

Clinico-Haemato-Biochemical Alterations and Therapeutic Management of Bovine Theileriosis

Krishna D. Prajapati^{1*}, Sunant K. Raval¹, Ghanashyam C. Mandali¹, Neha Rao², Shailesh K. Bhavsar³, Munja Bharai¹

ABSTRACT

Theileriosis is an economically important haemoprotozoan disease of cattle caused by *Theileria* spp. It causes production losses and mortality in dairy industry. A total of 135 cattle suspected of theileriosis were screened based on clinical signs and blood smear examination (Giemsa's stain). Out of them, 64 animals were diagnosed with theileriosis. Twenty-four cattle out of them were selected and divided into four equal groups, viz., B, C, D, and E, each comprised of six animals for four different treatment protocols to evaluate their therapeutic efficacy, while group A of six healthy cattle served as control. The most common clinical findings of theileriosis infected cattle (n = 24) in descending order were anorexia (100%), dullness and depression, decreased milk production, weakness, salivation, enlarged pre-scapular lymph nodes, pale conjunctival mucous membrane, presence of ticks over the body, emaciation, nasal discharge, cessation of rumination, lacrimation, diarrhea and sternal recumbency (8.33%). The mean rectal temperature, heart rate, and respiratory rate were $104.5 \pm 0.33^\circ \text{F}$, $79.5 \pm 2.85/\text{min}$ and $42.83 \pm 2.67/\text{min}$, respectively. The haematological findings revealed decreased values of haemoglobin, packed cell volume, total erythrocytes count (TEC), lymphocytes, and mean corpuscular hemoglobin concentration (MCHC), and increased values of neutrophils, eosinophils, Mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) in theileriosis infected cattle as compared to healthy cattle. Biochemical findings revealed decreased values of total protein and albumin while increased total bilirubin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Gamma-glutamyl transferase (GGT) in theileriosis-infected cattle. These altered haemato-biochemical profiles were found to be normalized by the 14th day in different treatment groups. The therapeutic efficacy of Buparvaquone 2 dose (Group B) and Buparvaquone plus Oxytetracycline LA (Group D) was better (100.00%) than the Arteether (Group C, 66.66%) and Arteether plus Oxytetracycline LA (Group E, 83.33%) in theileriosis infected cattle, hence may be advocated to the field veterinarians for effective treatment of theileriosis in cattle.

Keywords: Buparvaquone, Cattle, Clinical signs, Giemsa staining, Haemato-biochemical assay, Theileriosis, Therapeutic trials.

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INTRODUCTION

Bovine theileriosis is one of the most prevalent and economically significant diseases of cattle (Forsyth *et al.*, 1997). Exotic and crossbred cattle are more susceptible than the indigenous breeds of cattle. Out of six identified *Theileria* species, *Theileria annulata* and *Theileria parva* are the most important and pathogenic species which cause theileriosis in bovine (Kohli *et al.*, 2014). *Theileria* spp. is an obligate intracellular protozoan parasite. The vector responsible for transmission is biting tick *Hyalomma anatolicum*. The hot and humid climate favours the development and survival of ticks (Kohli *et al.*, 2014). *Theileria* sporozoites are transmitted to susceptible animals via the saliva of feeding ticks. *Theileria* sporozoites undergo a complex life cycle involving the replication of schizonts in the leukocytes and piroplasm in the erythrocytes (Gachohi *et al.*, 2012). The clinical signs of theileriosis in cattle are elevated body temperature 105°F , anorexia, swelling of superficial lymph nodes, weight loss, drop in milk yield, nasal discharge, lacrimation, dyspnoea, anaemia, and in later stage some time diarrhea and dysentery, with the presence of ticks on the body (Radostitis *et al.*, 2008). Infected cattle remain a carrier for a lifetime since organisms localize in the macrophages and lymphoid tissues.

Theileriosis in cattle is treated worldwide with different allopathic medicines like parvaquone, buparvaquone,

¹Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand-388001, India.

²Department of Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand-388001, India.

³Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand-388001, India.

