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Comparative efficacy of two antibabesial treatment protocols against canine babesiosis in and around Navsari, Gujarat

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Abstract
Canine babesiosis is an emerging tick borne infectious disease with wide range of clinical manifestations from subclinical to life-threatening conditions. Considering the importance of disease in canines, the present study was carried out to evaluate the comparative efficacy of two antibabesial treatment protocols against canine babesiosis in and around Navsari during a period from October 2022 to June 2023. Out of 1040 cases of dogs registered at Veterinary Clinical Complex, Veterinary College, Navsari, 52 cases were suspected for canine babesiosis based on clinical symptoms and haematological findings. Simultaneously, confirmation was made by commercially available rapid antibody diagnostic test kit. Of 29 confirmed cases, 12 cases were randomly distributed to two treatment protocols (6 each) under Group-II (*Imidocarb dipropionate*) and Group-III (Modified MCD protocol using combination of metronidazole, clindamycin and doxycycline), whereas, six healthy dogs (Group-I) were kept as control. Various haemato-biochemical and vital parameters were compared between and within a treatment groups at different time intervals. Therapeutic efficacies of both treatment groups were estimated based on percentage of reduction (PR) in clinical score. Overall incidence of canine babesiosis was 2.79 percent. The incidence of canine babesiosis among suspected cases was 55.77 percent. Important haemato-biochemical and vital parameters showed improvement towards normalcy in both treatment groups on day 14. The clinical scores of Group-II and Group-III were significantly reduced from day 0 to day 14. Based on percent reduction of clinical score, better treatment response was observed in Group-III (81.02%) with modified MCD protocol than Group-II (75.19%) with Inj. Imidocarb dipropionate.

Keywords: Canine babesiosis, Imidocarb, Modified MCD protocol

Introduction
Canine babesiosis is an emerging tick borne infectious disease caused by parasites of the genus *Babesia*, belonging to the family *Babesiidae* (Karasova *et al*., 2022) [10]. It can infect a wide variety of domestic and wild animals as well as humans (Schnittger *et al*., 2012) [24]. This disease in dogs is caused by two groups of the *Babesia* spp., *viz.*, small (1.0-2.5 µm) and large (2.5-5.0 µm) sized parasites. Among these, *B. canis* and *B. gibsoni* are the most frequently found *Babesia* species in dogs. The disease is characterized by a wide range of clinical manifestations; from subclinical to life-threatening conditions. Clinically, the disease is characterized by various types of anaemia, pyrexia, anorexia, icterus, tachypnoea, tachycardia, splenomegaly with or without multiple organ dysfunctions (Mittal *et al*., 2019) [18]. The variable prevalence of canine babesiosis was observed in all over the world. In India, state wise prevalence varies from 6-64% with geographic location (Kumar *et al*., 2015; Mahalingaiah *et al*., 2017) [12, 15]. Particularly in Gujarat, Bilwal (2016) [4] and Murabiya *et al*. (2018) [19] reported the overall prevalence of canine babesiosis 30.38% and 16.27%, respectively. The incidence of canine babesiosis depends on various factors including the species of *Babesia* involved, age, breed, concurrent infections, immune and physiological status of the host (Neelawala *et al*., 2021) [20]. Since beginning, microscopic blood smear examination is considered as gold standard method for diagnosis of haemoprotozoan infections and later on conventional PCR was used for confirmatory diagnosis (Reddy *et al*., 2016) [22]. But, the cases caused by the small sized organism and/or relatively low or intermittent parasitemia in chronically infected or subclinical carrier dogs results into lower incidence of canine babesiosis based on these techniques (Mittal *et al*., 2019) [18].

Therefore, various sero-diagnostic tests have been developed for diagnosis of the disease, such as the indirect fluorescent antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA) and the immunochromatographic test (ICT) (Verdida *et al*., 2005) [27] with native or recombinant proteins as antigens. Due to high specificity and sensitivity of ICT with advantage of being simple, economical, rapid and easy to manipulate even in laboratory or field conditions, it is being used for on spot rapid diagnosis (Luo *et al*., 2012) [14].

