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Efficacy of different treatment regimens against Oriental theileriosis in naturally infected cattle

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Abstract

Oriental theileriosis caused by *Theileria orientalis* is a baffling problem among cattle in the humid tropics. The pathogenesis of this disease is mainly due to the extravascular haemolysis leading to anaemia in infected cattle. There are limited options available for treatment and control of this disease. The present study was conducted in 30 cattle clinically infected with *T. orientalis* using three different treatment protocols to evaluate their efficacy. Treatment with buparvaquone and long acting oxytetracycline was found to be effective in terms of remission of clinical signs. Complete elimination of the organism was not observed with any of the three treatment protocols tested, indicating the persistence of carrier state in infected cattle.

Key words: Cattle, Buparvaquone, Theileriosis, Treatment

Theileriosis is one of the most economically important diseases of bovines. *Theileria* is an obligatory intracellular protozoan parasite that infects both domestic and wild animals. *Theileria annulata* and *T. parva* were considered as most pathogenic species due to their ability to transform the host lymphoid cells. Tropical theileriosis caused by *T. annulata* was distributed in southern Europe, Africa, Middle East, Asia and Caribbean countries (Jabbar *et al*., 2015). *Theileria parva* was distributed in eastern, central and southern Africa. Oriental theileriosis caused by *Theileria orientalis* is worldwide in distribution and it has been reported from more than 40 countries. Most of the outbreaks of *T. Orientalis* have been reported from Japan, Korea, Australia, New Zealand and China (Gebrekidan *et al*., 2020). Recently several outbreaks associated with *T. orientalis* have been reported from Asia-pacific countries (Jenkins and Bogema, 2016). In India, increased incidence of *T. orientalis* infection has been reported from different states during the last 10 years (Aparna *et al*., 2011; Kakati *et al*., 2015; Selim *et al*., 2020). *Theileria orientalis* exerts its pathological effects mainly through erythrocyte destruction leading to severe anaemia in infected animals (Eamens *et al*., 2013). The alterations in haematological parameters are considered as good tools for diagnosis and monitoring the effectiveness of therapy.

The treatment for theileriosis includes buparvaquone and/or oxytetracycline along with supportive therapy to combat anaemia in infected animals. Buparvaquone is a second generation hydroxynaphthoquinone and the drug of choice for treatment of theileriosis (Ibrahim *et al*., 2020). For treatment of *T. orientalis* infection, primaquine phosphate, halofuginone, oxytetracycline, buparvaquone and imidocarb were tried either alone or in combinations with variable results (Watts *et al*., 2016). Ozawa *et al*. (1988) found that treatment of *T. sergenti* infected cattle with buparvaquone @ 2.5mg/kg IM resulted in reduction in parasitemia and remission of clinical signs. Jackson (2018) reported that combination of buparvaquone and oxytetracycline was effective in the treatment of *T. orientalis* infection. Most of the research on *T. orientalis* is concentrated on molecular characterisation of the parasite. Literature on haematological parameters and treatment of *T. orientalis* infected animals is limited. Hence the present study was aimed to evaluate the efficacy of three treatment regimens against oriental theileriosis.

Materials and Methods

The present study was conducted in cattle presented to veterinary hospitals suspected for oriental theileriosis. A total of 30 cattle showing the clinical signs such as fever, inappetance, lethargy, anaemia and decreased milk production and positive for *T. orientalis* by blood smear examination and polymerase chain reaction (PCR) were included in this study. Detailed anamnesis was collected from all the cases and clinical examination was performed. Mild to moderate tick infestation was noticed on all animals. Peripheral blood smears were prepared and stained with Giemsa stain. About 2 ml of

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Table 1. Treatment protocols adopted in three different groups

Group	Treatment Protocol
Group I	Inj. Buparvaquone # @ 2.5 mg/kg body weight single dose I.M.
Group II	Inj. Buparvaquone @ 2.5 mg/kg body weight single dose I.M. and Inj.Oxytetracycline LA† @ 20mg/kg body weight I.M and repeated after 48 h
Group III	Inj. Buparvaquone @ 2.5 mg/kg body weight single dose I.M. and Inj. Marbofloxacin* @ 2 mg/kg body weight for 4 days I.M.

Zubion, Intas Pharmaceutical Ltd, Ahmedabad
†Steclin LA, Zydus AH, Ahmedbad
* Marbomet, Intas Pharmaceutical Ltd, Ahmedabad

blood was collected by jugular venepuncture in EDTA vials, and complete blood count was carried out on an automated haematology analyser. The remaining whole blood samples were used for molecular confirmation. Blood smears and whole blood were collected before treatment and seven days after treatment. Six healthy cross bred cattle were selected from same area as control group based on clinical examination and negative on blood smear examination and in PCR.