Corresponding Author: Krishna D. Prajapati, Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand-388001, India, e-mail: krishnaprajapati9697@gmail.com

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halofuginone lactate, oxytetracycline, and diminazene aceturate (Ngumi *et al.*, 1992). Arteether is a synthetic derivative of artemisinin, a natural product of the Chinese plant *Artemisia annua*, popularly known as sweet wormwood. It is currently used as a second-line drug in severe malaria

cases as schizonticide specifically indicated for the treatment of chloroquine-resistant *Plasmodium falciparum* malaria and cerebral malarial cases. It has also been used in bovine theileriosis (Khawale *et al.*, 2019). Suppose the infected animals are not treated properly and efficiently. In that case, it may have a huge economic impact on the livestock sector due to heavy morbidity and mortality, affecting around 80% world population (Kasozi *et al.*, 2014). Therefore, this study was planned to diagnose tropical theileriosis in cattle using clinical signs and Giemsa staining of blood smears and to evaluate the therapeutic efficacy of different regimens through clinico-haemato-biochemical observations and recovery rates after treatment.

MATERIALS AND METHODS

The present study was undertaken from January to June 2021 on 135 cattle suspected of having theileriosis and presented at the Veterinary Clinical Complex of the College, Anand, and nearby villages of the Anand district. Blood smear examination of all these animals was done initially, which revealed the presence of *Theileria* spp. piroplasm in 64 cattle (Figure 1). Of these, 24 cattle selected for therapeutic trial were divided into four equal groups, viz., B, C, D, and E, having six animals in each. Group A included six healthy animals from the same area as the control. Blood samples were collected from the jugular vein in sterile plastic K₃-Ethylenediamine tetraacetic acid (K₃EDTA) vials and serum clot activators from all healthy and affected animals before the initiation of treatment and on the 14th day of treatment for haemato-biochemical analysis.

Animals of Group B were treated with Buparvaquone @2.5 mg/kg b.wt., IM, repeated after 48 hours; Group C treated with Arteether @5 mg/kg b.wt., IM for 3 consecutive days; Group D treated with buparvaquone @ 2.5 mg/kg b.wt., IM, and 24 hrs later Inj. Oxytetracycline (long-acting) @20 mg/kg b.wt., IM, and Group E cattle were treated with Arteether @5 mg/kg for three consecutive days followed by Inj. Oxytetracycline (long-acting) @20 mg/kg b.wt., IM.



Figure 1: Blood smear of cattle shows *theileria* piroplasm like a signet ring or half-moon shape

Apart from these, all groups received supportive therapy, viz., haematinic preparation-Feritas (Iron + folic acid + vitamin B₁₂) orally, Meloxicam @ 0.2 mg/kg b.wt., IM, Chlorpheniramine maleate @ 30 to 50 mg/kg b.wt., IM, and vitamin B complex along with appropriate fluid therapy. The infected cattle were monitored from pre-treatment to 14 days post-treatment. The therapeutic efficacy of different treatment regimens was evaluated based on clinical signs, blood smear examinations, and haemato-biochemical assay before and after treatment.

The haematological analysis of whole blood was done using an Automatic Whole Blood Analyzer (Abacus Junior Vet-5). The serum biochemical parameters studied using standard assay kits with the help of Clinical Biochemistry Auto-analyser (CKK 300) included glucose, total protein, albumin, total bilirubin, AST, ALT, GGT, serum creatinine, and BUN. The clinical signs and therapeutic efficacy data were analyzed using descriptive statistics and those on haemato-biochemical assays by using one-way ANOVA and paired 't' test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Clinical Findings

The clinical findings of 24 theileriosis affected cattle recorded in decreasing frequency were anorexia (100%), followed by dullness and depression (95.83%), reduced milk production (87.50%), weakness (58.33%), salivation (58.33%), enlarged pre-scapular lymph nodes (54.16%), pale conjunctival mucous membrane (54.16%), presence of ticks over the body (54.16%), emaciation (41.66%), nasal discharge (37.5%), cessation of rumination (33.33%), lacrimation (28.83%), diarrhea (12.50%) and sternal recumbency (8.33%). The mean values of rectal temperature, heart rate, and respiratory rate observed were $104.5 \pm 0.33^\circ\text{F}$, 79.5 ± 2.85 per min and 42.83 ± 2.67 per min, respectively. Similar findings were also reported earlier by Naik *et al.* (2016), Kumar *et al.* (2018), and Nagar *et al.* (2019). The clinical parameters in treatment groups B, C, D, and E, treated with Buparvaquone, Buparvaquone plus oxytetracycline LA, arteether, and arteether plus oxytetracycline LA were more or less similar to the overall findings as the animals were randomly and equally allotted to four treatment groups.

