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Sandeep
Post-Graduation Student, Department of Medicine Veterinary, College Hebbal, Bengaluru, Karnataka, India

Basavaraj Vadari
Post-Graduation Student, Department of Parasitology Veterinary, College Hebbal, Bengaluru, Karnataka, India

Corresponding Author:
Sandeep
Post-Graduation Student, Department of Medicine Veterinary, College Hebbal, Bengaluru, Karnataka, India

Therapeutic management of canine babesiosis

Sandeep and Basavaraj Vadari

Abstract
The present study was conducted at Department of Veterinary Medicine, Veterinary College Hospital, Hebbal, Bengaluru with an objective of studying the incidence of canine babesiosis by blood smear examination and to compare efficacy of different treatment regimen. The study was conducted for a period of six months from July 2022 to Dec 2022. During this study period incidence rate accounts to 1.55 per cent (298/19,166) by microscopic examination. In the clinical trial, all the four-treatment regimen were found to be effective in treatment of canine babesiosis. But on comparison of all the four treatments, treatment regimen III *i.e.*, {Berenil + triple drug therapy (doxycycline-clindamycin -metronidazole) and *Calotropis procera*} was found to be more efficacious than other three groups which indicates that inclusion of *Calotropis procera* as an additive has got a beneficial effect in the treatment of canine babesiosis. As regards to the treatment regimen V where Buparvoquone is replaced by *Calotropis procera*, the treatment may require to be continued for some more days for complete recovery and restoration of haemto-biochemical parameters to normalcy.

Keywords: Babesiosis, doxycycline, caleotropies procera, clindamycin, metroidazole, *Babesia canis vogeli*, *Babesia gibsoni* and *B. canis*

Introduction
Canine babesiosis, a tick borne protozoan disease caused by apicomplexan parasites of the genus *Babesia*, is characterized by fever, anemia and haemoglobinuria. *Babesia gibsoni* and *B. canis* comprise the two main species causing natural infections in dogs in a widespread geographic distribution including India. The latter is grouped into three phylogenetic groups and they vary in their geographical distribution, vector specificity and antigenic properties. These subspecies include *Babesia canis canis*, found in Europe; *Babesia canis vogeli*, in North and South Africa, North America and Brazil; and *B. canis rossi*, in South Africa (Uilemberg *et al.* 1989; Carret *et al*. 1999; Caccio *et al.* 2002; Matijatko, *et al.* 2009) [22, 1, 24, 9]. The clinical manifestation of the disease varies from subclinical to fatal depending upon pathogenicity of *Babesia* spp. (Solano-Gallego and Baneth 2011) [20]. According to Shah *et al*. (2011) [16], *Rhipicephalus sanguineus* and *Haemaphysalis longicornis* are the putative vectors of *B. canis vogeli* and *B. gibsoni* respectively in India. In India, tick borne diseases have been diagnosed by traditional methods using microscopic observation of the organism in blood smears which, however, does not permit reliable identification of the species. Molecular evidence of *B. canis vogeli and B. gibsoni* were reported from different cities of India including Delhi, Mumbai, Sikkim and Ladakh. In South India.

Material and Methods
A dogs with clinical signs suggestive of babesiosis presented to the Department of Veterinary Medicine, Veterinary College Hospital, Hebbal, Bengaluru with a history of anorexia, tick infestation, lethargy, weakness and red coloured urine (haemoglobinuria) were included for the study.

Collection of blood sample for haematological and microscopic examination and Biochemistry estimation
Two ml of whole blood sample was collected from the cephalic/saphenous vein in EDTA coated vacutainer tube (M/s Xline Medicals) as per the method of Ooms, (2004) [11]. Three ml of blood sample was collected from the cephalic vein/saphenous vein into serum vacutainer tube with clot activator. After 20 minutes of storage at room temperature, samples were centrifuged at 3,000 rpm for 10 minutes using high speed ultra-centrifuge and serum samples were collected and processed within 15 minutes for serum biochemistry.

Hydro-Alcoholic extraction of *Calotropis procera*

Hydro alcoholic extract was prepared according to standard procedures using the maceration method in the ratio of 60:40 and stored in an air tight bottle.

