RESEARCH ARTICLE

Therapeutic Efficacy of Clindamycin in Dogs Naturally Infected with *Babesia gibsoni*

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Abstract

As there are no anti-babesial therapies to eliminate the parasite from the animal's body to date, pets that survive acute infections are at risk for recurring clinical disease and serve as reservoir hosts. This study was undertaken to evaluate the therapeutic efficacy of clindamycin for the treatment of dogs naturally infected with *Babesia gibsoni*. Dogs of various breeds and age groups of either sex diagnosed with *Babesia gibsoni* infection by blood smear examination and confirmed by PCR were selected for the study. Positive cases (n=12) were divided into two equal groups to evaluate the therapeutic efficacy of two drugs, *viz.,* Gr. A (Diminazene aceturate @ 3.5 mg/kg b.wt., i/m once) and Gr. B (Clindamycin @ 11 mg/kg b.wt., i/v q24 h for 10 days) both with supportive treatment. All animals showed clinical cure with improvement in appetite and physical activity by day seven post-treatment with gradual increase in haematological parameters including platelet count and serum biochemistry values, and antioxidant level, *i.e.,* TA, GSH and SOD, and reduced oxidative stress, *i.e.,* LPO, in both the treated groups till day 21 post-treatment. The finding suggests that clindamycin along with supportive therapy might be useful for the treatment of dogs naturally infected with *B. gibsoni* infection as a substitute of Diminazene aceturate.

Key words: *Babesia gibsoni*, Biochemical, Canine, Clindamycin, Haematology, Oxidative stress.

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Introduction

Canine babesiosis is a clinically important and well-known haemoprotozoan disease of dogs. Newly recognized *Babesia* with zoonotic potential continues to emerge around the world and the substantial economic impact of babesiosis is ongoing on livestock and companion animals especially in the tropics and subtropics (Kivaria *et al.*, 2007). *Babesia* causes massive injuries to the host depending on the virulence and pathogenicity of the parasite. Canine babesiosis is caused by tick-transmitted apicomplexan parasites of *Babesia* species, which parasitize erythrocytes. The currently recognized species include *B. canis, Babesia vogeli, Babesia rossi, B. gibsoni, Babesia conradae* and *Babesia vulpes* (also termed *Theileria annae* and *Babesia microti*-like) (Baneth, 2018). Babesiosis primarily affects erythrocytes leading to progressive anaemia, but can involve multiple organs and can range from a relatively mild to a fatal per-acute disease. There is strong evidence of the role played by erythrocytic peroxidation in the pathogenesis of several haemoparastic infections (Nazifi *et al.,* 2008). Oxidative stress in babesiosis may cause damage to erythrocytes that result in their increased susceptibility to phagocytosis (Tvedten, 2004).

Nowadays, so many chemotherapies and vaccinations are applied to treat and eradicate babesiosis without success. Since the parasites are not eliminated by any of the anti-babesial therapies tested to date, pets that survive acute infections are at risk for recurring clinical disease and serve as reservoir hosts (Stegeman *et al.*, 2003). Currently, the most common conventional antibabesial drugs used in *B. gibsoni* infection are Diminazene aceturate (Berenil) and Imidocarb dipropionate (Imizol) often with toxic effects including vomiting and diarrhea and fatal nervous complications after 24-48 h of overdose (Boozer and Macintire, 2003). A new therapeutic modality for canine babesiosis using drugs such as clindamycin, atovaquone and multiple drug combinations has been suggested (Kumara, 2016). Some studies have

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reported the efficacy of clindamycin-metronidazole-doxycycline (DOXY), but this regimen takes a long time and often requires supportive therapy (Nandini *et al*., 2016). However, relapses after administering the combinations of antibabesial drugs and high therapeutic costs still pose significant challenges to veterinarians. So, there is a need to evaluate a therapeutic regimen to treat this condition in India. Hence, the present study was undertaken to evaluate the therapeutic efficacy of clindamycin for the treatment of dogs naturally infected with *Babesia gibsoni* infection.

