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Research Article

EVALUATION OF THREE DIFFERENT THERAPEUTIC PROTOCOLS IN DOGS INFECTED WITH *BABESIA GIBSONI*

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ABSTRACT

Current study was conducted to compare the efficacies of three treatment regimens for babesiosis in dogs. A total of 18 *Babesia gibsoni* infected dogs diagnosed based on microscopy and/or 18S PCR assay were randomly assigned to three treatments: Group I [n=6, Imidocarb dipropionate (Id) @ 6 mg/kg IM, 2 doses 14 days apart]; Group II [n=6, Diminazene aceturate (Da) @ 3.5 mg/kg IM on day 1 followed by Id@ 6 mg/kg IM on day 2]; and Group III [n=6, Da @ 3.5 mg/kg IM on day 1, Id @ 6 mg/kg IM on day 2 followed by Clindamycin (C) @ 30 mg/kg, PO q 12 h for 14 days]. Clinico-pathological examinations and parasite detections were done before and at day 21 post treatments for determination of efficacy. Major clinico-pathological abnormalities at initial presentation included tachypnea, anorexia, abnormal mucous membrane colour, lethargy, fever, thrombocytopenia and anaemia. Dogs treated in group III showed significant ($P<0.05$) remissions in abnormalities related with most of the observed parameters as compared with dogs treated in Group I or II. Complete parsitological clearance was not observed and 4 dogs out of 18 treated were found to be positive for *B. gibsoni* (2 in group I, 1 in group II and 1 in group III) on microscopy, whereas all dogs were still positive on PCR testing. Based on the clinic-pathological recovery, it can be suggested that for better results therapy undertaken in group III can be used under field conditions.

Keywords: *Babesia Gibsoni*, Clindamycin, Dog, Diminazene, Imidocarb

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Canine babesiosis is an important haemoprotozoal disease caused by parasites of the genus *Babesia* with *B. gibsoni* as one of the major causative agents in India (Mittal *et al.*, 2019) and other parts of the world (Solano-Gallego *et al.*, 2016).The disease is transmitted by ticks and the most frequent clinico-pathologic abnormalities included fever, lethargy, pale mucosa, lymphadenopathy, haemolytic anaemia, thrombocytopenia and splenomegaly (Mittal *et al.*, 2019).

Diagnosis is primarily based on characteristic clinico-pathological abnormalities and detection of piroplasm within infected erythrocytes by microscopy or parasite DNA by molecular techniques such as polymerase chain reaction (PCR) assay (Singh *et al.*, 2014; Mittal *et al.*, 2019). Many drugs or drug combinations have been used in the management of canine babesiosis with some drugs being reported to reduce the clinico-pathologic severity and the mortality associated with the disease (Baneth, 2018). Synergistic effects of certain anti-babesial drugs, such as atovaquone and azithromycin, have shown promising results (Lin *et al.*, 2012; Baneth, 2018) but atovaquone is not available in India. Therefore, the present study was planned to compare three different therapeutic protocols in clinical management of babesiosis in dogs naturally infected with *B. gibsoni*.

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MATERIALS AND METHODS

Therapeutic protocol and selection criteria of dogs

A total of 18 dogs diagnosed to be suffering from *B. gibsoni* based on clinical signs, haematological abnormalities, conventional microscopy and PCR assay were enrolled for the treatment trial excluding the dogs with any co-morbidity or concomitant infection of other vector-borne haemoparasites (as detected by microscopy). Dogs were either presented to the Teaching Veterinary Hospital, GADVASU or two privately run veterinary clinics from Ludhiana, Punjab. A systematic clinical examination was performed, and abnormalities were recorded before start of therapy and at day 21 of treatment. The permission for sampling and other procedures was duly approved by the Institutional Animal Ethics Committee of the university (vide memo no. IAEC/2018/ 1090-1125). The dogs were randomly divided into three groups -

- Group I (n=6): Imidocarb dipropionate (Id) (6 mg/kg body weight, IM, 2 doses 14 days apart).
- Group II (n=6): Diminazene aceturate (Da) (3.5 mg/kg body weight, IM on day 1 followed by Id (6 mg/kg body weight, IM) on day 2.
- Group III (n=6): Da (3.5 mg/kg body weight, IM on day 1) and Id (6 mg/kg body weight, IM on day 2) followed by Clindamycin (C) (30 mg/kg body weight,

PO every 12 h for 14 days).

