polymerase chain reaction (PCR)

Two primer pairs (N516: 5′-GTAACCTTTAAAAACGT and N517: 5′-GTTACGAACATGGGTTT) were used to amplify a 721 bp fragment from the gene encoding 30-KDa major *T. annulata* merozoite surface antigens (Tams 1) according to d'Oliveira method [19].

PCR was performed using 25 µl volume as follows: 12 µl of H2O, 2 µl of each primer, 4 µl of genomic DNA and 5 µl of Maxime PCR PreMix Kit (i-Taq) (iNtRON Biotechnology, Korea) containing 1x reaction buffer (10x), 2.5 U of i-TaqTM DNA Polymerase (5U/ µl), 2.5 mM of each dNTPs and 1x Gel loading buffer. The amplification was performed with an initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 3 min, 55°C for 1 min, 72°C for 2 min and final extension step at 72°C for 10 min. PCR products were separated on 1.5% agarose gel.

2.5 Drug and Treatment

Cows that confirmed diseased with tropical theileriosis either by microscopic examination or by PCR were classified into two groups; 68 of infected animals were treated with Buparvaquone (T), while 28 of infected animals were not treated (NT) as control group.

Buparvaquone (Butalex®) (Eagle Vet. Tech Co., Ltd., Korea) is a clear dark-red solution for

injection deep intramuscularly at a dose rate of 2.5 mg/kg body weight. Each millilitre contains 50 mg buparvaquone. The withdrawal period of Bupaquone for meat and milk consumption is 42 days and 48 days, respectively. The animals' weights were determined before injection of the drug as a therapeutic dose then the animals were divided into three groups according to their gestation periods; (A) 6 months, (B) 7 months and (C) 8 months. The cows those were positive for both blood smears and PCR (n= 96) were divided into two groups, treated (T, n=68) and non-treated (NT, n=28).

A single dose of 2 .5 mg per kg b.w. (1 ml Bupaquone per 20 kg/b.w.) was used. The follow up was carried out after the first treatment and then 48 hours intervals as necessary. Each animal was re-examined at 4 and 6 weeks after the treatment using blood films and PCR.

2.6 Statistical Analysis

The IBM SPSS 21 Package was utilized in the analysis. Descriptive statistics were determined for all quantitative variables. Data were analysed using Chi-square test to show the differences between the treated group and non-treated group. Differences were considered significant at $p \leq 0.05$.

3. RESULTS

3.1 The Prevalence of *Theileria* spp. Piroplasms before Treatment

Generally, the disease under investigation was detected in most animals with variations observed among different gestation periods. The overall prevalence was 91% using blood smear (BF), 75% using PCR and 96% were found positive for both tests (Fig. 2) (Table 2). Therefore, the cows that confirmed positive for *Theileria* infection, based on the results of the blood smear or PCR (n=96), were subjected to the treatment trial (Table 2).

3.2 Prevalence of *Theileria* spp. Piroplasms after Treatment

The recovery percentages in the treated groups (T), after 4 weeks of treatment for groups TA, TB, and TC were 18.7%, 32.1% and 58.3%, respectively. These percentages increased after 6 weeks of treatment to 81.3%, 57.1% and 66.7% for the respective groups (Table 3). Similarly, the recovery percentages increased in the non-treated group (NT) from 0% for NTA, NTB, and NTC after 4 weeks to 33.3%, 38.5% and 50% for the respective groups after 6 weeks (Table 3).

The results of blood smear and PCR showed that the percentages of infected cases in the treated groups after 4 weeks of treatment were 81.3%, 67.9% and 41.7% for groups TA, TB and TC, respectively. These percentages were decreased after 6 weeks of treatment to 18.7%, 42.9 % and 28.6% for the same groups. Similarly, the infected cases decreased in the non-treated group (NT) from 100% for NTA, NTB, and NTC after 4 weeks to 66.7%, 61.5% and 50% for the same groups after 6 weeks (Table 3).

The Chi-square analysis showed a significant variation between the treated group and non-treated group ($p = 0.000$), where 38.2% of animals recovered after 4 weeks of treatment, while none of the non-treated animals recovered. A significant variation was also observed after 6 weeks of treatment ($p = 0.014$), where 66.2% of animals recovered, while in non-treated animals the recovery percentage was 39.3% (Table 4).