Materials and Methods

The study was conducted following approval of IAEC (No. CVSC/CAU.IAEC/16-17/P-14, dated 24/09/2017) during June 2017 to May 2018 on dogs (n=1200) of various breeds and age groups of either sex presented to the Veterinary Clinical Complex, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl from different parts of Mizoram (India) with clinical signs suggestive of babesiosis, *viz*., weakness, anorexia, pale mucous membranes, fever and jaundice. For diagnosis, blood drop was collected from the tips of the ear of infected dog and smears were prepared on clean grease-free glass slides. The smears were stained with Giemsa stain and examined under oil immersion objective, and the results were confirmed by PCR assay.

Molecular Diagnosis (PCR)

Genomic DNA was extracted from 100 µL of EDTA-blood samples, using a commercial DNA extraction kit (DNeasy Blood and Tissue kits, QIAGEN, Germany) following the manufacturer's recommendations with minor modifications and stored at -20˚C till use. The PCR assay was optimized targeting a portion of the 18S rRNA gene to amplify *Babesia* species. The sequences of the primers used are as shown in Table 1.

PCR assay in a final volume of 25 µL was carried out in a PCR thermal cycler (Applied Biosystems, USA). *Taq* PCR Master Mix (QIAGEN) 12.5 µL included *Taq* DNA polymerase, QIAGEN PCR Buffer, $MgCl_2$ and ultrapure dNTPs at optimized concentrations, 1 µL of template DNA isolated samples. The volume was made up to 25 µL with nuclease-free water. The PCR cycling conditions were initial denaturation at 95˚C for 5 min, 35 cycles each of denaturation at 95˚C for 30 sec, annealing at 54˚C for 30 sec, and extension at 72˚C for 30 min, and the final extension was performed at 72˚C for 5 min. The PCR products obtained were checked for amplification by electrophoresis on a 1.5% agarose gel and visualized using a gel documentation system (Syngene, UK). Positive PCR products were sequenced at the outsource. DNA sequences were compared for similarity with sequences available in GenBank®, using the BLAST program. The species identity found was determined according to the closest BLAST match with an identity of 97-100% to an existing GenBank® accession.

Therapeutic Protocol

All dogs had not received any antiprotozoal drugs before their admission to the animal hospital. After diagnosis, 12 positive cases were randomly divided into two equal groups. Group A (n=6) dogs were given standard therapy, *i.e.* Diminazine aceturate @ 3.5 mg/kg b.wt., i/m once, along with symptomatic therapy. Group B (n=6) dogs were treated with selected medicine, *i.e.* Clindamycin @ 11 mg/kg b.wt., i/v q24 h for 10 days along with supportive therapy, *viz.,* Inj. Ringers Lactate 20-50 mL/kg b.wt., and Inj. DNS 5% 20-50 mL/kg b.wt., i/v; corticosteroids Inj @ 0.5-1 mg/kg b.wt., q12-24 h, and Inj. Pantoprazole @ 1 mg/kg b.wt. OD, and Syp. aRBCe pet or Sharkoferol, and Inj. Ferritas were given according to the symptoms. For comparison, six healthy dogs that came for a general health check-up were also taken as a control group (Gr. C).

Blood samples (10 mL; 2 mL for CBC and 8 mL for serum biochemistry and oxidative stress indices) were collected with or without an EDTA tube at days 0, 7, 14 and 21 after the first administration of drug. Blood smears were also prepared to examine the parasitemia microscopically. Complete blood count analysis was performed using an automated blood cell counter (MS4 S2, France). Serum biochemistry, *viz*., alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, total protein, albumin/globulin ratio and total bilirubin were analyzed with the help of biochemistry analyzer (automated Fuji Drichem 4000i biochemistry analyzer, Japan). The oxidative stress indices were measured with the help of a commercial kit (Cayman Chemical, USA). The therapeutic evaluation was done based on the improvement of clinical signs, absence of organism in blood smear, negative result of PCR, and haemato-biochemical and oxidative stress changes.