The hydro alcoholic extract was prepared by the method described by Siddiqui *et al.* (2017) [17] with slight modifications. According to this technique, the freshly prepared powder of flowers (25 g) of *Calotropis procera* was immersed in hydro-ethanol solution (40 per cent distilled water and 60 per cent ethanol) in a flask stoppered tightly with cotton plug and was kept at room temperature for 48 hours at 150 rpm in an orbital shaker. The contents of the flask were filtered through muslin cloth. The residue left in the flask was rinsed with a small quantity of hydro-alcoholic solvent and filtered through the muslin cloth. The filtrate thus obtained was filtered through Whatman No. 1 filter paper. The final filtrate, so obtained was transferred to a previously weighed large petri dish and was kept for evaporation of solvent at room temperature. After complete evaporation, the petri dish was once again weighed to determine the amount of extract and per cent extractability was determined. The dried extract was removed and lyophilized in a freeze drier to remove trace quantities of moisture if left. Then, freeze dried root extract was kept in air tight bottle.

Clinical trial

Dogs which were positive for babesiosis by blood smear examination, were randomly grouped into four groups of eight animals each and were treated with four different treatment regimen which are as follows:

Group I

Apparently healthy dogs which did not have any clinical symptoms suggestive of babesiosis, negative for babesiosis by microscopic examination of stained blood smears and PCR, were grouped as control group.

Group II

Animals which were positive for canine babesiosis by microscopic examination, were treated with Berenil injection containing Diminazine aceturate (70 mg/ml) at a dose rate of 3.5 mg/kg body weight administered intramuscularly along with Triple drug therapy *i.e.*, clindamycin, doxycycline and metronidazole administered at a dose rate of 25 mg/kg, 5 mg/kg and 10 mg/kg respectively b.i.d per orally for 10 days and with supportive therapy

Group III

Animals which were positive for canine babesiosis by microscopic examination, were treated with Berenil at a dose rate of 3.5 mg/kg body weight administered intramuscularly along with triple drug therapy *i.e.*, clindamycin, doxycycline and metronidazole administered at a dose rate of 25 mg/kg, 5 mg/kg and 10 mg/kg respectively *b.i.d* per orally for 10 days and hydro-alcoholic extraction of *Calotropis procera* @ 0.3 mg/kg b.i.d. per oral for 10 days and with supportive therapy.

Group IV

Animals which were positive for canine babesiosis by microscopic examination, were treated with Buparvoqone at a dose rate 2.5 mg/kg body weight administered intramuscularly along with Azithromycin at a dose rate 10 mg/kg per orally for 10 days and with supportive therapy.

Group V

Animals which were positive for canine babesiosis by microscopic examination, were treated with Azithromycin at a dose rate 10 mg/kg body weight administered per orally for 10 days along with hydro-alcoholic extraction of *Calotropis procera* at a dose rate 0.3 mg/kg b.i.d. per oral for 10 days and with supportive therapy.

The efficacy of different treatment regimen, was evaluated by reversal of clinical signs and evaluation of blood sample on 5th, 10th, 15th day of post treatment for haematological and serum biochemical parameters. The haematological and serum biochemistry results were compared with the zero-day values to evaluate therapeutic efficacy of different treatment regimen within the group and with the control group values to evaluate the therapeutic efficacy between the groups.

Statistical analysis

The data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994) [19] using Graph Pad Prism (version 8.01) statistical software by using two-way ANOVA test at 95% confidence level.

Result and Discussion

Incidence of Babesiosis in Dogs

During this study, a total of 19,166 cases were presented to Department of Veterinary Medicine, Veterinary College Hospital, Hebbal, Bengaluru, from July, 2022 to December, 2022. Three hundred and ninety five blood samples collected from dogs with clinical signs suggestive of babesiosis, were examined by microscopic examination of Giemsa-stained blood smear. Two hundred ninety eight (298) out of 19,166 cases were found positive for *Babesia* organisms accounting to an incidence of 1.55% (Table 1, Fig. 1).

Similar observation has been reported by Wadhwa *et al.* (2011) [23] who recorded an incidence of 1.34 per cent from Himachal Pradesh, Reddy *et al.* (2014) [13] who reported 2.1 per cent in Andhra Pradesh and Preena *et al.* (2021) [12] who reported an incidence of 1.11 per cent in Kerala.

Whereas Kumar *et al.* (2009) [7] in Punjab, Selvaraj *et al.* (2010) [15] in Madras, Singh *et al.* (2012) [18] in Punjab, Sahu *et al.* (2014) [14] in Bhubaneswar reported a higher incidence of 11.6, 8.7, 5.82 and 10.54 respectively, in dogs by microscopic examination of Giemsa-stained peripheral blood smear.