The DNA was extracted from 100 µL of whole blood by DNeasy Blood and Tissue kit (QIAGEN, Germany) as per the manufacturer's instructions. The species specific primers described by Tanaka et al. (1993) were used for confirmation of *T. orientalis* with the PCR protocol recommended by Goud and Vijayakumar (2020).

Thirty animals clinically infected with *T. orientalis* were divided randomly into three groups and subjected to three different treatment protocols as shown in Table 1. Supportive therapy comprised of Meloxicam (Melonex) @ 0.2 mg/kg body weight IM, Ferrous fumerate + Vit B12+Folic acid @ 1 bolus PO BID daily for one week (Bol. Ferritas). The efficacy of treatment was evaluated based on remission of clinical signs, molecular detection of parasite and changes in haematological parameters after seven days of treatment.

Statistical analysis

IBM-SPSS software version 26.0 was used for statistical analysis. Haematological parameters in control and diseased animals were analysed by ANOVA before treatment. The haematological parameters in treatment groups before and after treatment were analysed by Paired 't' test as per the principles of Snedecor and Cochran (1994).

Results and Discussion

Examination of Giemsa stained blood smears revealed presence of *Theileria* piroplasms in all the thirty cattle. The piroplasms were pleomorphic in morphology, but majority appeared as thin and thick rods with trailing cytoplasm (Fig. 1). This was in accordance with Jackson (2018). Species specific PCR targeting the MPSP gene

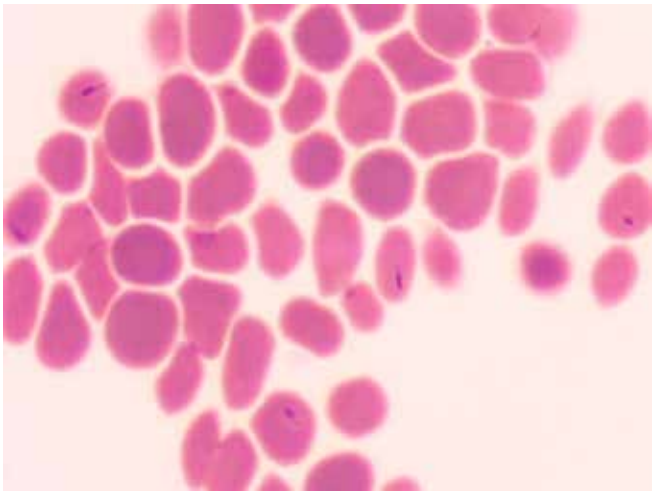
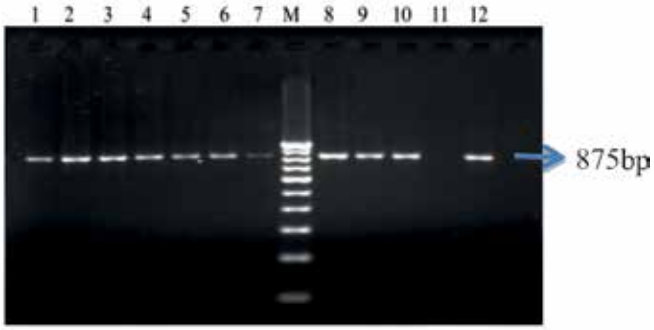


Fig. 1. Theileria orientalis piroplasms in blood smear



Lane M : 100 bp Ladder
Lane 1 to 10 samples positive for T. orientalis
Lane 11: Negative control, Lane 12: Positive control

Fig. 2. Agarose gel electrophoresis of PCR amplified products of T. orientalis

of *T. orientalis* using the MPSP- F/MPSP-R primers amplified 875 bp product specific for *T. orientalis* from all thirty DNA samples (Fig.2).

The common clinical signs observed in *T. orientalis* infected cattle were fever, pale mucous membranes, variable degree of weakness, depression and lethargy in affected cattle due to anaemia.

The mean haematological values of control group and treatment groups before treatment were shown in Table 2. In the present study, the cattle infected with *T. orientalis* showed significant decrease ($p<0.05$) in TEC, haemoglobin and VPRC (Volume of Packed Red Cells), this finding was in agreement with (Selim *et al*., 2020). The decreased TEC, haemoglobin and VPRC observed in present study was attributed to both extravascular and intravascular haemolysis in *T. orientalis* infected animals (Lawrence *et al*., 2018; Agina *et al*., 2020). In addition along with the parasitized erythrocytes, non parasitized erythrocytes were also removed from circulation in *T. orientalis* infected animals leading to severe anaemia in affected animals.

The present finding of non significant changes in mean MCV, MCH and MCHC values in *T. orientalis* infected animals compared to healthy control group was in agreement with Sivakumar *et al*., (2017). Significant increase in lymphocyte count was observed in *T. orientalis* infected animals as compared to healthy animals in the present study, which was in agreement with Selim *et al*. (2020).