Each dog was scored for severity of clinical signs using a categorized scoring system from 0 to 3 (from low to high severity) as described in Table 1 (adapted from Miró *et al.*, 2009). By adding the scores awarded to each clinical sign, an overall clinical score was calculated (maximum possible score = 28).

Therapeutic evaluation

For therapeutic evaluation, blood and serum samples from all the dogs before and at day 21 of therapy were collected and subjected to haematobiochemical analysis and detection of parasite (microscopically as well as by PCR assay). Haemoglobin (Hb; g/dL), haematocrit (%), total erythrocyte count (TEC; $\times 10^6/\mu L$) and platelet count ($\times 10^3/\mu L$) were estimated using automatic haematoanalyzer. Biochemical parameters including total bilirubin (mg/dL), total protein (g/dL), albumin (g/dL), alanine aminotransferase (ALT; U/L), gamma glutamyltransferase (GGT; U/L), blood urea nitrogen (BUN; mg/dL) and creatinine (mg/dL) were analyzed using automated clinical chemistry analyzer.

The clinical response to treatment was assessed by examining changes in clinical score after 21 days as score percentage reductions (PR) calculated as described by Hernández *et al.* (2015) using the following equation:

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

Statistical analysis

Data were analyzed using Minitab statistical software (Version 14.2, State College, PA, USA). Pre- and post-treatment numerical data of each parameter within each group were analyzed by Wilcoxon signed-rank tests and the differences were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Globally, *B. gibsoni* is known as a major pathogen responsible for canine babesiosis (Mittal *et al.*, 2019). Dogs in present study with *B. gibsoni* infection were presented with a wide variety of clinical manifestations. The major abnormalities included anorexia (94.4%), fever (94.4%), abnormal mucous membrane colour (88.9%), tick infestation (83.3%), lethargy (77.8%), lymphadenopathy (72.2%), pigmenturia (66.7%), weight loss (61.1%), vomiting (55.6%), melena (33.3%), and diarrohea (22.2%). An overall reduction in the number of dogs with abnormal clinical signs following treatment at 21 days, however, the maximum remission in the clinical signs were noted in dogs treated with combination of Da+Id+C. In this group, all dogs recovered from fever, pigmenturia and diarrhoea/melena, whereas in group I and II, none of the dogs showed complete remission of any of the presenting clinical manifestation. Reduction in clinical score was maximum (>80%) in dogs belonging to group III as compared with group II (65.3%) and group I (51.1%).

On physical examination, all infected dogs were tachypneic (>34 breaths/minute), 88.9% had abnormal mucous membranecolour (pale in 11 and icteric in 5 dogs), 77.8% had fever (>102.5°F), and 72.2% had tachycardia (>120 beats/minute). The mean clinical score of dogs in treatment group I was 10.8±2.1 (median 9.5; range 6 to 19), in treatment group II was 11.3±1.0 (median 10.5; range 9 to 16), and in treatment group III was 10.7±1.5 (median 8.5; range 8 to 16). Clinical signs and other physical examination findings observed in this study were similar to the previous studies (Solano-Gallego *et al.*, 2016; Mittal *et al.*, 2019). With respect to physical parameters, 14 dogs were still tachypneic (6 in group I, 5 in group II and 3 in group III), 6 had abnormal mucous membranes (2 in group I, 3 in group II and 1 in group III), 6 had fever (3 in group I and II each), and 5 had tachycardia (1 in group I and 2 each in group II and III). Changes in vital parameters recorded at day 0 and day 21 and dogs in group III showed significant improvement in temperature and respiration rate whereas heart rate reduced to normal range but difference observed was non significant as shown in Table 2. Improvement observed in other two groups were non significant.