haematological Findings

The results of haematological findings in all five groups of animals before and after treatment are shown in Table 1. The mean (\pm SE) values of haemoglobin, packed cell volume, total erythrocytes count, and lymphocytes were significantly decreased ($p < 0.05$) in theileriosis infected cattle as compared to healthy cattle group A. The total leucocytes count, monocytes and eosinophils were non-significantly increased, while neutrophils increased significantly ($p < 0.05$) in theileriosis infected cattle as compared to healthy cattle. However, there was a significant ($p < 0.05$) increase in MCH and a non-significant increase in MCV, while MCHC

was non-significantly decreased in theileriosis infected cattle compared to healthy cattle. On 14th day of different treatments, the values of most of these haematological parameters were improved as compared to '0' day values and were found to be normal or nearer to normal, except few parameters in certain groups which were still statistically different from the day '0' values and also of a healthy control group (Table 1). These observations suggested the beneficial effect of specific and supportive therapies used in theileriosis-infected cattle.

These findings concurred well with the earlier reports of Tuli *et al.* (2015), Saravanan *et al.* (2017), Brahambhatt *et al.* (2019), and Goud *et al.* (2021). The lower haemoglobin, PCV, and TEC values observed in theileriosis-infected animals might be due to the toxic metabolites of *Theileria* spp. (Radostits *et al.*, 2008), which destruct the erythrocytes. Persistent loss of blood caused by blood-sucking ticks is also an important factor. The higher value of total leucocyte counts might be due to leukocytosis (Pandey *et al.*, 2017).

Lymphocytopenia might be due to the destruction of lymphocytes in lymphoid organs and the infiltration of these cells into various organs (Goyal, 2018). Neutrophilia observed in theileriosis-infected cattle may be due to the release of endogenous corticosteroid in acute disease or stress or inflammation (Ugalmugle *et al.*, 2010). The higher value of MCV, MCH, and the lower value of MCHC observed may be due to macrocytic hypochromic anaemia in theileriosis-infected cattle (Durrani and Kamal 2008).

Biochemical Findings

The results of biochemical findings observed in different groups of animals before and after treatment are presented in Table 2. The mean (\pm SE) values of total protein and albumin were decreased non-significantly, while glucose was decreased significantly ($p < 0.05$) in theileriosis-infected cattle as compared to the healthy group. Similar findings were reported by Khan *et al.* (2011) and Kachhawa *et al.* (2016). The lower value of total protein and albumin might be due to

Table 1: Haematological findings of healthy (Gr A) and theileriosis infected cattle under different treatment regimens (Gr. B, C, D, E) (mean \pm SE, n = 6 each)

