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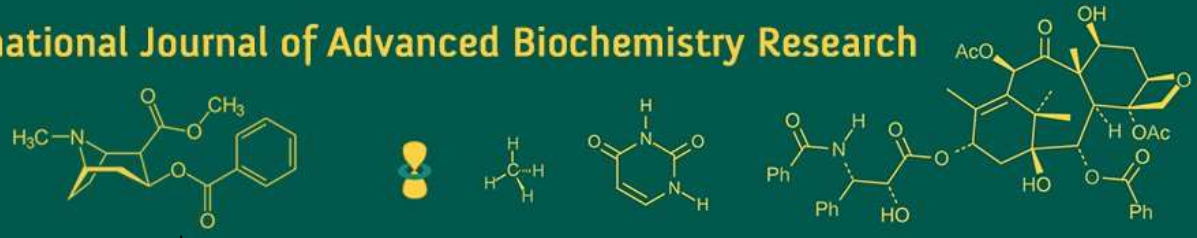

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Comparison of the efficacy of different therapeutic regimen for the treatment of IMHA caused by *B. gibsoni* in dogs

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Abstract
The present study was conducted in dogs presented to small animal OPD, Department of Veterinary Medicine with clinical signs of anorexia, pyrexia, lethargy, pale mucous membrane, lymphadenopathy, ascites, vomiting, diarrhoea, icterus, hematuria suggestive of canine babesiosis. Eighteen dogs were primarily screened for *Babesia gibsoni* infection by blood smear examination and then confirmed by PCR. Further these dogs were subjected to saline agglutination test, spherocytosis in blood smear examination and Coombs test for diagnosis of immune mediated hemolytic anemia (IMHA). Dogs in Group I were treated with a combination of Doxycycline, Metronidazole and Clindamycin at a dose rate of 5 mg/kg, 15 mg/kg, 25 mg/kg respectively *b.i.d* per orally for 21 days, dogs in Group II were treated with a combination of Doxycycline, Metronidazole and Clindamycin at a dose rate of 5 mg/kg, 15 mg/kg, 25 mg/kg respectively *b.i.d* per orally for 21 days and prednisolone at a dose rate of 2 mg/kg body weight per orally *b.i.d* for first 5 days followed by 1 mg/kg body weight per orally *b.i.d* for next 5 days and drug was tapered to 0.5 mg/ kg body weight per orally *b.i.d* for another 5 days and dogs in Group III were treated with a combination of Doxycycline, Metronidazole and Clindamycin at a dose rate of 5 mg/kg, 15 mg/kg, 25 mg/kg respectively *b.i.d* per orally for 21 days and a combination of Azathioprine at a dose rate of 2mg/kg body weight per orally *s.i.d* for the first 5 days followed by 1 mg/kg per orally *s.i.d* for next 5 days and 0.5 mg/kg per orally *s.i.d* for another 5 days and Prednisolone at a dose rate of 1.5 mg/kg body weight per orally *b.i.d* for a period of 5 days followed by 1 mg/kg body weight per orally *b.i.d* for next 5 days followed by 0.5 mg/kg body weight orally *b.i.d* for another 5 days. Though all the therapeutic regimens were efficacious in the treatment of IMHA caused by *B. gibsoni*, treatment with combination of Doxycycline, Metronidazole, Clindamycin and Prednisolone was found to be more efficacious as all the dogs in this group were negative for Coombs test post therapy with no abnormal elevation in biochemical parameters post therapy.