With respect to the severity of haemato-biochemical abnormalities in *B. gibsoni* infected dogs, the most obvious alterations were observed in haematological parameters. About 83.3% of the dogs had a moderate to severe reduction in platelet count ($<150 \times 10^3/\mu L$). Similarly, moderate to severe reductions were observed with respect to Hb (<8 g/dL; in 72.2% dogs), TEC ($<3.5\times10^6/\mu L$; in 77.8% dogs) and haematocrit (<25%; in 61.1% dogs). However, the measured biochemical parameters did not alter much; highest abnormalities (moderate to severe) were seen in BUN (>40 mg/dL; in 50% dogs), albumin (<2 g/dL; in 44.4% dogs) and ALT (>80 U/L: in 44.4% dogs). Percentage of dogs with moderate to severe changes with respect to other biochemical parameters (such as total bilirubin, GGT, total protein and creatinine) were <34%. Similar observations were also documented previously (Baneth, 2018; Mittal *et al.*, 2019) and these abnormalities may be attributed to various reasons including antibody mediated cytotoxic destruction of erythrocytes, formation of auto-antibody against infected red blood cells, hypoxic injury and systemic inflammatory response that lead to multiple organ

Table 1

Clinical score system used to grade clinical signs in dogs with *B. gibsoni* infection (maximum 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
Diarrohea	Absence	Occasional	Frequent	Haematochezia
Melena	Absence	-	Occasional	Frequent

Table 2

Pre- and post-treatment physical examination findings in *B. gibsoni* infected dogs treated with imidocarb dipropionate (group I), diminazene aceturate-imidocarb dipropionate (group II) and diminazene aceturate-imidocarb dipropionate-clindamycin (group III).

Parameter	Reference range	Group I (n=6)		Group II (n=6)		Group III (n=6)	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Body temperature (°F)	100.5-102.5	102.7 (100.9-105.6)	102.4 (100.6-105.0)	103.9 (102.6-105.1)	102.6 (102.0-102.9)	103.9[a] (102.9-104.9)	102[b] (102.0-102.9)
Heart rate (beats/minute)	70-120	130.0 (86-160)	107.0 (92-122)	148.5 (76-160)	111.0 (102-156)	143.0 (124-162)	112.0 (98-150)
Respiratory rate (breaths/minute)	18-34	59.0 (51-99)	55.0 (40-78)	86.0[a] (80-95)	44.0[b] (35-60)	63.0[a] (52-95)	34.1[b] (30-51)

Values are median (range); Within each group, pre- and post-treatment values with different superscripts differ significantly (P<0.05).

dysfunction (Mittal *et al.*, 2019).

Post therapy, haemato-biochemical abnormalities showed a general trend towards their reduced frequency and severity (Table 3). Except for the platelet count, the remission in the severity of these abnormalities for the various parameters were appreciable more in group III in which all the dogs with moderate to severe haemato-biochemical abnormalities had either recovered completely or their number had reduced at 21 days post-treatment when compared with group I and II. The severity of abnormalities had reduced in both group II and group III; none of the dogs in group II had abnormal levels of BUN; and none of the dogs in group II and III had abnormal levels of creatinine following the treatment. In group I, rather the number of dogs with moderate to severe abnormalities with respect to ALT, GGT and total protein had increased.

A significant improvement (P<0.05) was observed in all the haematological parameters in dogs treated with Da+Id+C combination, whereas, only Hb and platelet count were improved in dogs treated with Da+Id (Table 3). Dogs treated with Da+Id showed significant improvements (P<0.05) in their post-treatment values of total protein and BUN (Table 3). In dogs treated with Da+Id+C combination, the post-treatment levels of total bilirubin decreased significantly (P<0.05) from that of pre-treatment levels. There was no haemato-biochemical improvement noticed in dogs treated with Id.