Haematology	Stage of treatment	Haematological profile in Groups				
		A	B	C	D	E
Hb (gm/dl)	0 day	10.73 ^b \pm 0.39	5.95 ^a \pm 0.64	6.54 ^a \pm 0.98	5.93 ^a \pm 0.57	6.28 ^a \pm 0.82
	14 th day	10.80 ^b \pm 0.44	7.72 ^a \pm 0.69 ^{**}	7.19 ^a \pm 1.08 [*]	7.45 ^a \pm 0.28	7.53 ^a \pm 0.74 [*]
PCV (%)	0 day	32.34 ^b \pm 0.69	20.19 ^a \pm 2.39	20.87 ^a \pm 3.29	20.23 ^a \pm 2.19	24.87 ^{ab} \pm 4.05
	14 th day	32.73 ^b \pm 0.89	25.97 ^{ab} \pm 2.60 ^{**}	23.71 ^a \pm 3.15	25.58 ^{ab} \pm 0.74	25.81 ^{ab} \pm 3.48
TEC (x 10 ⁶ / μ L)	0 day	7.21 ^b \pm 0.09	4.59 ^a \pm 0.66	4.09 ^a \pm 0.68	4.05 ^a \pm 0.36	4.98 ^a \pm 1.95
	14 th day	7.44 ^c \pm 0.46	6.29 ^b \pm 0.12 [*]	5.00 ^a \pm 0.48 [*]	5.53 ^{ab} \pm 0.28 [*]	5.38 ^{ab} \pm 0.30
TLC (x 10 ³ / μ L)	0 day	7.20 \pm 1.01	9.79 \pm 1.44	9.64 \pm 2.08	9.81 \pm 0.39	8.60 \pm 1.52
	14 th day	7.37 \pm 0.46	8.12 \pm 0.62	9.43 \pm 1.40	7.51 \pm 1.07	8.19 \pm 0.76
Lymphocytes (%)	0 day	60.10 ^b \pm 2.50	46.98 ^a \pm 4.57	47.66 ^a \pm 8.82	44.73 ^a \pm 6.62	45.78 ^a \pm 1.55
	14 th day	61.73 ^b \pm 1.39	60.57 ^b \pm 3.29	51.69 ^a \pm 2.42	59.73 ^b \pm 2.08	58.56 ^{ab} \pm 2.49 ^{**}
Monocytes (%)	0 day	1.95 \pm 0.35	3.22 \pm 0.65	2.75 \pm 0.72	4.82 \pm 2.03	3.62 \pm 0.67
	14 th day	1.93 \pm 0.34	2.40 \pm 0.70	2.24 \pm 0.63	2.40 \pm 0.61	2.90 \pm 0.79
Neutrophils (%)	0 day	35.08 ^a \pm 01.82	46.67 ^b \pm 5.08	47.36 ^b \pm 9.37	48.05 ^b \pm 6.98	48.20 ^b \pm 1.99
	14 th day	34.68 ^a \pm 1.25	35.65 ^{ab} \pm 3.61	44.09 ^b \pm 2.72	36.40 ^{ab} \pm 2.29	37.07 ^{ab} \pm 3.09 [*]
Eosinophils (%)	0 day	1.15 \pm 0.37	3.08 \pm 0.82	2.21 \pm 0.45	2.27 \pm 0.32	2.37 \pm 0.30
	14 th day	1.52 \pm 0.78	1.32 \pm 0.23	1.89 \pm 0.39	1.43 \pm 0.18	1.40 \pm 0.51
Basophils (%)	0 day	0.05 \pm 0.03	0.05 \pm 0.05	0.03 \pm 0.03	0.13 \pm 0.13	0.03 \pm 0.21
	14 th day	0.13 \pm 0.07	0.07 \pm 0.03	0.08 \pm 0.03	0.03 \pm 0.03	0.07 \pm 0.03
MCV (fl)	0 day	47.83 \pm 0.48	52.00 \pm 1.03	51.37 \pm 3.49	51.06 \pm 1.35	50.15 \pm 1.68
	14 th day	47.17 \pm 1.66	48.21 \pm 1.13 ^{**}	49.71 \pm 2.26	49.52 \pm 1.56	49.77 \pm 2.06
MCH (pg)	0 day	14.87 ^a \pm 0.44	18.30 ^b \pm 0.69	16.72 ^b \pm 0.50	17.25 ^b \pm 0.49	17.06 ^b \pm 0.72
	14 th day	15.11 \pm 0.31	14.36 \pm 1.00 ^{**}	15.41 \pm 0.74	14.72 \pm 0.35 ^{**}	14.87 \pm 0.62 ^{**}
MCHC (g/dL)	0 day	33.28 \pm 1.52	29.53 \pm 0.55	28.26 \pm 1.48	29.58 \pm 0.71	29.49 \pm 3.85
	14 th day	34.75 \pm 1.57	31.75 \pm 2.00	30.93 \pm 1.64	31.09 \pm 1.21	30.87 \pm 2.58

Means bearing different superscripts (a,b) within the row differ significantly between groups ($p < 0.05$). * $p < 0.05$, ** $p < 0.01$ between 0 and 14th day for a particular parameter.