Keywords: Immune, mediated, haemolytic, anemia

Introduction
Canine babesiosis is a serious disease that affects domestic and wild canids worldwide. It is caused by intraerythrocytic protozoa of the genus Babesia, family Babesiidae, order Piroplasmida, within the phylum Apicomplexa. The most common species that cause canine babesiosis include *Babesia canis* and *Babesia gibsoni*, which could be differentiated based on their size within the parasitized erythrocytes. *B. gibsoni* is distributed throughout the world including Middle East, Northern Africa, and South Asia (Salem and Farag., 2014) [14].
Clinical signs and physical examination of dogs infected with *Babesia gibsoni* include pyrexia, lymph node enlargement, dullness, depression, inappetance, pale mucous membrane, tachycardia, tachypnoea, epistaxis, ascites, loss of body weight, hepatomegaly, splenomegaly, nervous deficits, constipation, diarrhoea, icterus, and nephropathy (Varshney *et al.*, 2008, Reddy *et al.*, 2016, Teodorowski *et al.*, 2022) [19, 23, 18].
The complications of babesiosis in dogs include immune mediated haemolytic anemia, haemoconcentration, coagulopathy, acute renal failure, acute respiratory distress syndrome, myocardial pathology, pancreatitis, multiple organ dysfunction syndrome and systemic inflammatory response syndrome (Vishwakarma and Nandini 2019; Halder and Gupta 2021) [11, 20, 7].

Immune-mediated haemolytic anemia (IMHA) is one of the commonly diagnosed canine autoimmune diseases and a model of acute and clinically relevant anemia. Impaired immune tolerance leads to premature destruction of red blood cells (RBCs). IMHA can be idiopathic (primary) or secondary to underlying disease condition such as parasitic, infectious, neoplastic or drug induced (Maney and Marincheva, 2018) [9]. The antigen antibody mediated destruction of RBC in IMHA can cause severe anemia, affecting the oxygen carrying capacity of blood leading to tissue hypoxia, MODS, cardiovascular abnormalities and collapse. Therefore identifying the underlying cause, early diagnosis and treatment prevents the progression of life threatening IMHA.

India being a tropical country, the prevalence of tick borne diseases is higher and in turn the possibility of occurrence of secondary IMHA is high. Therefore the present study was carried out to determine the efficacy of different therapeutic regimen for the treatment of immune mediated hemolytic anemia caused by *B. gibsoni.*

Materials and Methods

Dogs presented to the Department of Veterinary Medicine, Veterinary College Hospital, Hebbal, Bengaluru with a history of tick infestation, high fever, pale / icteric mucous membrane inappetence, anaemia, lethargy, vomiting, diarrhoea, hematuria were screened by saline agglutination test, spherocytosis and Coombs test for diagnosing IMHA. These dogs were screened for *B. gibsoni* infection by microscopic examination of blood smears and PCR. Eighteen dogs infected with *B. gibsoni*, positive for saline agglutination test, spherocytosis and Coombs test were selected for the study.

Eighteen dogs that were positive for IMHA by saline agglutination test, spherocytosis in the blood smear, positive for Coombs test were included in this study. These dogs were positive for *Babesia gibsoni* by PCR. They were randomly grouped into three groups of six dogs each and were treated with three treatment regimen as follows.

Group I: Six dogs affected with immune mediated hemolytic anemia caused by *Babesia gibsoni* were treated with a combination of Doxycycline, Metronidazole and Clindamycin at a dose rate of 5 mg/kg, 15 mg/kg, 25 mg/kg respectively *b.i.d* per orally for 21 days.

Group II: Six dogs affected with immune mediated hemolytic anemia caused by *Babesia gibsoni* were treated with a combination of Doxycycline, Metronidazole and Clindamycin at a dose rate of 5 mg/kg, 15 mg/kg, 25 mg/kg respectively *b.i.d* per orally for 21 days and prednisolone at a dose rate of 2 mg/kg body weight per orally *b.i.d* for first 5 days followed by 1 mg/kg body weight per orally *b.i.d* for next 5 days and drug was tapered to 0.5 mg/ kg body weight per orally *b.i.d* for another 5 days.

Group III: Six dogs affected with immune mediated hemolytic anemia caused by *Babesia gibsoni* were treated with a combination of Doxycycline, Metronidazole and Clindamycin at a dose rate of 5 mg/kg, 15 mg/kg, 25 mg/kg respectively *b.i.d* per orally for 21 days and a combination of Azathioprine at a dose rate of 2mg/kg body weight per orally *s.i.d* for the first 5 days followed by 1 mg/kg per orally *s.i.d* for next 5 days and 0.5 mg/kg per orally *s.i.d* for another 5 days and Prednisolone at a dose rate of 1.5 mg/kg body weight per orally *b.i.d* for a period of 5 days followed by 1 mg/kg body weight per orally *b.i.d* for next 5 days followed by 0.5 mg/kg body weight orally *b.i.d* for another 5 days.