Table. 1: Primers used in the PCR assay of *Babesia* species

Target organism	Primer	Sequence	Fragment length (bp)	Reference
Piroplasma	Piroplasmid-F Piroplasmid-R	CCAGCAGCCGCGGTAATTCCTTTC GCAGTAGTTYGTCTT-TAACAAATCT	400	Tabar *et al*. (2008)
Babesia spp.	Babesia18S-F Babesia18S-R	CCGTGCTAATTGTAGGGCTAATACA GCTTGAAACACTC-TARTTTTCTCAAAG	551	Almeida *et al*. (2012)

Statistical Analysis

The data were analyzed by using two-way ANOVA and the groups/treatment means were compared using Duncan's *post hoc* multiple range tests, using Statistical Package SPSS 16 (SPSS, Science, Chicago, USA).

Results and Discussion

Identification *of Babesia* Species

On viewing the thin peripheral blood smears using an oil-immersion lens, typical intraerythrocytic ring-form trophozoites, tetrads, and paired pyriform (Fig. 1A-D) were observed without any pigments and few merozoites. The parasitemia level was calculated to be as high as 55.2±0.12 % in a blood smear. All the samples were further confirmed by PCR (Fig. 2). By BLAST analysis, the DNA sequences obtained from *Babesia*-PCR showed that all the samples were 97-100 % identical with available sequences of *Babesia gibsoni* (AB478321.1, KY021187.1, MF140997.1, KY433318.1 and KP666168.1). From the study, it was confirmed that the overall incidence of canine babesiosis caused by *Babesia gibsoni* was 1.25% (15/1200).

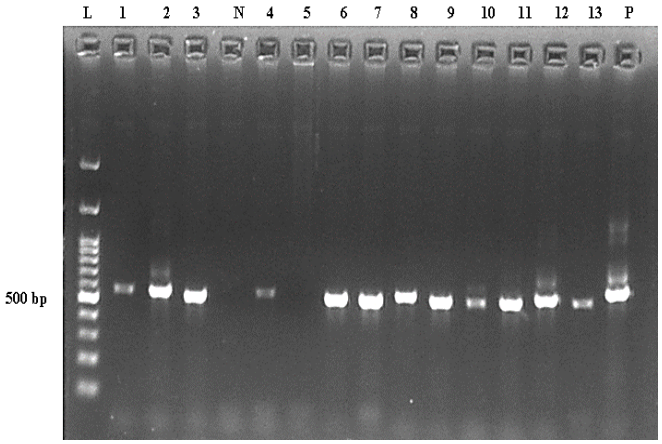

Fig. 2: Gel electrophoresis showing amplification of *Babesia* spp. L: Gene Ruler 100 bp Ladder, Lane-P: positive control, Lane-N: negative control, Lanes-1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13 showing positive amplification of *Babesia* spp.

In dogs, babesiosis is reported worldwide (Garcia, 2006) including in various parts of India (Gonde and Chhabra, 2017) with a variable prevalence. In the present study, the overall incidence of babesiosis in and around Aizawl was observed