These variations in the prevalence of canine babesiosis could be attributed to tick population, seasonal variation, immune status of the host, other managemental practices and agroclimatic conditions in different geographical locations where highly variable conditions of temperature and humidity are known to prevail (Gonde *et al.* 2017) [4].

Table 1: Percentage of incidence of *Babesia* by blood smear examination

Methods	Total number of samples tested	Number of sample Positive	Percentage of incidence
Microscopic examination of stained blood smears	19166	298	1.55%

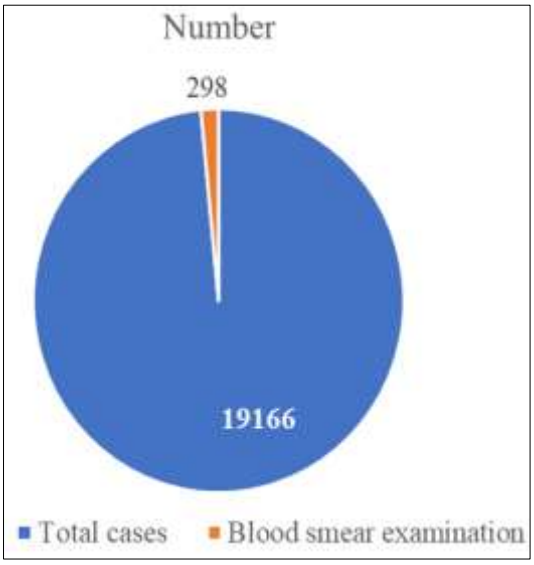

Fig 1: Percentage of incidence of *Babesia* by blood smear examination

Clinical trial

Within the group

Group II- Treatment with diminazene aceturate with oral combination of Triple drug therapy (doxycycline-clindamycin -metronidazole)

Haemato-biochemical changes

Eight dogs suffering from babesiosis were treated with diminazene aceturate with oral combination of doxycycline-clindamycin -metronidazole for 10 days**.** All the dogs showed complete clinical recovery by 15th day. On treatment, there was gradual and significant increase in the mean values of TEC, Hb, PCV, PLT and Total protein, which were observed on 5th, 10th and 15th day as compared to zero (Table 12). Significant decrease in the mean values of TLC and ALT, was observed on 5th, 10th and 15th day as compared to day zero. Total bilirubin, BUN and creatinine value also decreased on treatment though not significant. Similarly, by 15th day post treatment all the dogs of the group were almost normal and the hemato-biochemical parameters were in the normal range. Our observation is an agreement with the Lin and Huang (2010) [8], Ming and Hui (2010) [10] Suzuki *et al.* (2007) [21] and Halder and Gupta (2022) who conducted clinical trial with the above-mentioned protocol and they noticed improvement in clinical signs and hemato-biochemical parameters.

All the dogs of this group showed improvement in clinical signs and haemato-biochemical parameters, indicating that this treatment is effective and could be used for treatment of canine babesiosis.

Table 2: Mean±SE of haemato-biochemical values of dogs of Group II treated with diminazene aceturate with oral combination of Triple drug therapy (doxycycline- clindamycin -metronidazole)

Parameters	Zero day	5th day	10th day	15th day
TEC (x10^6 /µL)	2.12±0.25^a	3.12±0.24^b	4.57±0.31^c	5.65±0.19^d
TLC (x10^3 /µL)	31.13±0.39^a	26.46±0.78^b	14.35±1.03^c	10.66±0.37^d
Hb (g/dL)	4.90±0.49^a	6.99±0.46^b	10.09±0.40^c	12.54±0.30^d
PCV (%)	14.88±4.19^a	21.03±1.30^b	31.35±1.89^c	37.38±1.15^d
Platelet (x10^3 /µL)	40.63±2.04^a	83.63±6.03^b	203.13±5.53^c	229.8±4.67^d
ALT (U/L)	140.7±5.51^a	118±2.75^b	73.5±5.62^c	48±5.39^d
Creatinine (mg/dL)	1.30±0.04^a	1.24±0.05^a	1.20±0.02^a	1.15±0.06^a
BUN (mg/dL)	22.84±0.57^a	20.57±0.58^a	20.36±0.63^a	18.62±0.54^a
Total protein (g/dL)	5.33±0.09^a	5.63±0.09^a	6.08±0.05^b	6.44±0.08^c
Total bilirubin (mg/dL)	0.59±0.08^a	0.55±0.02^a	0.47±0.03^a	0.38±0.06^a

$^{a, b, c, d}$ Mean values in a row with different superscripts differ significantly ($p \leq 0.05$)

Group III-Treatment with diminazene aceturate with oral combination Triple drug therapy (doxycycline-clindamycin -metronidazole) and *Calotropis procera* in dogs with babesiosis.