Results and Discussion

Group I

Haematological parameters

Dogs of Group I showed pre therapeutic mean WBC values of 24.12±4.675 × 10^3 /µL which was significantly increased when compared to control group (12.13±0.885 × 10^3 /µL). The post therapeutic value showed a significant decrease with the mean value of 12.93±2.23 × 10^3 /µL after therapy. The results are depicted in Table 1.

The pre therapeutic mean RBC value in dogs of Group I was found to be 2.05±0.20 ×10^6 /µL which was significantly lower as compared to control group (6.805±0.397 × 10^6 / µL). The mean RBC value showed a significant increase after treatment with the mean value of 5.36±0.423 ×10^6 /µL (Table 1).

Dogs of Group I had a pre therapeutic mean Hb values of 4.96±0.282 g/ dL which was significantly lower when compared to the control group (14.47±1.083 g/dL). However, after treatment, the Hb values showed significant increase to 11.45±1.266 g/dL (Table 1).

From the table 1 it is evident that the dogs of Group I had a significantly lower platelet count with a mean value of 58.50±25.06 × 10^3 /µL as compared to the apparently healthy dogs in the control group (265.2±19.19 × 10^3 /µL). The post therapeutic value had a significant increase with the mean value of 340.0±107.7 × 10^3 /µL (Table 1).

The pre therapeutic mean PCV in group I was 16.17±0.885 percent which was significantly reduced as compared with the mean value of dogs in the control group (48.05±2.525 percent). There was significant increase in the PCV in the mean value reaching 36.20±4.016 percent) (Table 1).

Table 1: Mean±SE of haematological values of dogs in Group I before and after therapy

Parameter	Healthy dogs (n=6)	Infected dogs before therapy(n=6)	After therapy (n=6)
TLC (×10^3 / µL)	12.13±0.885 ^{a}	24.12±4.675 ^{b}	12.93±2.23 ^{ac}
TEC (×10^6/ µL)	6.805±0.397 ^{a}	2.05±0.20 ^{b}	5.36±0.423 ^{c}
Hb (g/dL)	14.47±1.083 ^{a}	4.96±0.282 ^{b}	11.45±1.266 ^{ac}
Platelet count (×10^3/ µL)	265.2±19.19 ^{a}	58.50±25.06 ^{b}	340.0±107.7 ^{ac}
PCV (%)	48.05±2.525 ^{a}	16.17±0.885 ^{b}	36.20±4.016 ^{bc}

Note: Mean±SE bearing different superscripts within a row are statistically different at $P< 0.05$.

Biochemical parameters

In the dogs of this group, the mean pre therapeutic BUN was 24.63±3.05 mg/dL which was significantly elevated as compared with the mean value of healthy dogs (14.72±2.324 mg/dL). On 21st day post therapy the BUN significantly reduced, with a mean value of 12.78±1.132 mg/dL. The results are depicted in Table 2.

Dogs of Group I had pre therapeutic mean creatinine value of 1.51±0.110 (mg/dL) which was significantly higher as compared with the control group (0.93±0.033 mg/ dL). On day 21 after therapy the mean creatinine value statistically decreased to 0.93±0.083 mg/dL (Table 2).

The mean pre therapeutic total protein value was 5.48±0.212 g/dl which was non significantly decreased as compared with control group (6.25±0.283g/dL). On the 21st day post treatment, there was non significant increase in the mean value reaching 6.03±0.209 g/dL (Table 2).

Dogs the of Group I had mean pre therapeutic mean albumin value of 1.33±0.06 g/dL which was significantly reduced when compared to the control group 2.60±0.109 g/ dL. The post therapeutic value showed a significant elevation with a mean value of 2.51±0.074 g/ dL (Table 2).

In the dogs of this Group the mean value of globulin was 4.15±0.194 g/dL which was non significantly higher than healthy dogs (3.65±0.338 g/ dL). There was non significant reduction with the mean value of 3.51±0.195 g/ dL after therapy (Table 2).