Table 2: Biochemical findings in healthy (Gr A) and theileriosis infected cattle under different treatment regimens (Gr. B, C, D, E) (mean \pm SE, n = 6 each)

Biochemical findings	Stage of treatment	Biochemical profile in Groups				
		A	B	C	D	E
Total protein (gm/dL)	0 day	7.05 \pm 0.38	5.48 \pm 0.44	5.69 \pm 0.31	6.27 \pm 0.54	6.25 \pm 0.65
	14 th day	7.33 \pm 0.22	7.10 \pm 0.45*	5.71 \pm 0.46	6.51 \pm 0.54	6.26 \pm 0.64
Albumin (gm/dL)	0 day	3.63 \pm 0.28	2.81 \pm 0.31	2.88 \pm 0.39	3.08 \pm 0.16	2.97 \pm 0.19
	14 th day	3.62 \pm 0.20	3.31 \pm 0.13	3.13 \pm 0.22	3.32 \pm 0.09*	3.18 \pm 0.23
Total bilirubin (mg/dL)	0 day	0.34 \pm 0.07	0.97 \pm 0.12	0.87 \pm 0.16	0.86 \pm 0.14	0.81 \pm 0.31
	14 th day	0.27 \pm 0.04	0.31 \pm 0.12**	0.56 \pm 0.12*	0.33 \pm 0.07**	0.39 \pm 0.15
Glucose (mg/dL)	0 day	54.17 ^b \pm 2.09	39.83 ^a \pm 3.06	40.17 ^a \pm 2.77	38.33 ^a \pm 3.03	39.67 ^a \pm 2.73
	14 th day	55.83 ^c \pm 1.30	51.00 ^{bc} \pm 2.34**	44.17 ^a \pm 2.21	50.17 ^b \pm 1.33*	45.67 ^{ab} \pm 1.49
AST (U/L)	0 day	85.14 ^a \pm 2.48	122.02 ^b \pm 8.73	108.81 ^b \pm 6.95	128.85 ^b \pm 7.51	113.71 ^b \pm 6.09
	14 th day	85.81 ^a \pm 2.13	90.75 ^{ab} \pm 3.71*	98.46 ^b \pm 3.43	92.15 ^{ab} \pm 2.63**	95.73 ^b \pm 3.21**
ALT (U/L)	0 day	28.54 \pm 2.07	38.39 \pm 4.58	38.82 \pm 4.07	40.42 \pm 3.02	38.46 \pm 1.68
	14 th day	27.99 \pm 2.52	29.72 \pm 2.39*	34.53 \pm 3.80*	30.67 \pm 1.85*	32.68 \pm 2.67**
GGT (U/L)	0 day	22.28 ^a \pm 1.91	53.80 ^b \pm 3.76	52.58 ^b \pm 3.36	46.34 ^b \pm 5.19	46.66 ^b \pm 3.72
	14 th day	23.08 ^a \pm 2.32	36.02 ^b \pm 1.59**	49.92 ^c \pm 4.20	38.97 ^b \pm 2.44	39.93 ^b \pm 3.93**
Creatinine (mg/dL)	0 day	1.15 \pm 0.14	1.13 \pm 0.16	1.00 \pm 0.13	1.14 \pm 0.07	1.03 \pm 0.15
	14 th day	0.74 \pm 0.10	1.05 \pm 0.13	0.87 \pm 0.11	1.05 \pm 0.18	0.85 \pm 0.10
BUN (mg/dL)	0 day	12.11 \pm 0.52	11.76 \pm 0.49	11.19 \pm 0.34	11.06 \pm 0.38	11.88 \pm 1.19
	14 th day	10.99 \pm 0.15	10.85 \pm 0.61*	10.78 \pm 0.24	10.97 \pm 0.41	10.45 \pm 0.40

Means bearing different superscripts (a,b) within the row differ significantly between groups ($p < 0.05$). * $p < 0.05$, ** $p < 0.01$ between 0 and 14th day for a particular parameter.

the harmful effect of toxic metabolites of *Theileria* spp. and due to liver failure (Aulakh and Singla, 2006). Hypoglycemia observed may be due to the utilization of glucose by *Theileria* spp. present in the blood (Sandhu *et al.*, 1998). In the present study, gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST) were increased significantly ($p < 0.05$), but total bilirubin and alanine aminotransferase (ALT) were increased non-significantly in theileriosis infected cattle as compared to healthy cattle. The higher values of serum enzyme activity found in theileriosis-infected cattle might be due to muscular trauma resulting from prolonged recumbency and hepatic dysfunction (Abubakar *et al.*, 2019). These findings concurred well with reports of Acharya *et al.* (2017) and Abubakar *et al.* (2019).