Standard and classic treatment with diminazene aceturate fails to consistently eliminate the organism from the bloodstream. Later on, Imidocarb dipropionate was used as a drug of choice for the large piroplasms (Weingart *et al.*, 2023) [28] with effect against *Ehrlichia canis* (Mathe *et al*., 2006) [17]. But, both drugs are toxic to kidney, brain and liver (Kaur *et al.*, 2022) [11]. Now-a-days, mono and/or combination therapy including drugs such as clindamycin, atovaquone, azithromycin, metronidazole, doxycycline and enrofloxacin appears to be effectively ameliorating clinical signs and prevents re-occurrence of the disease (Almendros *et al*., 2020) [1]. Considering these facts, the present study was planned to evaluate the efficacy of two antibabesial treatment protocols in dogs.

Materials and Methods

A total 1040 dogs were registered at Veterinary Clinical Complex (VCC), College of Veterinary Science & A. H., Kamdhenu University, Navsari over a period from October, 2022 to June, 2023 for various purposes. Of which, 52 dogs were suspected for canine babesiosis based on clinical symptoms and haematological findings such as high body temperature, pale mucous membrane, vomiting, yellow coloured urine, distended abdomen/ascites, anaemia, leucopenia and/or thrombocytopenia *etc*.. The commercially available test kit (®PetX Rapid Test Kit) was procured to detect the presence of antibodies against *Babesia canis* (BC Ab) and *Babesia gibsoni* (BG Ab) in dog's serum, plasma or whole blood and the test was performed as per protocol given by the manufacturer. Among 29 confirmed cases, 12 dogs were randomly distributed to two treatment protocols (6 each) for their evaluation, whereas, six apparently healthy dogs found negative on diagnostic tests were kept as control as per the details given below:

Table 1: Therapeutic Protocol

Group	Therapeutic Protocol	No. of cases
Group-I	Healthy control (No treatment given)	6
Group-II	Inj. Imidocarb dipropionate @ 6.6 mg/kg body weight subcutaneously, repeated after 14 days (if required) along with symptomatic and supportive treatment.	6
Group-III	Modified MCD protocol: Doxycycline @ 5 mg/kg body weight OD + Clindamycin @ 22 mg/kg body weight OD + Metronidazole @ 20 mg/kg body weight OD. For first three days intravenous administration was done followed by oral administration up to two weeks.	6

Further, infected dogs in treatment Group-II and III were given following symptomatic and supportive medications as per the requirement at the dose rate as given below:

Inj. DNS/RL/NS intravenously @ 15-20 ml/kg body weight; Inj. Metoclopramide @ 0.4 mg/kg body weight intravenously or orally two times a day; Inj. Pentoprazole @ 0.5-1 mg/kg body weight intravenously or orally two times a day; Inj. Tranexamic acids @ 10 mg/kg body weight intravenously or orally two times a day; Inj. N-acetyl cysteine (NAC) @70 mg/kg body weight intravenously once a day; Vitamin-B complex injections along with oral haematinics as required; If dog having PCV < 20% and Hb < 3 gm/dl, then blood transfusion to be done depending on availability of donor.

Various haematological parameters were analyzed in automatic cell counter (MEK-6420P, Nihon Kohden India). While, blood glucose (mg/dl) was estimated using commercially available glucometer (Dr. Morepen, Gluco-one BG 03, INDIA). Whereas, various biochemical parameters were estimated using commercially available test kits in semi-automatic serum biochemical analyzer (Microlab-300, Q Line BIOTECH, Netherland).

Percentage of Reduction (PR) in clinical score

The dogs under two treatment protocols (Group-II & Group-III) were clinically examined and scored before and after treatment as per the guideline given in Table-2.