Similarly, the severity of abnormalities in haemato-biochemical parameters had shifted by some degree from 'moderate or severe' towards 'mild' side. Dogs treated with Da+Id+C triple combination had recovered from almost all the clinicopathologic abnormalities associated with babesiosis (overall reduction of clinical scores around 80%). Although the median values for some of the physical and haemato-biochemical parameters had either remained within normal reference range or had returned to normal ranges in different treatment groups, but if clinical signs of individual dogs are assessed, it was observed that out of the 6 dogs treated in group III , 3 recovered completely from all the initially observed clinical signs, whereas only 1 dog

Table 3

Pre- and post-treatment haemato-biochemical findings in *B. gibsoni* infected dogs treated with imidocarb dipropionate (group I), diminazene aceturate-imidocarb dipropionate (group II) and diminazene aceturate-imidocarb dipropionate-clindamycin (group III).

Parameter	Reference range	Group I (n=6)		Group II (n=6)		Group III (n=6)	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Haemoglobin (g/dL)	12-18	8.2 (5.8-14.3)	8.0 (4.4-17.0)	6.9[a] (2.5-10.0)	10.9[b] (7.9-12.5)	4.9[a] (4.0-6.2)	9.9[b] (8.2-12.8)
Haematocrit (%)	37-55	26.8 (20.7-44.0)	28.5 (15.1-50.8)	22.7 (20.2-25.6)	31.8 (24.6-36.2)	21.5[a] (20.0-25)	31.5[b] (26.0-36.0)
TEC ($\times 10^6/\mu L$)	5.5-8.5	3.6 (2.3-6.7)	4.0 (2.3-7.1)	2.7 (2.1-3.4)	3.5 (2.4-4.2)	2.8[a] (2.3-3.9)	4.7[b] (3.4-5.8)
Platelet count ($\times 10^3/\mu L$)	200-900	149.5 (45-334)	198.5 (67-392)	61.0[a] (40-101)	118.5[b] (109-214)	48.5[a] (12-104)	110.0[b] (99-294)
Total bilirubin (mg/dL)	0.1-0.6	0.2 (0.1-7.1)	0.3 (0.1-5.2)	0.9 (0.2-1.9)	0.8 (0.1-1.2)	1.5[a] (0.2-2.2)	0.6[b] (0.1-1.3)
ALT (U/L)	8-58	58.0 (26-150)	38.0 (5-98)	91.5 (44-153)	70.5 (54-100)	63.5 (25-102)	54.0 (34-85)
GGT (U/L)	1-12	10.0 (5-21)	12.5 (5-96)	18.0 (5-28)	15.0 (6-21)	12.0 (5-58)	5.7 (5-56)
Total protein (g/dL)	5.5-7.5	4.8 (3.7-6.3)	5.0 (2.9-6.7)	4.8[a] (4.0-6.8)	5.9[b] (4.8-6.9)	4.7 (4.2-5.4)	5.8 (4.7-6.5)
Albumin (g/dL)	2.6-4	1.7 (1.3-2.8)	2.0 (1.1-2.9)	2.2 (1.8-3.5)	2.4 (2.4-3.5)	2.0 (1.8-2.4)	2.6 (2.1-3.0)
BUN (mg/dL)	8.8-26	30.0 (13-68)	13.0 (5-41)	48.0[a] (12-81)	28.0[b] (7-45)	21.0 (15-42)	25.0 (5-33)

* Fielder (2016); Values are median (range);TEC: Total erythrocyte counts; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transferase; BUN: Blood urea nitrogen. Within each group, pre- and post-treatment values with different superscripts differ significantly ($P<0.05$).