Animals in group I, were treated with buparvaquone single dose IM. Buparvaquone exerts its anti-theilerial activity by competitively inhibiting the cytochrome bc 1 complex leading to inhibition of electron transport chain of the parasite but not the host (Fry and Pudney, 1992). Out of the 10 animals in group I, marked improvement was noticed after 48 hours of treatment in seven animals. The remaining three animals were still positive for *T. orientalis* by blood smear examination after 48 hours and these animals were administered a second dose of buparvaquone. Among these three animals, two animals recovered and the remaining one animal continued to harbour *Theileria* piroplasms. Rare *Theileria* piroplasms were detected in three animals in this group after seven days of treatment. This finding was in agreement with Playford and McFadden (2015) who also stated that buparvaquone reduces the level of parasitaemia in *T. orientalis* infected animals but did not result in complete elimination of *T. orientalis* piroplasms. Mhadhbi *et al*. (2015) stated that resistance to buparvaquone was observed in *T. annulata* due to mutations in cytochrome b region leading to treatment failures in infected animals. These types of studies are warranted in *T. orientalis* infected animals to elucidate the reasons for treatment failure. Four animals in this group were positive for *T. orientalis* in MPSP PCR after seven days.

The animals in group II, were treated with buparvaquone and oxytetracycline LA, intramuscularly. Oxytetracycline inhibits the mitochondrial/ cytoplasmic

Table 2. Haematological parameters in control group and *Theileria orientalis* infected cattle

Haematological parameter	Control group (n=6)	Clinically affected animals		
		Group I (n=10)	Group II (n=10)	Group III (n=10)
Total Erythrocyte count ($x10^6/\mu L$)	8.23 ± 0.64^a	3.64 ± 0.13^b	4.14 ± 0.52^b	4.4 ± 0.23^b
Haemoglobin (g/dl)	10.85 ± 1.13^a	5.96 ± 031^b	6.17 ± 0.65^b	6.32 ± 0.39^b
VPRC (per cent)	35.88 ± 3.99^a	17.94 ± 0.63^b	19.91 ± 2.19^b	20.04 ± 0.98^b
MCV (fl)	43.88 ± 3.39	49.94 ± 2.61	49.76 ± 2.78	46.19 ± 1.80
MCH (pg)	13.22 ± 0.93	16.55 ± 1.07	15.49 ± 0.97	14.41 ± 0.59
MCHC (per cent)	30.33 ± 0.51	33.30 ± 1.42	31.25 ± 0.91	31.38 ± 1.15
Platelet count ($x10^3/\mu L$)	280.17 ± 42.63	246.60 ± 32.97	235.80 ± 52.60	230.61 ± 44.21
Total leucocyte count ($x10^3/\mu L$)	9.93 ± 0.98	13.02 ± 1.94	15.22 ± 2.36	15.00 ± 2.47
Lymphocytes ($x10^3/\mu L$)	2.95 ± 0.54^a	8.54 ± 2.10^b	11.11 ± 2.57^b	10.35 ± 1.66^b
Monocytes ($x10^3/\mu L$)	0.82 ± 0.09^a	0.43 ± 0.07^b	0.67 ± 0.07^a	0.49 ± 0.06^a
Granulocytes ($x10^3/\mu L$)	6.17 ± 1.44	4.07 ± 0.86	3.45 ± 0.43	4.16 ± 0.84

Means within a row with different superscripts (a,b) significantly differ ($p<0.05$)

Table 3. Rate of remission of clinical signs in treatment groups

Group	Drugs used	No. of cattle showing remission of clinical signs (per cent)	
		After 48 h of treatment	After seven days of treatment
Group I (n=10)	Buparvaquone	7 (70%)	9 (90%)
Group II (n=10)	Buparvaquone + Oxytetracycline LA	10 (100%)	10 (100%)
Group III (n=10)	Buparvaquone + Marbofloxacin	9 (90%)	9 (90%)

protein synthesis in susceptible organisms and it has been used along with buparvaquone for the treatment of tropical theileriosis (Saravanan *et al*., 2017). All the ten animals in this group showed marked clinical improvement after 48 hours of last treatment. But stray theilerial piroplasms were observed in three animals after 48 hours and also after seven days of treatment. Four animals in this group were positive in MPSP PCR for *T. orientalis* after seven days of treatment. This was in agreement with Ha *et al*. (2019) who stated that animals treated with combination of buparvaquone and oxytetracycline remained positive in MPSP PCR even after 4 months, though the total erythrocyte count increased within a few days while other haematological parameters took long time to return to normal.