Table 2: Clinical scoring system for dogs infected with babesiosis (max. score= 28)

Clinical sign	Severity Grade			
	0	1	2	3
Appetite	Normal	Reduced	Anorexia	-
Lethargy	Absence	Reduced	Mild	Severe
Fever (>102.5°F)	Absence	102.5 – 104°F	>104°F	-
Weight loss	Absence	Reduced (<10%)	Mild (10 - 20%)	Severe (>20%)
Mucous membranes	Normal	Light (pale)	Mild (anaemic)	Severe (icteric)
Lymphadenopathy	Absence	Localized (<2 enlarged nodes)	Localized (>2 enlarged nodes)	Generalized
Urine colour	Normal	Light yellow	Dark yellow	Haemoglobinuria
Vomiting	Absence	Occasional	Frequent	Haematemesis
Diarrhoea	Absence	Occasional	Frequent	Haematochezia
Melena	Absence	-	Occasional	Frequent

The percentage reduction (PR) in clinical score after completion of treatment on 14th day was calculated as per method given by Kaur *et al.* (2022) [11] using the following equation:

$$PR = \frac{\text{Pre-treatment clinical score - Post treatment clinical score}}{\text{Pre-treatment clinical score}} \times 100$$

Thereafter, percentage reduction in clinical score between

treatment groups were compared using student 'T' test. The data pertaining to various haematobiochemical and vital parameters of dogs under different treatment protocols were analysed by analysis of variance (ANOVA) and significant mean differences were analysed using Duncan's multiple range test (DMRT) in IBM SPSS statistical software version 20.0

Results and Discussion

Of 1040 registered cases, 52 cases were suspected based on based on clinical symptoms and haematological findings such as high body temperature, pale mucous membrane / anaemia, leucopenia and/or thrombocytopenia *etc.* Among suspected cases, 29 dogs were found positive on rapid antibody diagnostic test with 2.79% overall incidence of canine babesiosis. Of which, 12 positive dogs were randomly divided into two treatment protocols (6 each) *i.e.* Group-II and Group-III. As per the details given in Table-3a and 3b, various haemato-biochemical parameters were compared between and within treatment groups including healthy dogs under control group.

Table 3a: Comparison of various haemato-biochemical parameters between healthy and different treatment groups at different time intervals (n=6)

Sr. No.	Parameters	Time interval	Treatment groups			*p* value