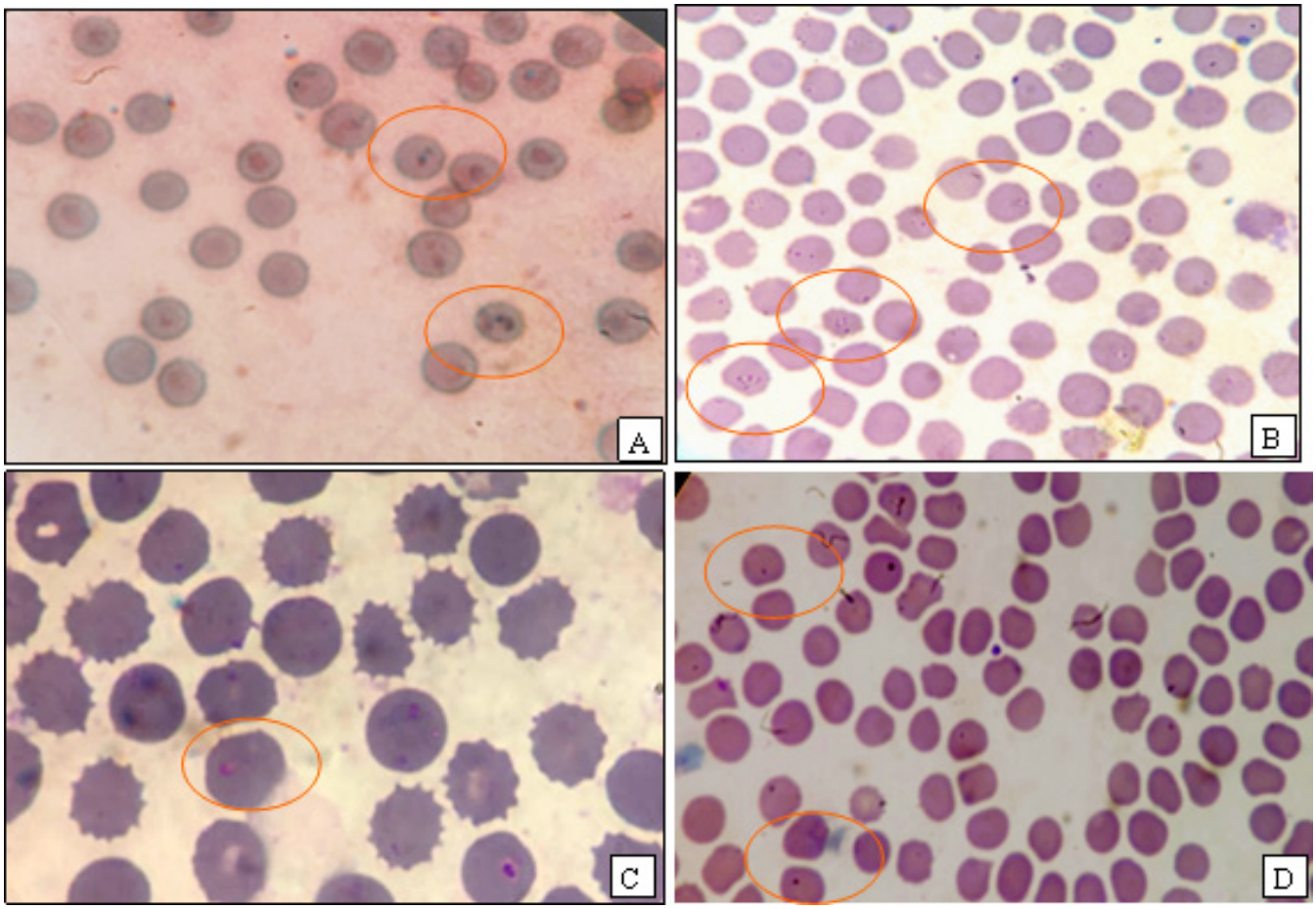

Fig. 1: Photomicrographs of red cells infected with *Babesia gibsoni* in a thin peripheral blood smear of the infected dogs. A. Pear shaped, B. Tetrad form, C. Ring form trophozoites, D. Dot form.

as 1.25% which corroborated with findings of Vatsya *et al.* (2010) and Wadhwa *et al.* (2011) in various part of northern India, *viz.,* Uttar Pradesh, Uttarakhand and Kangra Valley of Himachal Pradesh. In contrast to the present findings, a relatively higher prevalence of 8.49% was reported in southern India (Varshney *et al.,* 2003), 8% in Nagpur (Jumde *et al.,* 2011), and 48.57% in Guwahati (Bhattacharjee and Sarmah, 2013). The varying incidence of babesiosis in different parts of India might be due to sample size and clinical status of animals screened, geographical area, climatic conditions, which directly influence the tick population and time of sample collection. The PCR based prevalence rate of *B. gibsoni* infection ranging from 3.3 to 55% (Talukder *et al.,* 2013; Laha *et al.,* 2014) has been reported worldwide. Higher detection of canine babesiosis by PCR-based assays as compared to microscopy, as observed in the present study, indicates the higher sensitivity level of PCR (Laha *et al.,* 2014).