The pre therapeutic mean ALT value in dogs of Group I was found to be 71.43±6.111 IU/L which was significantly higher as compared to the control group (30.27±3.718 IU/L). On 21st day post therapy, the mean ALT value showed significant decrease to 34.13±2.035 IU/L (Table 2).

Dogs of Group I had pre therapeutic mean ALP value of 327.8±22.72 IU/L which was significantly higher as compared to the healthy dogs in control group (114.8±15.23 IU/L). The post therapeutic value showed a significant decrease with the mean value of 130.1±20.64 IU/L after therapy (Table 2).

In the dogs of Group I the mean pre therapeutic total bilirubin value was 1.26±0.424 mg/dL which was non significantly higher than control group with a mean value of 0.48±0.054 mg/ dL. On the 21st day after treatment, there was non significant decrease in total bilirubin value with a mean value of 0.38±0.147 mg/dL (Table 2).

Table 2: Mean±SE of serum biochemical values of dogs in Group I before and after therapy

Parameter	Healthy dogs	Infected dogs before therapy	After therapy
BUN (mg/dL)	14.72±2.324 [a]	24.63±3.045 [b]	12.78±1.132 [ac]
Creatinine (mg/dL)	0.93 ±0.033 [a]	1.51±0.110 [b]	0.93±0.088 [ac]
Total protein (g/dL)	6.25±0.283 [a]	5.48±0.212 [a]	6.03±0.209 [a]
Albumin (g/dL)	2.60±0.109 [a]	1.33±0.066 [b]	2.51±0.074 [ac]
Globulin (g/dL)	3.65±0.338 [a]	4.15±0.194 [a]	3.51±0.195 [a]
ALT (IU/L)	30.27±3.718 [a]	71.43±6.111 [b]	34.13±2.035 [ac]
ALP (IU/L)	114.8±15.23 [a]	327.8±22.72 [b]	130.1±20.64 [ac]
Total Bilirubin (mg/dL)	0.48±0.054 [a]	1.26±0.420 [a]	0.38±0.147 [a]

Group II

Haematological parameters

Dogs of Group II had pre therapeutic mean TLC value of 23.65±6.744 × 10^3 /µL which was significantly higher as compared to the healthy dogs in control group (12.13±0.885 × 10^3 /µL). The post therapeutic value showed a significant decrease with the mean value of 13.87±1.687 × 10^3 /µL after therapy. The results are depicted in Table 3.

The pre therapeutic mean RBC value in dogs of Group II was found to be 2.72±0.266 ×10^6 /µL which was significantly lower as compared to control group (6.805±0.397 × 10^6 / µL). The mean RBC value showed a significant increase after treatment with the mean value of 5.86±0.334 ×10^6 /µL (Table 3).

Dogs of Group II had a pre therapeutic mean Hb values of 6.48±0.406 g/ dL which was significantly lower when compared to the control group (14.47±1.083 g/dL). However, after treatment, the Hb values showed significant increase to 12.98±0.839 g/dL (Table 3).

From the table 14, it is evident that the dogs of Group II had a significantly lower platelet count with a mean value of 83.00±14.68 × 10^3 /µL as compared to the apparently healthy dogs in the control group (265.2±19.19 × 10^3 /µL). The post therapeutic value had a significant increase with the mean value of 337.8±98.67× 10^3 /µL (Table 3).

The pre therapeutic mean PCV in group II was 19.38±1.971 percent which was significantly reduced as compared with the mean value of dogs in the control group (48.05±2.525 percent). There was significant increase in the PCV in the mean value reaching 40.78±2.550 percent post therapy (Table 3).

The pre treatment erythrocyte indices such as MCV, MCH and MCHC were 70.75±4.640 fl, 25.77±3.437 pg, 36.13±3.204 mg/dl respectively which had no significant difference as compared to the control group (70.80±1.598 fl, 21.35±1.329 pg and 30.11±1.553 mg/dL respectively). On the 21st day after treatment, the corresponding values were 70.23±4.564 fl, 22.29±1.544 pg and 31.77±0.389 mg/dL respectively. No statistical significance was observed between day 0 and day 21 (Table 3).