			Group-I (Control)	Group-II	Group-II	
1.	Hb (g/dl)	0 Day	12.6 ± 0.32^{a}	8.23 ± 0.50^{b}	8.62 ± 0.91^{b}	0.000**
		7 Day	12.6 ± 0.32^{a}	8.88 ± 0.50^{b}	9.32 ± 0.63^{b}	0.000**
		14 Day	12.6 ± 0.32^{a}	9.63 ± 0.54^{b}	9.87 ± 0.71^{b}	0.003**
		p value	--	0.188	0.522	--
2.	TEC ($\times10^{6}$/µl)	0 Day	6.02 ± 0.24^{a}	4.45 ± 0.21^{b}	4.31 ± 0.31^{by}	0.000**
		7 Day	6.02 ± 0.24^{a}	4.94 ± 0.31^{b}	$5.48\pm0.30a^{bx}$	0.049*
		14 Day	6.02±0.24	5.37±0.36	5.78 ± 0.28^{x}	0.325
		p value	--	0.122	0.007**	--
3.	PCV (%)	0 Day	39.68 ± 0.86^{a}	27.16 ± 1.55^{b}	28.84 ± 3.52^{b}	0.003**
		7 Day	39.68 ± 0.86^{a}	28.80 ± 1.58^{b}	32.14 ± 2.47^{b}	0.002**
		14 Day	39.68 ± 0.86^{a}	32.09 ± 1.91^{b}	33.19 ± 1.69^{b}	0.007**
		p value	--	0.144	0.500	--
4.	TLC ($\times10^{3}$/µl)	0 Day	11.60 ± 1.80^{b}	19.85 ± 2.15^{ax}	19.65 ± 4.06^{a}	0.047*
		7 Day	11.60 ± 1.80^{b}	15.37 ± 0.77^{ay}	16.05 ± 1.07^{a}	0.006**
		14 Day	11.60±1.80	13.47 ± 1.16^{y}	14.13±0.63	0.148
		p value	--	0.023*	0.301	--
5.	Neutrophils (%)	0 Day	68.33 ± 1.99^{b}	73.00 ± 3.09^{abx}	78.00 ± 1.51^{ax}	0.031*
		7 Day	68.33±1.99	71.33 ± 1.65^{xy}	72.33 ± 1.33^{y}	0.247
		14 Day	68.33±1.99	65.17 ± 1.64^{y}	68.50 ± 2.20^{y}	0.420
		p value	--	0.047*	0.005**	--
6.	Lymphocytes (%)	0 Day	27.5 ± 1.95^{a}	23.00 ± 3.49^{aby}	19.50 ± 1.34^{by}	0.048*
		7 Day	27.5±1.95	25.33 ± 1.93^{xy}	24.17 ± 1.25^{xy}	0.410
		14 Day	27.5±1.95	32.17 ± 1.74^{x}	28.67 ± 2.06^{x}	0.234
		p value	--	0.035*	0.004**	--
7.	Monocytes (%)	0 Day	2±0.00	2.00±0.37	1.17±0.31	0.077
		7 Day	2±0.00	1.67±0.33	1.83±0.40	0.741
		14 Day	2±0.00	1.33±0.21	1.67±0.42	0.255
		p value	--	0.342	0.454	--
8.	Eosinophils (%)	0 Day	2.17±0.17	2.00±0.37	1.33±0.21	0.090
		7 Day	2.17±0.17	1.67±0.33	1.67±0.33	0.391
		14 Day	2.17±0.17	1.33±0.21	1.17±0.17	0.053
		p value	--	0.342	0.371	--
9.	Platelet counts ($\times10^{3}$/µl)	0 Day	314.87 ± 16.88^{a}	165.33 ± 11.30^{by}	126.33 ± 10.62^{by}	0.000**
		7 Day	314.87 ± 16.88^{a}	216.17 ± 13.62^{bx}	217.83 ± 9.83^{bx}	0.000**
		14 Day	314.87 ± 16.88^{a}	220.50 ± 8.05^{bx}	237.83 ± 11.98^{bx}	0.000**
		p value	--	0.006**	0.000**	--
10.	MCV (fl)	0 Day	66.18±1.36	60.92±1.31	59.55±8.33	0.615
		7 Day	66.18±1.36	58.51±1.72	58.85±3.99	0.099
		14 Day	66.18±1.36	59.97±1.76	57.66±2.40	0.067
		p value	--	0.578	0.406	--