Treatment Outcomes

Effect of treatment on clinical score and parasitaemia:

The clinical score of both the treated groups A & B was significantly ($p<0.01$) improved on day 7 (3.83±0.48 and 3.50±0.22) as compared to day 0 (6.53±1.00 and 6.83±0.79 respectively). Similarly, it was further significantly ($p<0.01$) improved on day 14 (1.67±0.21 and 1.63±0.22) and day 21 (0.98±0.21 and 0.67±0.20 respectively) after treatment (Table 2). All the treated dogs recovered rapidly, and the *Babesia* protozoan burden significantly ($p<0.01$) decreased in both the treated Groups (from 55.2±0.12% and 55.3±0.21% on day 0 to 2.00±0.02% and 1.00±0.02%, on day 21, respectively) after treatment (Table 2). Similarly, body temperature was also significantly ($p<0.01$) decreased after the 7 days of therapy, returned to normal after 14 days therapy, and

Table. 2: Therapeutic efficacy of diminazene aceturate and clindamycin on the patient's body temperature and *Babesia gibsoni* parasitemia level over 21 days following administration of therapy

Days	*Clinical score*		Parasitemia (%)		Temperature(° F)	
	Gr. A	Gr. B	Gr. A	Gr. B	Gr. A	Gr. B
Day 0	6.53 ± 1.00^{A}	6.83 ± 0.79^{A}	55.2 ± 0.12^{A}	55.3 ± 0.21^{A}	104.2 ± 0.40^{A}	104.1 ± 0.32^{A}
Day 7	3.83 ± 0.48^{B}	3.50 ± 0.22^{B}	32.0 ± 0.45^{B}	28.0 ± 0.32^{B}	$102.3\pm\pm0.17^{B}$	102.1 ± 0.17^{B}
Day 14	1.67 ± 0.21^{C}	1.63 ± 0.22^{C}	12.0 ± 0.12^{BC}	10.0 ± 0.23^{C}	101.8 ± 0.12^{BC}	101.3 ± 0.15^{BC}
Day 21	0.98 ± 0.21^{D}	0.67 ± 0.20^{D}	2.0 ± 0.02^{D}	1.0 ± 0.02^{D}	101.2 ± 0.22^{CD}	101.2 ± 0.21^{CD}

Values with different superscripts within column differ significantly ($p<0.05$).

Table. 3: Therapeutic effect of Diminazene aceturate (Gr. A) and Clindamycin (Gr. B) on haematological parameters (Mean ± SE) in dogs naturally infected with *Babesia gibsoni* in relation to healthy control dogs

Parameters	Group	Days post-treatment			
		0	7	14	21
HB (g/dL)	Gr. A	$9.10^{aA}\pm0.35$	$10.13^{bA}\pm0.28$	$11.26^{cA}\pm0.28$	$12.97^{d}\pm0.25$
	Gr. B	$7.98^{aA}\pm0.50$	$10.13^{bA}\pm0.29$	$11.00^{bA}\pm0.36$	$13.10^{c}\pm0.32$
	Control	$13.32^{B}\pm0.28$	$13.30^{B}\pm0.28$	$13.21^{B}\pm0.28$	13.12 ± 0.28
PCV (%)	Gr. A	$25.65^{aA}\pm1.30$	$29.47^{bA}\pm1.06$	$32.12^{bA}\pm0.59$	$37.62^{c}\pm0.56$
	Gr. B	$26.90^{aA}\pm1.58$	$30.65^{bA}\pm0.97$	$33.85^{bA}\pm0.78$	$37.57^{c}\pm0.95$
	Control	$37.63^{B}\pm1.71$	$36.83^{B}\pm1.71$	$37.53^{B}\pm1.71$	37.93 ± 1.71
TEC ($x10^{6}$/ µL)	Gr. A	$3.57^{aA}\pm0.07$	$3.80^{bA}\pm0.06$	$4.46^{cA}\pm0.04$	$5.55^{dA}\pm0.10$
	Gr. B	$3.50^{aA}\pm0.06$	$3.79^{bA}\pm0.09$	$4.57^{cA}\pm0.08$	$5.71^{dA}\pm0.09$
	Control	$6.18^{B}\pm0.14$	$6.28^{B}\pm0.14$	$6.48^{B}\pm0.14$	$6.08^{B}\pm0.14$
THR ($x10^{3}$/µL)	Gr. A	$162.17^{aA}\pm13.46$	$193.83^{bA}\pm7.90$	$221.17^{bA}\pm3.06$	$285.33^{c}\pm10.01$
	Gr. B	$145.17^{aA}\pm17.85$	$163.17^{aA}\pm20.77$	$215.83^{bA}\pm8.48$	$288.83^{c}\pm11.44$
	Control	$323.50^{B}\pm31.88$	$318.50^{B}\pm31.68$	$329.50^{B}\pm31.48$	333.50 ± 31.38
TLC ($x10^{3}$/µL)	Gr. A	17.70 ± 5.99	17.30 ± 3.92	14.69 ± 1.19	$15.48^{A}\pm0.96$
	Gr. B	19.58 ± 2.86	16.05 ± 2.44	15.68 ± 1.19	$17.00^{B}\pm1.56$
	Control	12.62 ± 0.75	12.32 ± 0.65	12.42 ± 0.70	$12.82^{A}\pm0.65$