Table 3: Mean±SE haematological values of dogs in Group II before and after therapy

Parameter	Healthy dogs (N=6)	Before therapy (n=6)	After therapy (n=6)
TLC (×10^3/ µL)	12.13±0.885[a]	23.65±6.744[b]	13.87±1.687[ac]
TEC (×10^6/ µL)	6.805±0.397 [a]	2.72±0.266 [b]	5.86±0.334 [ac]
Hb (g/dL)	14.47±1.083 [a]	6.48±0.406 [b]	12.98±0.839 [ac]
Platelet count (×10^3/ µL)	265±19.19 [a]	83.00±14.68 [b]	337.8±98.67 [ac]
PCV(%)	48.05±2.525 [a]	19.38±1.971 [b]	40.78±2.550 [ac]

Biochemical parameters

In the dogs of this group, the mean pre therapeutic BUN was 30.63±2.065 mg/dL which was significantly elevated as compared with the mean value of healthy dogs (14.72±2.324 mg/dL). On 21st day post therapy the BUN reduced significantly, with a mean value of 14.50±0.542 mg/dL. The resuls are depicted in Table 4.

Dogs of Group II had pre therapeutic mean creatinine value of 1.86±0.152 mg/dL which was significantly higher as compared with the control group (0.93±0.033 mg/dL). On day 21 after therapy, the mean Creatinine level decreased statistically to 0.88±0.083 (Table 4).

The mean pre therapeutic total protein value was 5.96±0.210 g/dL which was non significantly decreased as compared to the control group (6.25±0.283g/dL). On the 21st day post treatment, there was non significant increase in the mean value reaching 6.41±0.558 g/dL (Table 4).

Dogs of Group II had mean pre therapeutic albumin value of 1.85±0.170 g/dL which was non significantly reduced when compared to the control group 2.60±0.109 g/dL. The post therapeutic value showed a significant elevation with a mean value of 3.26±0.345 g/dL (Table 4). In the dogs of this Group the mean value of globulin was 4.11±0.284 g/dL which was non significantly increased when compared to the healthy dogs (3.65±0.338 g/dL). There was non significant reduction with the mean value of 3.15±0.269 g/dL after therapy (Table 4).

The pre therapeutic mean ALT value in dogs of Group II was found to be 74.32±5.610 IU/L which was significantly increased as compared to the control group (30.27±3.718 IU/L). On 21st day post therapy, the mean ALT value showed significant decrease with a mean value of 31.33±1.797 IU/L (Table 4).

In the dogs of Group II the mean pre therapeutic total bilirubin value was 1.26±0.424 IU/L was non significantly higher than control group with a mean value of 0.48±0.054 IU/L. On the 21st day after treatment, there was non significant decrease in total bilirubin value with a mean value of 0.36±0.066 IU/L (Table 4).

Dogs of Group II had pre therapeutic mean ALP value of 418.4±73.50 IU/L which was significantly higher as compared to the healthy dogs in control group (114.8±15.23 IU/L). The post therapeutic value showed a non significant decrease with the mean value of 223.2±79.95 IU/L after therapy (Table 4).

Table 4: Mean±SE serum biochemical values of dogs in Group II before and after therapy

Parameter	Healthy dogs (n=6)	Infected dogs before therapy (n=6)	After therapy (n=6)
BUN (mg/dL)	14.72±2.324 [a]	30.63±2.065 [b]	14.50±0.542 [ac]
Creatinine (mg/dL)	0.93±0.033 [a]	1.86±0.152 [b]	0.88±0.083 [ac]
Total protein (g/dL)	6.25±0.283 [a]	5.96±0.210[a]	6.41±0.558 [a]
Albumin (g/dL)	2.60±0.109 [a]	1.85±0.170 [a]	3.26±0.345 [ab]
Globulin (g/dL)	3.65±0.338 [a]	4.11±0.284 [a]	3.15±0.269 [a]
ALT (IU/L)	30.27±3.718 [a]	74.32±5.610 [b]	31.33±1.797 [ac]
ALP (IU/L)	114.8±15.23 [a]	418.4±73.50 [b]	223.2±79.95 [ab]
Total Bilirubin (mg/dL)	0.48±0.054 [a]	1.26±0.424 [a]	0.36±0.066 [a]

Note: Mean±SE bearing different superscripts within a row are statistically different at $P< 0.05$.