Means with different superscript a, b and c along a raw differ significantly at $p<0.05$
Means with different superscript x, y and z along a column differ significantly at $p<0.05$
* indicates significant at $p<0.05$ ** indicates highly significant at $p<0.01$

Table 3b: Comparison of various haemato-biochemical parameters between healthy and different treatment groups at different time intervals (n=6)

Sr. No	Parameters	Time interval	Treatment groups			*p* value
			Group-I (Control)	Group-II	Group-II	
11.	MCH (pg)	0 Day	20.10±0.37	18.45±0.38	20.16±2.02	0.343
		7 Day	20.10±0.37^{a}	18.04±0.48^{b}	17.02±0.69^{b}	0.000**
		14 Day	20.10±0.37^{a}	18.01±0.49^{b}	17.09±0.90^{b}	0.001**
		p value		0.749	0.200	
12.	MCHC (g/dl)	0 Day	31.75±0.28	30.30±0.39	30.43±1.71	0.558
		7 Day	31.75±0.28^{a}	30.85±0.26^{ab}	29.23±0.97^{b}	0.030*
		14 Day	31.75±0.28^{a}	30.06±0.42^{b}	29.58±0.64^{b}	0.014*
		p value		0.320	0.766	
13.	Glucose (mg/dl)	0 Day	98.89±2.14^{a}	57.80±3.36^{bz}	60.98±4.72^{by}	0.000**
		7 Day	98.89±2.14^{a}	81.65±4.60^{by}	73.84±4.17^{by}	0.001**
		14 Day	98.89±2.14^{ab}	106.81±4.77^{ax}	87.93±4.16^{bx}	0.012*
		p value		0.000**	0.002**	
14.	SGPT (IU/L)	0 Day	40.46±1.58^{b}	289.13±26.64^{ax}	324.67±32.99^{ax}	0.000**
		7 Day	40.46±1.58^{b}	191.87±16.38^{ay}	195.38±24.76^{ay}	0.000**
		14 Day	40.46±1.58^{b}	136.94±15.36^{ay}	128.05±21.45^{ay}	0.001**
		p value		0.000**	0.000**	
15.	SGOT (IU/L)	0 Day	32.76±2.21^{b}	54.20±2.99^{ax}	52.19±4.04^{ax}	0.000**
		7 Day	32.76±2.21^{b}	46.13±1.83^{axy}	45.71±1.87^{axy}	0.000**
		14 Day	32.76±2.21^{b}	39.43±3.17^{aby}	41.94±2.43^{by}	0.038*
		p value		0.006**	0.043*	
16.	ALP (IU/L)	0 Day	67.72±1.24^{a}	237.40±16.13^{bx}	244.07±24.06^{bx}	0.000**
		7 Day	67.72±1.24^{a}	191.23±16.42^{bx}	170.51±10.56^{by}	0.000**
		14 Day	67.72±1.24^{a}	143.66±9.77^{by}	139.25±9.86^{by}	0.000**
		p value		0.001**	0.001**	
17.	Total protein (g/dl)	0 Day	6.05±0.34	6.29±0.23	5.97±0.07	0.620
		7 Day	6.05±0.34	6.69±0.26	6.36±0.14	0.247
		14 Day	6.05±0.34	6.67±0.24	6.68±0.22	0.202
		p value		0.447	0.210	
18.	Albumin (g/dl)	0 Day	4.27±0.21^{a}	2.00±0.40^{by}	2.09±0.37^{by}	0.000**
		7 Day	4.27±0.21^{a}	2.29±0.20b^{xy}	2.67±0.24^{bxy}	0.000**
		14 Day	4.27±0.21^{a}	3.12±0.18^{bx}	3.02±0.09^{bx}	0.000**
		p value		0.033*	0.046*	
19.	BUN (mg/dl)	0 Day	21.13±1.02^{b}	107.68±5.58^{ax}	97.49±17.49^{ax}	0.000**
		7 Day	21.13±1.02^{b}	86.42±8.66^{ax}	63.25±10.61^{ax}	0.000**
		14 Day	21.13±1.02^{b}	29.13±3.70^{ay}	20.09±1.89^{by}	0.040*
		p value		0.000**	0.001**	
20.	Creatinine (mg/dl)	0 Day	0.88±0.05^{b}	2.14±0.20^{a}	2.09±0.16^{ax}	0.000**
		7 Day	0.88±0.05^{b}	1.88±0.13^{a}	1.91±0.25^{ax}	0.001**
		14 Day	0.88±0.05^{c}	1.70±0.09^{a}	1.17±0.08^{by}	0.000**
		p value		0.145	0.045*	

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Means with different superscript x, y and z along a column differ significantly at $p<0.05$
* indicates significant at $p<0.05$ ** indicates highly significant at $p<0.01$

Haematological parameters: Initially, the mean haemoglobin, total erythrocyte count, packed cell volume and platelet count in both treatment group was significantly lower than corresponding means of control group ($p<0.01$) indicated macrocytic and hypochromic anaemia and thrombocytopenia. Similarly, moderate to severely decreased values of Hb, TEC, PCV and platelet count in Babesiosis infected dogs under the different treatment groups than corresponding reference values were recorded by scientists (Bilwal, 2016; Chauhan *et al.*, 2022; Kaur *et al*., 2022) [4, 5, 11]. Such decreased Hb, TEC and PCV levels in infected dogs might be due to direct mechanical disruption of RBCs by parasite and mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes leading to intravascular hemolysis which releases reactants in circulation further initiate immune or non-immune mediated destruction of red blood cells results into severe anemia (Niwetpathomwat *et al*., 2006) [21]. While, the pathogenesis of thrombocytopenia during Babesiosis may be due to platelet sequestration in the spleen or immune mediated platelet destruction and development of disseminated intravascular coagulation (Gonmei *et al*., 2020) [8]. Further, the findings of increased TLC in both treatment groups than control is in agreement with previous reports (Bilwal, 2016; Gonmei *et al.*, 2020; Anju *et al*., 2022) [4, 8, 2]. On contrary, two reports mentioned TLC within normal range (Roopali *et al.*, 2018) [23]. In differential leukocytic count, neutrophilia was observed in *Babesia* infected dogs under both treatment groups along with comparative lymphopenia. Such findings are in agreement with previous reports (Bilwal 2016; Gonmei *et al.*, 2020; Anju *et al*., 2022) [4, 8, 2]. Other blood parameters showed non-significant alterations in *Babesia* infected dogs under both treatment groups.