Serum creatinine and blood urea nitrogen showed non-significant differences in theileriosis infected and healthy cattle. On day 14th of treatment in theileriosis infected cattle with four different regimens, the values of these biochemical parameters had reverted to normal or near-normal levels, except a few parameters like glucose and GGT, which still differed significantly from the day '0' values, and from the healthy group in some specific treatment protocols (Table 2).

Therapeutic Management

The therapeutic efficacy of different treatment regimens in 4 groups of theileriosis-infected cattle (6 in each) was evaluated based on blood smear examinations for parasitemia before

and after treatment. Out of 24 cattle treated, 21 cases (87.5%) were cured successfully. In Group B (Inj. Buparvaquone 2 dose 48 hr apart) and Group D (Inj. Buparvaquone plus Inj. Oxy LA next day) cattle, the clinical and parasitological recovery was recorded in all animals on 14th day with 100% cure rate. The recovery was also noted by elimination of parasites in the blood smear examination on the 14th day of treatment in Group C (Inj. Arteether daily for 3 days) and Group E (Inj. Arteether for 3 days plus Inj. Oxy LA 4th day), but at the lower rate of 66.66% (4/6) and 83.33% (5/6), respectively, as two and one animal in these groups did not recover completely by the respective treatment protocol (Table 3).

Overall, the efficacy of treatment regimens recorded in this study was 100.00, 66.66, 100, and 83.33% in Group B, C, D, and E, respectively. Many earlier workers have also reported similar efficacy of Buparvaquone and Arteether in theileriosis-infected cattle (Kohli *et al.*, 2014; Kumar *et al.*, 2016; Khan *et al.*, 2017; Siddiqui *et al.*, 2017; Nagar *et al.*, 2019; Khawale *et al.*, 2019).

CONCLUSION

From the study, it is concluded that the parenteral administration of Buparvaquone (2.5 mg/kg b.wt. twice 48 hrs apart) and Buparvaquone plus Oxytetracycline LA (20 mg/kg b.wt. 24 hr later) are therapeutically better regimens than the Arteether (5 mg/kg b.wt. for 3 days) and Arteether plus



Table 3: Post-treatment detection of parasitemia and therapeutic efficacy of different regimens in theileriosis infected cattle

Groups / Drugs	Case no.	Parasitemia		Treatment result	Cure rate (%)
		0 day	14 th day		
Group B Inj. Buparvaquone two dose 48 hours apart	1	+++	-	Cured	6/6 (100.0%)
	2	++	-	Cured	
	3	++++	-	Cured	
	4	+	-	Cured	
	5	+++	-	Cured	
	6	+++	-	Cured	
Group C Inj. Arteether daily for 3 days	1	++	+	Not cured	4/6 (66.66%)
	2	++	-	Cured	
	3	+++	++	Not cured	
	4	++	-	Cured	
	5	++	-	Cured	
	6	+++	-	Cured	
Group D Inj. Buparvaquone plus Inj. Oxy (LA) next day	1	++	-	Cured	6/6 (100.0%)
	2	+++	-	Cured	
	3	++	-	Cured	
	4	+++	-	Cured	
	5	+++	-	Cured	
	6	+++	-	Cured	
Group E Inj. Arteether for 3 days plus Inj. Oxy (LA) 4 th day	1	++	-	Cured	5/6 (83.33%)
	2	+++	-	Cured	
	3	++++	+	Not cured	
	4	+	-	Cured	
	5	++	-	Cured	
	6	+++	-	Cured	

Oxytetracycline LA (20 mg/kg b.wt. on 4th day) at the given dose rates in the treatment of theileriosis infected cattle.

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