Animals in group III, were treated with buparvaquone and marbofloxacin on first day and marbofloxacin repeated for another three days. Pulmonary edema has been reported during necropsy examination of animals that died due to oriental theileriosis by Aparna *et al*. (2011) and Kakati *et al*. (2015). Marbofloxacin was effective and recommended for the treatment of respiratory infection in livestock (Hayashi *et al*., 2019) hence, marbofloxacin was used along with buparvaquone in group III for the treatment of *T. orientalis* infected animals. All the ten animals in this group showed clinical improvement after 48 hours of last treatment. But stray theilerial piroplasms were detected in four animals after 48 hours of last treatment. These four animals were positive in blood smear and also in MPSP PCR after seven days of treatment. Similarly, Al-Hosary *et al*., (2010) reported 93.33 per cent recovery rate in tropical theileriosis infected animals treated with combination of buparvaquone and marbofloxacin.

The clinical recovery rate in different treatment groups I, II and III was 70 per cent, 100 per cent and 90 per cent, respectively after 48 hours of last treatment. In treatment group I, the clinical recovery rate increased to 90 per cent after seven days of treatment.

Microscopic examination of stained blood smears revealed presence of *Theileria orientalis* piroplasms in three animals in each group I and II, whereas piroplasms were detected in four animals of group III, after 48 h of last treatment. After seven days of treatment, stray *Theileria* were detected in the blood smears of above positive cattle (Table 3).

It was observed that treatment regimen of group II was found to be comparatively more effective based on the remission of clinical signs than group I and III (Table 4).

There was no significant difference between all the haematological parameters before and after treatment in any of the three treatment groups (Table 5). This might be due to persistence of long term carrier state in *T. orientalis* infected animals (Kubota *et al*., 1996).

Table 4. Efficacy of treatment regimens after seven days of treatment

Parameters	Group I	Group II	Group III
Microscopic detection of *T. orientalis* piroplasms (%)	30	30	40
Molecular detection of *T. orientalis* by MPSP PCR (%)	40	40	40
Remission of clinical signs (%)	90	100	90

Table 5. Haematological parameters in *Theileria orientalis* infected animals before and after treatment

Haematological parameter	Group I (n=10)		Group II (n=10)		Group III (n=10)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Total Erythrocyte count ($x10^6/\mu L$)	3.64±0.13	3.94±0.26	4.14±0.52	4.91±0.46	4.40±0.23	4.78±0.27
Haemoglobin (g/dL)	5.96±0.31	6.21±0.23	6.17±0.65	6.82±0.56	6.32±0.39	7.09±0.36
VPRC (per cent)	17.94±0.63	18.96±0.84	19.91±2.19	21.89±2.21	20.04±0.98	20.36±1.10
MCV (fl)	49.94±2.61	49.02±2.40	49.76±2.78	44.74±1.52	46.19±1.80	42.82±1.11
MCH (pg)	16.55±1.07	15.72±0.90	15.49±0.97	14.17±0.77	14.41±0.59	14.92±0.53
MCHC (per cent)	33.30±1.42	32.13±1.00	31.25±0.91	31.66±1.02	31.38±1.15	34.95±1.01
Platelet count ($x10^3/\mu L$)	246.60± 32.97	357.00± 52.24	235.80± 52.60	439.70± 60.36	230.61± 44.21	253.90± 37.14
Total leucocyte count ($x10^3/\mu L$)	13.02±1.94	12.62±1.89	15.22±2.36	11.47±2.03	15.00±2.47	11.71±1.32
Lymphocytes ($x10^3/\mu L$)	8.54±2.10	8.50±1.68	11.11±2.57	7.21±1.96	10.35±1.66	7.99±1.24
Monocytes ($x10^3/\mu L$)	0.43±0.07	0.54±0.10	0.67±0.07	0.55±0.11	0.49±0.06	0.56±0.08
Granulocytes ($x10^3/\mu L$)	4.07±0.86	3.59±0.46	3.45±0.43	3.71±0.59	4.16±0.84	3.22±0.38

Note: No significant difference was observed between before and after treatment values

Conclusion

Theileria orientalis is a haemoprotozoan parasite of economic significance throughout the world. The development of effective therapeutics and control strategies is of great importance due to recent spread of disease to Asia-pacific region. The results of present study revealed that, even though clinical recovery was noticed in *T. orientalis* infected animals, complete elimination of the piroplasms was not noticed in any of the three treatment combination tested. Based on remission of clinical signs buparvaquone along with oxytetracycline was found to be more effective than buparvaquone alone or in combination with marbofloxacin. Since there is persistency of the organisms in the blood even after treatment, it poses a risk of disease getting transmitted to other animals in the herd through vector ticks and hence is of great significance.

Ethical approval and consent statement

Verbal consent was taken from the owner of the animals before drawing blood from animals. There is no specific law in India that requires permission from the ethics committee for collecting less than 5 ml of blood and further, the blood samples were collected from the infected animals presented to veterinary hospitals as a part of clinical diagnosis by qualified veterinarians.

Conflicts of interest

There was no conflict of interest reported by the author(s).

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