Haemato-biochemical changes

Eight dogs suffering from babesiosis were treated with diminazene aceturate with oral combination of Triple drug therapy (doxycycline- clindamycin -metronidazole) and *Calotropis procera* for 10 days. By 15th day post treatment all the animals showed compete recovery. During the course of treatment, there was gradual and statistically significant increase in the mean values of TEC, Hb, PCV, PLT and Total protein which were observed on 5th, 10th and 15th day as compared to day zero (Table 13). Significant decrease in the mean values of TLC, ALT and BUN which was observed on 5th, 10th and 15th day as compared to day zero. Total bilirubin and creatinine also decreased though not statically significant. By 15th day post treatment all the hemato-biochemical parameters were in the normal range.

Thus, based on the regression of the clinical signs and improvement in haemato biochemical parameters it can be concluded that treatment with diminazene aceturate with oral combination Triple drug therapy and *Calotropis procera* for 10 days is efficacious and could be used as treatment of choice for the treatment of babesiosis of in dogs.

All the dogs show improvement in clinical signs and haemato-biochemical parameters.

Table 3: Mean±SE of haemato-biochemical values of dogs of Group III treated with diminazene aceturate with oral combination Triple drug therapy and *Calotropis procera*

Parameters	Zero day	5th day	10th day	15th day
TEC (x10^6 /µL)	1.53±0.15^a	2.90±0.17^b	4.47±0.37^c	5.69±0.29^d
TLC (x10^3 /µL)	31.43±0.43^a	27.70±0.76^b	14.51±0.33^c	10.55±0.47^d
Hb (g/dL)	4.44±0.12^a	6.53±0.24^b	9.98±0.29^c	12.89±0.39^d
PCV (%)	12.13±0.81^a	20.75±0.90^b	31.95±0.94^c	38.10±0.76^d
Platelet (x10^3 /µL)	40±2.21^a	89±1.76^b	223.13±2.70^c	252.7±1.87^d
ALT (U/L)	218.5±6.75^a	144±8.04^b	94±8.04^c	45.25±7.52^d
Creatinine (mg/dL)	1.34±0.09^a	1.23±0.03^a	1.21±0.05^a	1.11±0.09^a
BUN (mg/dL)	37.16±0.88^a	25.60±0.80^b	19.32±1.34^c	19.11±0.56^d
Total protein (g/dL)	5.30±0.08^a	5.80±0.03^b	6.09±0.05^{cb}	6.46±0.08^d
Total bilirubin (mg/dL)	0.61±0.06^a	0.58±0.04^a	0.45±0.08^a	0.39±0.09^a

$^{a, b, c, d}$ Mean values in a row with different superscripts differ significantly ($p \leq 0.05$)

Group IV- Treatment with Buparvoquone with Azithromycin in dogs with babesiosis

5.4.1.3.2 Haemato-biochemical changes

Eight dogs suffering from babesiosis were treated with Buparvoquone and Azithromycin. On treatment gradual and significant increase in the mean values of TEC, Hb, PCV and PLT which were observed on 5th, 10th and 15th day as compared to day zero (Table 14). Whereas a significant decrease in the mean values of TLC and ALT which was observed on 5th, 10th and 15th day as compared to day zero. Further a gradual increase though not significant was noticed with total protein. Total bilirubin and creatinine an apparent decrease was observed. Our observations are in agreement with the Checa *et al.*, 2017 and Halder and Gupta 2021 who conducted clinical trial with above mentioned protocol and they noticed regression of clinical signs and improvement in the hemato-biochemical parameters.

This indicate that this could be another treatment regimen for treatment of babesiosis among dogs.

Table 4: Mean±SE of haemato-biochemical values of dogs of Group IV treated with Buparvoquone with Azithromycin