in group I and none of the dogs in group II showed complete recovery on follow-up examinations. According to the previous studies, Id is preferred for the treatment of canine babesiosis caused by large *Babesia* species such as *B. canis*. It is less effective in *B. gibsoni* infection; in most cases, the parasites are not totally eliminated (Baneth, 2018). It has a direct action against the parasite DNA that causes unwinding and denaturation (Wozniak *et al.*, 1997). The antibabesial property of Da, an aromatic diamidine, is not well understood (Baneth, 2018) and it has been proposed that Da disrupts the parasite's DNA synthesis and aerobic glycolysis (Plumb, 2015) in the same manner as reported for *Trypanosoma* and *Leishmania* species (Bitonti *et al.*, 1986). Studies have shown that Da alone cannot completely eliminate *B. gibsoni* from infected dogs, and relapses often occur (Wulansari *et al.*, 2003b; Matsuu *et al.*, 2008) unfortunately due to the possible development of Da resistance in *Babesia* parasites as suggested recently (Yamasaki *et al.*, 2017). Clindamycin is an immune enhancing lincosamide antibiotic which inhibits bacterial growth by hindering the RNA-dependent protein synthesis (Plumb, 2015). It could eliminate *Babesia* organisms from the peripheral blood and reduce clinicopathologic alterations in *B. gibsoni* infected dogs (Wulansari *et al.*, 2003b). However, it has been found that the activity of clindamycin against *B. gibsoni* was 16-24 times lower than that against *B. divergens* (Matsuu *et al.*, 2008). Nevertheless, it has also been suggested that clindamycin stimulate humoral and cellular immunity against *Babesia* infection and results in improvement in clinical condition (Wulansari *et al.*, 2003a). Although the efficacy of Da or clindamycin against *B. gibsoni* was not evaluated individually in this study, but the ability of each drug alone to successfully treat *B. gibsoni* infections is still controversial as suggested in previous studies (Matsuu *et al.*, 2008). One previous study had evaluated the effect of Da+Id+C combination against *B. gibsoni* infection in dogs (Lin *et al.*, 2012); higher recovery rates and shorter administration periods were observed as compared with atovaquone and azithromycin combination. In line with

these findings, the present study also reported better clinicopathologic cure in dogs infected with *B. gibsoni* which might be attributed to the synergistic effect of Da+Id+C triple combination (Baneth, 2018).

Effect of treatment on parasitological cure

Of the 18 dogs with *B. gibsoni* infections, 14 dogs (4 in group I and 5 each in group II and III) became negative microscopically for *B. gibsoni* at 21 days post-treatment. However, all the 18 dogs were still positive for *B. gibsoni* at 21 days post-treatment as revealed by the presence of 672 bp amplicon by 18S PCR assay. It may be assumed that the treated dogs might have undergone into a sub-clinical/chronic phase of the infection following treatment which might had resulted in to non-detection of the parasite by microscopy but not by PCR assay. Furthermore, there are documentations that PCR assay is more sensitive than microscopy in detection of sub-clinical/chronic infections in *B. gibsoni* infected dogs (Abd Rani *et al.*, 2011; Singh *et al.*, 2014). Although, we were not able to examine all the dogs by microscopy and PCR assay at 12-13 weeks post-treatment, but the results obtained from some of the cases (1 from Group II and 2 from Group III) showed parasitic clearance by both microscopy and PCR assay after 12-13 weeks of treatment. When the results of these diagnostic tests along with clinicopathologic finding were compared between group II and III, a better efficacy of Da+Id+C combination against *B. gibsoni* infections was observed.

In conclusion, although complete elimination of *B. gibsoni* from the blood could not be achieved with any of the three treatment protocols at 21 days post-treatment, yet highest efficacy of Da+Id+C combination in terms of remission of clinicopathologic abnormalities makes it a good alternative available with veterinary practitioners under field conditions for the treatment of dogs with *B. gibsoni* infections without any adverse effects.

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