Biochemical parameters: As a result of disease, various biochemical parameters such as SGOT, SGPT, ALP, BUN, and Creatinine were found significantly increased in dogs infected with babesiosis under different treatment groups ($p<0.01$). Similarly, reports mentioned significantly increased value of

SGPT, SGOT and ALP in *Babesia* infected dogs as compared to healthy dogs (Bilwal, 2016; Gonmei *et al*., 2020) [4, 8]. The increased values of above enzymes might be due to centrilobular hepatitis with hypoxic liver damage or damage to hepato-biliary system (Sindhu *et al*., 2020) [26]. Similar to our findings, significant increased values of BUN and creatinine in *Babesia* infected dogs than healthy dogs was also reported previously (Bilwal, 2016; Roopali *et al*., 2018; Anju *et al*., 2022; Kaur *et al*., 2022) [4, 23, 2, 11]. The sequential renal damage caused by inflammatory mediators in dogs with babesiosis leads to reduced renal tissue perfusion and glomerular filtration results elevated level of BUN and creatinine (Sindhu *et al*., 2020) [26]. Further, the mean values of total protein and albumin in *Babesia* infected dogs were found lower than control at day 0. Similarly, some reports also recorded significantly decreased values of total protein and albumin in dogs with babesiosis as compared to healthy dogs (Bilwal, 2016; Roopali *et al*., 2018; Gonmei *et al*., 2020; Sindhu *et al*., 2020) [4, 23, 8, 26]. The hepatopathy effects of babesiosis cause disruption of liver function which results into decrease albumin synthesis and consequently affect total protein levels (Gonmei *et al*., 2020; Sindhu *et al*., 2020) [8, 26]. The blood glucose was also found significantly lower in *Babesia* infected dogs as compare to control (p<0.01) as also reported by Sindhu *et al*. (2020) [26] and stated that the hypoglycemia in *Babesia* infected dogs might be due to anorexia, impaired hepatic function and increase glucose breakdown system (glycolysis).

Table 4: Pre and post treatment comparison of vital parameters (Mean±SE) between healthy and treatment groups (n=6)

Sr. No.	Parameters	Time interval	Treatment groups			*p* value
			Group-I (Control)	Group-II	Group-II	
1.	Rectal temp.(°F)	Pre	101.98±0.23^{b}	103.88±0.27^{ax}	104.05±0.49^{ax}	0.001**
		Post	101.98±0.23	101.85±0.08^{y}	102.1±0.13^{y}	0.542
		p value	--	0.001**	0.007**	--
2.	Heart rate (beats/min)	Pre	120.00±2.22^{b}	143.67±2.93^{ax}	147.67±4.29^{ax}	0.000**
		Post	120.00±2.22	121.5±6.56^{y}	123.83±5.29^{y}	0.864
		p value	--	0.041*	0.009**	--
3.	Respiration rate (breaths/min)	Pre	24.33±1.05^{b}	56.00±2.19^{ax}	55.33±6.22^{ax}	0.000**
		Post	24.33±1.05	24.5±2.55^{y}	28.17±1.99^{y}	0.324
		p value	--	0.000**	0.009**	--
4.	CRT (seconds)	Pre	1.50±0.18^{b}	2.83±0.31^{ax}	2.5±0.22^{a}	0.004**
		Post	1.50±0.18	1.92±0.20^{y}	1.83±0.31	0.440
		p value	--	0.020*	0.102	--
5.	Pulse rate (per minute)	Pre	121.83±1.92^{b}	158.33±7.09^{a}	151.67±8.32^{a}	0.002**
		Post	121.83±1.92	134.33±6.70	135.67±10.85	0.280
		p value	--	0.121	0.752	--