Group III

Haematological parameters

Dogs of Group III had pre therapeutic mean TLC value of 24.63±5.531 × 10^3 /µL which was significantly higher as compared to the healthy dogs in control group (12.13±0.885 × 10^3 /µL). The post therapeutic value showed a significant decrease with the mean value of 11.12±1.176 × 10^3 /µL after therapy. The results are depicted in Table 5.

The pre therapeutic mean RBC value in dogs of Group II was found to be 1.75±0.159 ×10^6 /µL which was significantly lower as compared to control group (6.805±0.397 × 10^6 / µL). The mean RBC value showed a significant increase after treatment with the mean value of 4.83±0.658 ×10^6 /µL (Table 5).

Dogs of Group III had a pre therapeutic mean Hb values of 4.95±0.409 g/ dL which was significantly lower when compared to the control group (14.47±1.083 g/dL). However, after treatment, the Hb values showed significant increase to 11.08±1.459 g/dL (Table 5).

From the table 16, it is evident that the dogs of Group III had a significantly lower platelet count with a mean value of 58.50±24.76 [b] × 10^3 /µL as compared to the apparently healthy dogs in the control group (265.2±19.19 × 10^3 /µL). The post therapeutic value had a significant increase with the mean value of 232.3±33.53 × 10^3 /µL (Table 5).

The pre therapeutic mean PCV in group III was 16.27±1.213 percent which was significantly reduced as compared with the mean value of dogs in the control group (48.05±2.525 percent). There was significant increase in the PCV in the mean value reaching 34.08±3.984 percent post therapy (Table 5).

The pre treatment erythrocyte indices such as MCV, MCH and MCHC were 93.73±3.018fl, 28.33±0.924 pg and 30.33±0.575 mg/dL respectively, which had no significant difference as compared to the control group (70.80±1.598 pg, 21.35±1.329 fl and 30.11±1.553 mg/dl respectively). After treatment, the corresponding values were 30.18±3.281 pg, 23.20±0.821 fl and 32.23±0.799 mg/dL respectively. No statistical significance was observed between day 0 and day 21 (Table 5).

Table 5: Mean Mean±SE of haematological values of dogs in Group III before and after therapy

Parameter	Healthy dogs	Before therapy	After therapy
TLC (×10^3 / µL)	12.13±0.885 [a]	24.63±5.531 [b]	11.12±1.176 [ac]
TEC (×10^6 / µL)	6.805±0.397 [a]	1.75±0.159 [b]	4.83±0.658 [c]
Hb (g/dL)	14.47±1.083 [a]	4.95±0.409 [b]	11.08±1.459 [ac]
Platelet count (×10^3 / µL)	265±19.19 [a]	58.50±24.76 [b]	232.3±33.53 [ac]
PCV (%)	48.05±2.525 [a]	16.27±1.213[b]	34.08±3.984 [bc]

Note: Mean±SE bearing different superscripts within a row are statistically different at $P< 0.05$.

Biochemical parameters

In the dogs of this group, the mean pre therapeutic BUN was 36.58±1.307 mg/dL which was significantly elevated as compared with the mean value of healthy dogs (14.72±2.324 mg/dL). On 21st day post therapy the BUN reduced significantly, with a mean value of 17.58±0.969 mg/dL. The results are depicted in the Table 6.

Dogs of Group III had pre therapeutic mean creatinine value of 1.73±0.098 mg/dL which was significantly higher as compared with the control group (0.93±0.033 mg/dL). On day 21 after therapy there was a significant decrease in the Mean Creatinine value to 0.88±0.113mg/dL (Table 6).

The mean pre therapeutic total protein value was 5.65±0.306 g/dL which was non significantly decreased as compared to the control group (6.25±0.283g/dL). On the 21st day post treatment, there was non significant decrease in the mean value reaching 5.31±0.407 g/dL (Table 6).