Table 2. Prevalence of *Theileria* spp. piroplasms in the blood smears and *T. annulata* using PCR in cows before treatment based on the gestation period

Gestation period (months)	Total No. of examined animal	No. of positive (%)*		Total No. of positive animals confirmed either by BF and/ or PCR
		BF	PCR (*T. annulata*)	
6	27	25 (92.6%)	19 (70.4%)	25 (92.6%)
7	41	39 (95.1%)	32 (78%)	41 (100%)
8	32	27 (84.4%)	24 (75%)	30 (93.8%)
Total	100	91 (91%)	75 (75%)	96 (96%)

**Percentages are out of the number of examined cows in each gestation period. BF: blood smear (A) 6 months, (B) 7 months and (C) 8 months*

Table 3. Prevalence of infected *Theileria* spp and recovered pregnant cows in treated and non-treated groups

Gestation period (Months)	Condition	No. of cases			
		Infected cases**		Recovered cases ***	
		4 weeks after treatment	6 weeks after treatment	4 weeks after treatment	6 weeks after treatment
A (n=25) *	TA(n=16)*	13(81.3%)	3(18.7%)	3(18.7%)	13(81.3%)
	NTA(n=9)*	9(100%)	6(66.7%)	0(0%)	3(33.3%)
B (n=41)*	TB(n=28)*	19(67.9%)	12(42.9%)	9(32.1%)	16(57.1%)
	NTB(n=13)*	13(100%)	8(61.5%)	0(0%)	5(38.5%)
C (n=30) *	TC(n=24)*	10(41.7%)	8(28.6%)	14(58.3%)	16(66.7%)
	NTC(n=6)*	6(100%)	3(50%)	0(0%)	3(50%)

**Total no. of the positive cows before treatment conformed by either blood smears (BF) and/or PCR*
*** no of the positive animal after treatment conformed by either blood smears (BF) and/or PCR*
**** no of the negative cows after treatment confirmed by both blood smears (BF) and PCR.*
(TA), (TB) and (TC) Treated cows form group A, B and C, respectively.
(NTA), (NTB) and (NTC) non-treated cows form group A, B and C, respectively

Table 4. Chi-square analysis for the effects of treatment with buparvaquone compared between treated and non-treated groups, 4 and 6 weeks after treatment

Gestation period (Months)	Condition	No. of cases							
		4 weeks after treatment		X2	P-value	6 weeks after treatment		X2	P-value
		Infected cases**	Recovered cases***			Infected cases**	Recovered cases***		
Total (n=96)*	T(n=68)*	42(61.8%)	26(38.2%)	14.682	0.00	23(33.8%)	45(66.2%)	5.90	0.014
	NT(n=28)*	28(100%)	0(0%)			17(60.7%)	11(39.3%)		

**Total no. of the positive cows before treatment conformed by either blood smears (BF) and/or PCR*
*** no of the positive animal after treatment conformed by either blood smears (BF) and/or PCR*
**** no of the negative cows after treatment confirmed by both blood smears (BF) and PCR.*
(T) treated animals, (NT) not-treated animals

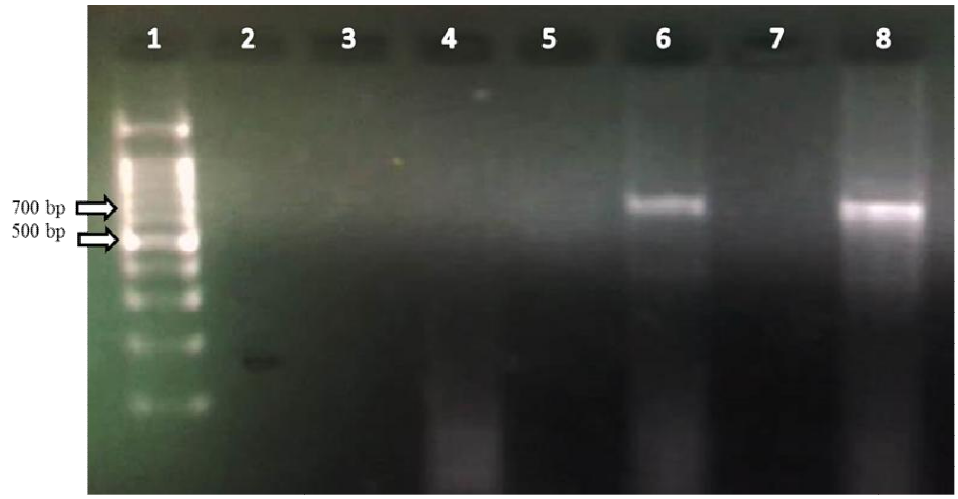

Fig. 2. PCR amplification of DNA of *T. annulata*. Lanes 1: DNA ladder, 2-5: DNA template giving negative result, 6: DNA template giving PCR product of 721 bp, 7: negative control, 8: positive control