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Means with different superscript x, y and z along a column differ significantly at p<0.05
* indicates significant at p<0.05 ** indicates highly significant at p<0.01

Vital parameters: Before the initiation of treatment, various vital parameters were recorded and compared with control group and their results are given in Table-4. The mean values of rectal temperature, heart rate, pulse rate and respiration rate in *Babesia* infected dogs was observed significantly higher than control (p<0.01) before treatment. The results are in accordance with findings of Bilwal (2016) [4] and Kaur *et al.* (2022) [11] who had also recorded significantly higher rectal temperature, heart rate, pulse rate and respiration rate in dogs with Babesiosis than healthy dogs. The production of cytokines and other vasoactive amines due to presence of parasites in the blood stream as foreign bodies might be the cause of fever or hyperthermia. The elevated heart rate and pulse rate in babesiosis infected dogs than healthy dogs might be due to anaemia and compensatory mechanism to pump more and more blood to maintain normal oxygen and nutritional support to tissue. The increase in respiration rate in infected dogs could be due to compensate increased oxygen demand in body as a result of reduced oxygen-caring capacity due to haemolysis in canine babesiosis. In *Babesia* infected dogs, the mean value of capillary refill time (CRT) was found also significantly higher than control (p<0.01). Only a single available report indicating non-significant increased capillary refill time in *Babesia* infected dogs than healthy dogs (Bilwal, 2016) [4] which might be due to impairment of the blood circulation and reduced tissue perfusion as a result of anemia and dehydration.

As a result of treatment of *Babesia* infected dogs under different treatment groups, altered haemato-biochemical and vital parameters showed improvement towards normalcy on day 14 but still did not come within normal range. Similarly, the improvement in altered haemato-biochemical and vital parameters towards normalcy was also reported by Lin and Huang (2010) [13], Chauhan *et al.* (2022) [5] and Kaur *et al.* (2022) [11] while evaluating different therapeutic protocols in Babesiosis infected dogs.

Therapeutic response: The therapeutic response to each treatment was assessed by examining changes in clinical score before (day 0) and after (day 14) treatment as percentage reductions (PR) score given in Table-5.

Table 5: Evaluation of therapeutic protocols based on percentage reduction (PR) of clinical score in *Babesia* infected dogs

Groups		Clinical Score		Percent Reduction (%)	*p*-value
		Before	After		
II	Total	118	43	75.19%	0.000**
	Mean±SE	21.5±0.76	5.33±0.49		
III	Total	123	39	81.02%	0.000**
	Mean±SE	22.83±0.87	4.33±0.49		

** indicates highly significant at p<0.01

Number of scientists evaluated different treatment protocols in

dogs infected with canine babesiosis. Earlier, while studying effect of doxycycline-enrofloxacin-metronidazole in combination with/without diminazene diaceturate to treat naturally occurring canine babesiosis, Lin and Huang (2010) [13] found 85.7% and 83.3% overall efficacy of a combination of doxycycline-enrofloxacin-metronidazole with and without administration of diminazene diaceturate, respectively and concluded that concomitant use of intramuscular diminazene diaceturate may not improve the efficacy of a doxycycline-enrofloxacin-metronidazole combination in management of canine babesiosis caused by *B. gibsoni*. Later on, Gonde (2014) [7] reported maximum efficacy (76.92%) in diminazene aceturate with oral combination of doxycycline-enrofloxacin-metronidazole (D-E-M) followed by diminazene aceturate (62.50%), single dose of long acting enrofloxacin with oral combination of doxycycline- metronidazole (50%) and oxytetracycline (50%). In an another study, Eldakhly (2021) [6] reported higher treatment efficacy in Group-I (56.2%) treated with Metronidazole @ 15mg/kg body weight orally q12h and Doxycycline @ 5mg/kg body weight orally q12h than Group-II (37.5%) treated with Quinine @ 30mg/kg body weight orally q12h with Clindamycin @ 25mg/kg body weight orally q12h for seven days. Chauhan *et al.* (2022) [5] also studied comparative therapeutic efficacy of Imidocarb and Diminazene aceturate against *Babesia gibsoni* in dogs. On the basis of clinical recovery and restoration of haemato-biochemical parameters, the best therapeutic response was observed in group T2 were treated with doxycycline @ 5mg/kg body weight orally q12 h for 30 days, metronidazole @ 15 mg/kg body weight orally q12 h for 30 days and Imidocarb @ 6.6 mg/kg body weight subcutaneously two doses 14 days apart than group T3 treated with doxycycline @ 5mg/kg body weight orally q12 h for 30 days, metronidazole @ 15 mg/kg body weight orally q12 h for 30 days and diminazene aceturate @ 5 mg/kg body weight intramuscularly two doses 48 h apart. While evaluating three different therapeutic protocols in dogs infected with *B. gibsoni*, Kaur *et al.* (2022) [11] also monitored the clinical response to treatment by examining changes in clinical score. They observed maximum reduction of clinical score in Group-III (Diminazene aceturate, Imidocarb dipropionate and Clindamycin) (>80%) followed by Group-II (Diminazene aceturate) (65.3%) and Group-I (Imidocarb dipropionate) (51.1%) and reported highest efficacy of Group-III in terms of remission of clinico-pathologic abnormalities.