Parameters	Zero day	5th day	10th day	15th day
TEC (10^6 /µL)	1.84±0.16^a	2.82±0.10^b	3.96±0.10^c	4.64±0.10^d
TLC (10^3 /µL)	29.79±0.32^a	25.49±0.64^b	15.78±0.28^c	14.35±0.2^c
Hb (g/dL)	4.80±0.11^a	6.88±0.21^b	9.08±0.20^c	10.53±0.18^d
PCV (%)	14.35±0.53^a	22.17±0.71^b	29.04±0.64^c	34.73±0.59^d
Platelet (10^3 /µL)	57.63±3.88^a	110.7±2.70^b	181.25±3.70^c	225.12±3.69^d
ALT (U/L)	132±9.64^a	104.2±5.93^b	68±8.01^c	43.75±4.89^d
Creatinine (mg/dL)	1.09±0.05^a	1.02±0.03^a	1.01±0.04^a	1.03±0.02^a
BUN (mg/dL)	21.33±0.95^a	20.30±1.60^a	19.34±0.51^a	19.12±0.42^a
Total protein (g/dL)	5.40±0.05^a	5.56±0.06^a	5.92±0.03^a	6.04±0.03^a
Total bilirubin (mg/dL)	0.49±0.04^a	0.42±0.03^a	0.39±0.02^a	0.35±0.05^a

$^{a, b, c, d}$ Mean values in a row with different superscripts differ significantly ($p \leq 0.05$)

Group V-Treatment with of *Calotropis procera* with Azithromycin in dogs with babesiosis

Haemato-biochemical changes

Eight dogs suffering from babesiosis were treated with *Calotropis procera* and Azithromycin. During the course of treatment, there is a significant increase in the mean values of TEC, Hb, PCV and PLT on 5th day when compare to day zero. On 10th and 15th day though there is an increase in the mean Hb, platelet and PCV values, significant increase is not consistent from from 5th to 10th and 10th to 15th day. There is a gradual decrease in the mean values of TLC, and ALT which was observed 5th, 10th and 15th day as compared to zero day, but significance is not consistent throughout the period of treatment. Total protein, Total bilirubin, BUN and creatinine were almost in the normal range throughout the treatment. When the mean values of all the hemato-biochemical parameters on 15th day observed, though there is improvement, the values have not reached the normal range of the species which means that treatment may required to be continued for few more days for complete recovery. Further it also indicates that *Calotropis procera* may not act as a replacement for Buparvoquone.

Table 5: Mean±SE of haemato-biochemical values of dogs of Group V treated with of *Calotropis procera* with Azithromycin

Parameters	Zero day	5th day	10th day	15th day
TEC (x10^6 /µL)	2.18±0.15^a	2.64±0.15^{ab}	2.98±0.19^b	3.30±0.16^b
TLC (x10^3 /µL)	25.20±0.56^a	22.43±0.65^b	20.80±1.04^{bc}	19.54±1.05^{cd}
Hb (g/dL)	5.18±0.24^a	6.60±0.30^b	7.34±0.27^{bc}	7.88±0.28^c
PCV (%)	15.5±1.23^a	20.36±0.89^b	22.31±0.70^{bc}	23.85±1.07^c
Platelet (x10^3 /µL)	54.63±2.67^a	74.88±1.87^b	85.88±1.83^{bc}	96.13±1.7^c
ALT (U/L)	85.50±3.52^a	74.75±5.81^{ab}	59.50±5.64^b	32.25±4.71^c
Creatinine (mg/dL)	1.04±0.03^a	1.01±0.01^a	1.03±0.02^a	1.00±0.04^a
BUN (mg/dL)	19.66±1.34^a	18.87±0.61^a	18.97±1.07^a	18.79±0.81^a
Total protein (g/dL)	5.90±0.03^a	5.98±0.06^a	5.91±0.09^a	6.09±0.15^a
Total bilirubin (mg/dL)	0.38±0.02^a	0.36±0.01^a	0.34±0.04^a	0.37±0.03^a

$^{a, b, c, d}$ Mean values in a row with different superscripts differ significantly ($p \leq 0.05$)

In the clinical trial all the four-treatment regimen were found to be effective in the treatment of canine babesiosis which is revealed by regression of clinical symptoms and improvement in the hematobiochemical values. On comparison of all the four-treatment regimen, treatment regimen III i.e., (Berenil + triple drug therapy (doxycycline- clindamycin -metronidazole) and *Calotropis procera*) was found to be more efficacious followed by treatment regimen II, IV and V. Addition/Inclusion of *Calotropis procera* with Berenil plus triple drug therapy has a beneficial effect in the treatment of canine babesiosis. Treatment regimen V where Buparvoquone is replaced by *Calotropis procera*, may required to be continued for some more days for complete recovery and restoration of haemato-biochemical parameters to normalcy. As there is paucity of information in the literature further study in this direction is required.

Conclusion

Treatment regimen III i.e., (Berenil + triple drug therapy (doxycycline- clindamycin -metronidazole) and *Calotropis procera*) was found to be more efficacious for treatment of canine babesiosis in the fiels level and more economical to use.

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