4. DISCUSSION

Ticks and tick-borne diseases (TBDs) are one of the most important impediments to livestock development in the Sudan [20]. Due to the rapid growth of human population in Khartoum State, there is a rising demand for milk and milk products. Thus, many farmers in Al Nuba town, Sudan which is located close to Khartoum State, increased the number of cattle population. However, TBDs are the main threat to these cattle in most locations within the town. One of the most important TBD of cattle in the Sudan is tropical theileriosis [21].

In this study, the overall prevalence rates of *Theileria* spp. piroplasms and *T. annulata* infection of cattle in Nuba farms were 91% and 75% using blood smear examination and PCR assay, respectively. In previous studies conducted in the same region, it was reported that 16.5% and 47% as prevalence of *Theileria* spp. piroplasms and the sero-positivity for *T. annulata* antibodies respectively [22]. Another study performed by [23] , who reported that the percentage of infected cattle with *T. annulata* was 48.1% using PCR, whereas it was 65.4% using reverse line blot (RLB) in Khartoum State. In the present study, there is a gradual increase in the prevalence rate compared with the previous study. This increase may be due to the introduction of exotic cattle without regulations mandatory to control ticks which act as vectors for the spread and raised prevalence rate of the disease.

The result of blood smears was higher compared with PCR. This finding suggests a possible infection with other *Theileria* species. Moreover, the blood smears can detect the piroplasms of all *Theileria* spp. in which case differentiation using blood smears is not possible, while PCR is specific for one species only [24,25].

The therapeutic efficacy of buparvaquone was investigated in several studies. Muhammad [26] reported that, 93% of animal treated with buparvaquone and oxytetracycline recovered. Similarly, Qayyum [27] stated that 81.73% effectiveness of buparvaquone and oxytetracycline in bovine theileriosis. Other studies used buparvaquone as a treatment for theileriosis recorded a recovery rate of 90% in Kenya [28] and 95.2% in Tanzania [29]. In this study, out of 68 animals treated cows with the therapeutic doses of buparvaquone, the recovery rates were 38.2% and 66.2% after 4 and 6 weeks of treatment, respectively. On the other hand, out of 28 non- treated animals the recovery was 0% and 39.3% at the same time. Although the recovery rate in the current study was lower than reported in previous studies, there was a significant difference between the treated and non-treated groups (control) after four and six weeks of treatment.

One obvious possible explanation for the differences in recovery rates between our and previous results is the stage of the disease. It is well known that the buparvaquone is usually not effective at the late stage of the theileriosis [30].

In addition, the prophylactic efficacy of buparvaquone and oxytetracycline was higher than the efficacy of buparvaquone only [27]. Moreover, drugs may also be less effective during pregnancy because of pharmacokinetic changes such as increased metabolism or excretion [31].

In this study, there is a clear variation in the recovery rates between the treated groups based on the gestation period, where the recovery rates for group TA, TB and TC were ranged between 18.7% and 58.3% after 4 weeks of treatment and between 81.3% and 57.1% after 6 weeks of treatment respectively. This result is indicating that pregnancy has an influence on the efficacy of buparvaquone. In human medicine, the dynamic physiological changes that occur during pregnancy influence the pharmacokinetic processes of drug absorption, distribution and elimination [32]. For example, a total mean of body water increases during pregnancy, which in turn alters drug concentrations and distribution [33]. On the other hand, the renal filtration rate usually increases during pregnancy, especially during the third trimester, leading to decreased drug concentration because of their increased clearance [32,33,34]. The major consequence of these physiological changes during pregnancy is that some drugs can be inadequate as results of changes in their concentration, which leads to ineffective treatment [32,31,34]. Therefore, the doses of these drugs, including buparvaquone, may need to be adjusted during pregnancy.

5. CONCLUSION

Theileriosis has an important economic impact in the study area and a large number of cattle have severed from the disease. Although the recovery rate in this study is low, buparvaquone is still therapeutically effective in bovine theileriosis and hence holds to be the drug of choice.

In pregnant cows, physiological changes may reduce the efficiency of buparvaquone. Thus, further work is needed to monitor the therapeutic concentration of buparvaquone during pregnancy. Future investigations are necessary to evaluate the protective effect of buparvaquone against new-born deaths.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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