Values with different superscripts within the rows (a,b,c) and columns (A.B.C) differ significantly ($p<0.05$) for a parameter.

then maintained normally thereafter. No side effects to Clindamycin were observed in treated dogs during the study period, and all the parameters were statistically similar at different time intervals in both the groups (Table 2).

Effect of treatment on haemato-biochemical values:

The Hb, PCV, TEC, and platelet count were significantly ($p<0.01$) lower in infected dogs on day 0 (day of presentation) as compared to the control group, which were improved significantly ($p<0.01$) on day 21 in both the treated groups (Table 3). Total leukocyte counts also showed a gradual decrease post-treatment as compared to day 0. Results indicated that both Diminazene aceturate and Clindamycin therapy improved the haematological values of babesiosis-infected dogs.

There was a significant increase ($p<0.01$) in the values of ALT and ALP on day 0 in dogs of both the infected groups as compared to the control group. There was a significant ($p<0.01$) decrease in the values of ALT and ALP on day 21 in both the treated groups. Dogs of both groups depicted steady non-significant (increase) improvement in the ALT and ALP values as compared to healthy ones (Table 4). The level of serum total protein, albumin and globulin were significantly lower ($p<0.01$) on day 0 in both the infected groups before treatment as compared to the healthy control group, which returned to normal range on day 21 post-treatment in both the groups (Table 4).

Haemato-biochemical analyses revealed reduced levels of Hb, PCV, TEC, thrombocyte count as well as serum protein, albumin, and elevated serum ALT, ALP and globulin levels. Parasitic migration out of the erythrocytes causes mechanical damage to the erythrocyte that exacerbates anaemia in *B. gibsoni* infected dogs due to the erythrophagocytic capacity of peripheral blood and bone marrow macrophages. The mechanism of thrombocytopenia is platelet sequestration in the spleen or immune-mediated platelet destruction and the development of disseminated intravascular coagulopathy (Baneth, 2018). On 7th day of treatment slight increase in Hb, PCV, TEC and platelet was evident, whereas ALT and ALP were found to be normal. On 21st day, animals were reassessed for haemato-biochemical parameters, which showed improvement. Wulansari *et al*. (2003) reported that clindamycin, a dose-dependent antibiotic with the property of immune-enhancing ability, gradually reduced the level of parasitemia and induced morphological changes in parasites. That is probably the reason for lack of adverse reactions and the dogs show clinical and haematological improvement from few days of treatment onwards and recovered uneventfully by the end of therapeutic protocol (Nandini *et al.,* 2016). Nevertheless, it is also suggested that clindamycin stimulate humoral and cellular immunity against babesia infection and results in improvement in clinical condition (Nandini *et al.,* 2016). Hepatic dysfunction was evident as serum enzymatic activity of ALT, AST and ALP was high in infected animals (Nel *et al.,* 2004; Matijatko *et al*., 2009). Intravenous DNS provides sufficient energy to hepatic cells.