Various reports showed complete clinical recovery and negative blood smear for presence of *Babesia* organism on use of single or two doses of Inj. Imidocarb dipropionate @ 6-6.6 mg/kg body weight intramuscularly or subcutaneously with supportive treatment with or without side effects like sialoree, vomiting and general weakness (Roopali *et al.*, 2018; Martinescu *et al.*, 2021) [23, 16].

Based on previous reports, two treatment protocols were evaluated during the present study. The clinical scores in Group-II and Group-III were significantly reduced to 43 (75.19%) and 39 (81.02%), respectively, on 14th day of treatment. Based on percent reduction of clinical score, better treatment response was observed in Group-III with modified MCD protocol than Group-II with Inj. Imidocarb dipropionate. Unfortunately, there was a death of one dog from Group-II treated with Imidocarb dipropionate after 20th day of treatment with multiple organ dysfunction syndrome (MODS) which might be a reason for reduced clinical score in treatment Group-II as compared to Group-III. Previously, various scientists used MCD protocol in cases of canine babesiosis with or without other drugs and found significant improvement in the various haematological parameters towards normalcy with clinical recovery and effective parasite clearance without history of relapse for longer period of time (Hyung-Mo *et al*., 2019; Almendros *et al*., 2020; Yadav *et al*., 2011) [9, 1, 29].

Baneth (2018) [3] stated that Imidocarb is having better treatment effect with direct action against the parasite DNA that causes unwinding and denaturation of large *Babesia* spp. but it is less effective against small *Babesia* spp. and toxic effects. Whereas, among the drugs used in MCD protocol, Clindamycin is an immune enhancing lincosamide antibiotic which inhibits bacterial growth by hindering the RNA-dependent protein synthesis and could known to eliminate *Babesia* organisms from the peripheral blood and reduce clinico-pathological alterations in *Babesia* infected dogs (Kaur *et al.*, 2022) [11]. While, Doxycycline is one of the tetracycline antibiotics having a prophylactic effect against large *Babesia* spp. and metronidazole is one of the anti-trichomonal agents having therapeutic effect against large *Babesia* spp. Combination of these drugs suppresses parasitemia effectively and resistance had not been reported yet (Hyung-Mo *et al.*, 2019) [9]. The incidence of canine babesiosis was comparatively higher in middle aged dogs (3-6 years), mainly in most commonly adopted breed (Labrador retriever) in a population under the study. The disease was more prevalent in males and in hot and humid period of summer season of south Gujarat, which favours seasonal activity of the brown dog ticks that spread the disease.

Conclusion

In a present study, canine babesiosis resulted into macrocytic and hypochromic anaemia, thrombocytopenia, splenomegaly and hepatomegaly with decreased haemoglobin and platelet count and increased liver and kidney enzymes. Both treatment groups showed improvement in all altered haemato-biochemical and vital parameters towards normalcy. Based on percent reduction of clinical score, better treatment response was observed in dogs treated with modified MCD protocol than Inj. Imidocarb dipropionate.

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