Effect of treatment on biomarkers of oxidative stress:

The total antioxidant (TA) and antioxidant indices namely SOD and GSH activity were significantly ($p<0.01$) lower on day

Table. 4: Therapeutic effect of Diminazene aceturate (Gr. A) and Clindamycin (Gr. B) on biochemical parameters (Mean ± SE) in dogs naturally infected with *Babesia gibsoni* in relation to healthy control dogs

Parameters	Group	Days post-treatment			
		0	7	14	21
ALT (U/L)	Gr. A	62.00^{bB}±3.27	59.50^{bB}±3.27	57.17^{abB}±2.82	48.00^{aB}±3.51
	Gr. B	59.83^{bB}±2.71	61.17^{bB}±2.30	56.17^{bB}±2.39	47.00^{aB}±2.41
	Control	33.17^{A}±2.11	33.27^{A}±2.51	32.47^{A}±2.31	32.57^{A}±2.31
ALP (U/L)	Gr. A	134.33^{cB}±3.91	121.83^{bcB}±3.70	109.50^{bB}±4.25	93.17^{aB}±7.08
	Gr. B	118.33^{bB}±5.84	114.67^{bB}±5.55	101.17^{abB}±5.09	87.50^{aB}±6.26
	Control	48.83^{A}±6.61	48.63^{A}±6.31	47.83^{A}±6.11	47.73^{A}±6.41
TP (g/dL)	Gr. A	5.03^{aA}±0.09	5.10^{aA}±0.06	5.68^{bA}±0.11	6.30^{c}±0.11
	Gr. B	5.08^{aA}±0.07	5.17^{aA}±0.06	6.03^{bA}±0.09	6.52^{c}±0.14
	Control	6.62^{B}±0.19	6.52^{B}±0.20	6.42^{B}±0.17	6.82±0.21
Albumin (g/dL)	Gr. A	1.75^{aA}±0.09	1.80^{aA}±0.08	2.33^{bA}±0.10	2.78^{c}±0.11
	Gr. B	1.85^{aA}±0.09	2.00^{aB}±0.06	2.53^{bA}±0.09	2.82^{c}±0.08
	Control	2.90^{B}±0.06	2.92^{C}±0.05	2.87^{B}±0.04	2.95±0.07
Globulin (g/dL)	Gr. A	3.28^{bA}±0.05	3.30^{bA}±0.04	2.90^{aA}±0.16	3.52^{b}±0.05
	Gr. B	3.23^{aA}±0.04	3.17^{aA}±0.04	3.50^{bB}±0.04	3.70^{c}±0.07
	Control	3.72^{B}±0.16	3.76^{B}±0.17	3.52^{B}±0.17	3.62±0.17

Values with different superscripts within the rows (a,b,c) and columns (A.B.C) differ significantly ($p<0.05$) for a parameter.

Table. 5: Therapeutic effect of clindamycin on oxidant-antioxidant status in naturally infected *Babesia gibsoni* infection in dogs

Parameters	Group	Days			
		0	7	14	21
TAS (mM)	Gr. A	0.96^{aA}±0.03	0.99^{aA}±0.03	1.48^{bA}±0.08	1.75^{c}±0.07
	Gr. B	0.91^{aA}±0.01	0.96^{aA}±0.02	1.48^{bA}±0.03	1.81^{c}±0.03
	Control	2.05^{B}±0.20	2.05^{B}±0.20	2.05^{B}±0.20	2.05±0.20
SOD (U/mL)	Gr. A	0.40^{aA}±0.02	0.42^{aA}±0.02	0.73^{bAB}±0.09	0.95^{c}±0.08
	Gr. B	0.39^{aA}±0.02	0.42^{aA}±0.02	0.61^{bA}±0.05	0.82^{c}±0.07
	Control	0.92^{B}±0.12	0.92^{B}±0.12	0.92^{B}±0.12	0.92±0.12
GSH (µM)	Gr. A	0.47^{aA}±0.01	0.49^{aA}±0.01	0.61^{bA}±0.02	0.68^{cAB}±0.02
	Gr. B	0.50^{A}±0.01	0.53^{A}±0.01	0.62^{A}±0.03	0.51^{A}±0.11
	Control	0.77^{B}±0.03	0.77^{B}±0.03	0.77^{B}±0.03	0.77^{B}±0.03
LPO (nM)	Gr. A	0.07^{aB}±0.01	0.07^{aB}±0.01	0.04^{b}±0.01	0.03^{b}±0.01
	Gr. B	0.07^{cB}±0.00	0.07^{cB}±0.00	0.05^{b}±0.00	0.04^{a}±0.00
	Control	0.03^{A}±0.01	0.03^{A}±0.01	0.03±0.01	0.03±0.01

Values with different superscripts within the rows (a, b, c) and columns (A.B.C) differ significantly ($p<0.05$) for a parameter.

0 in both the infected groups as compared to healthy dogs (Table 5). The TAS, SOD and GSH levels gradually increased on day 7 post-treatment onward. LPO was significantly ($p<0.01$) decreased on day 14 post-treatment and returned to the normal range comparable with the healthy control group (Table 5).

In recent years, many studies have focused on the assessment of the potential role of ROS in the pathogenesis of various parasitic infections. They often indicate that infections caused by various parasites are associated with a significant increase in lipid peroxidation (Crnogaj *et al.,* 2010). In the present study, LPO concentrations were significantly increased in dogs with babesiosis in comparison to the healthy group. Similarly, increases in LPO have also been reported in research conducted by other authors on dogs suffering from *B. canis* (Crnogaj *et al*., 2010) and *B. gibsoni* (Chaudhuri *et al.,* 2008). This increase in LPO concentrations might be an indication that oxidative stress plays a role in the pathogenesis of babesiosis in dog.

SOD is an antioxidant enzyme, known for its ability to scavenge toxins from body. In this study, SOD activity showed a significant decrease compared to control group. SOD plays an integral part in RBCs protection system against oxidative damage (Rezaei and Dalir-Naghadeh, 2006). Presence of haemoprotozoan inside RBCs considerably affects the key antioxidant system of red cells, leading to a state in which red cells bump into oxidant mediators' assault, which eventually will cause cell injury and hemolysis (Nazifi *et al.,* 2008). Chaudhuri *et al*. (2008) reported that SOD and catalase activities exhibit a significant rise in dogs naturally infected with *Babesia gibsoni*. They concluded that the increased level of SOD during parasitemia could be due to the high percentage of reticulocytes in the infected dogs, since the activity of enzyme is higher in reticulocytes than in mature erythrocytes (Yamasaki *et al*., 2008). Reduction in the level of TAC may probably be ascribed to the consumption of antioxidant enzymes as free radical scavengers during the oxidative process in natural *B. gibsoni* infection in dogs. All the levels were significantly ($p<0.05$) improved after therapy. Clindamycin stimulates humoral and cellular immunity against *Babesia* infection and results in improvement in oxidant and antioxidant parameters (Hwang *et al*., 2010). Wulansari *et al*. (2003) reported that clindamycin, a dose-dependent antibiotic with the properties of immune-enhancing ability, inactivated or damaged *B. gibsoni* organisms in infected dogs. Clindamycin which is a type of lincomycin-derived antibiotic stimulates both cellular and humoral immunity by damaging *Babesia gibsoni* and is effective against babesiosis (Dutta and Lodh, 2013).

Conclusion

The study demonstrated that clindamycin is an effective treatment for *B. gibsoni* infection without concurrent side effects. Therefore, the purpose and strategy of *B. gibsoni* infection therapy, rather than to quickly eliminate the parasites from hosts, have been to suppress the proliferation of parasites without any adverse side effects. Based on the results of this study, clindamycin appears to be an effective therapeutic regimen against *B. gibsoni* infection in dogs.

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