Dogs of Group III had mean pre therapeutic mean albumin value of 1.51±0.132 g/dL, which was significantly reduced when compared to the control group 2.60±0.109 g/dL. The post therapeutic value showed a significant increase with a mean value of 2.18±0.147 g/dL (Table 6).

In the dogs of this Group the mean value of globulin was 4.13±0.265 g/dL which had non significantly higher when compared to the healthy dogs (3.65±0.338 g/dL). There was non significant decrease with the mean value of 3.80±0.395 g/dL after therapy (Table 6).

The pre therapeutic mean ALT value in dogs of Group III was found to be 67.40±5.552 IU/L which was significantly increased as compared to the control group (30.27±3.718 IU/L). On 21st day post therapy, the mean ALT value showed significant elevation to 128.4±4.639 IU/L (Table 6).

Dogs of Group III had pre therapeutic mean ALP value of 273.9±41.26 IU/L which was significantly higher as compared to the healthy dogs in control group (114.8±15.23 IU/L). The post therapeutic value showed a significant increase with the mean value of 551.9±33.81 IU/L after therapy (Table 6).

In the dogs of Group III the mean pre therapeutic total bilirubin value was 1.115±0.1948 mg/dL which was significantly higher than control group with a mean value of 0.48±0.054 mg/dL. On the 21st day after treatment, there was significant decrease in total bilirubin value with a mean value of 0.500±0.894 mg/Dl (Table 6).

Dogs of Group III had pre therapeutic mean ALP value of 273.9±41.26 IU/L which was significantly higher as compared to the healthy dogs in control group (114.8±15.23 IU/L). The post therapeutic value showed a significant increase with the mean value of 551.9±33.81 IU/L after therapy (Table 6).

In the dogs of Group III the mean pre therapeutic total bilirubin value was 1.115±0.1948 mg/dL which was significantly higher than control group with a mean value of 0.48±0.054 mg/dL. On the 21st day after treatment, there was significant decrease in total bilirubin value with a mean value of 0.500±0.894 mg/dL (Table 6).

Table 6: Mean±SE of serum biochemical values of dogs in Group III before and after therapy

Parameter	Healthy dogs	Infected dogs before therapy	After therapy
BUN (mg/dL)	14.72±2.324 [a]	36.58±1.307 [b]	17.58±0.969 [ac]
Creatinine (mg/dL)	0.93±0.033 [a]	1.73±0.098 [b]	0.88±0.113 [ac]
Total protein (g/dL)	6.25±0.283 [a]	5.65±0.306 [a]	5.31±0.407 [a]
Albumin (g/dL)	2.60±0.109 [a]	1.51±0.132[b]	2.18±0.147[ac]
Globulin (g/dL)	3.65±0.338 [a]	4.13±0.265 [a]	3.80±0.395 [a]
ALT (IU/L)	30.27±3.718 [a]	67.40±5.552 [a]	128.4±4.639 [b]
ALP (IU/L)	114.8±15.23 [a]	273.9±41.26 [b]	551.9±33.81[bc]
Total Bilirubin (mg/dL)	0.48±0.054 [a]	1.115±0.1948 [b]	0.500±0.894 [ac]

Note: Mean±SE bearing different superscripts within a row are statistically different at $P< 0.05$.

Discussion

Therapeutic trial

Group I

In this group, all the dogs were treated with a combination of Doxycycline @ 5 mg/kg b.wt *per os b.i.d*, Clindamycin @ 25 mg/kg b.wt *per os b.i.d* and Metronidazole @ 15 mg/kg b.wt *per os b.i.d*, for a period of 21 days. Response to therapy was studied by improvement in haemato biochemical parameters and by negative saline agglutination test and Coombs test after treatment.

The dogs in Group I before therapy had significantly decreased mean concentration of Hb, TEC and PCV of 4.96±0.282 g/ dL, 2.05±0.20 × 10^6 / µL and 16.17±0.885 percent respectively. After treatment, there was significant increase in the mean Hb, TEC and PCV to 11.45±1.266 g/dL, 5.36±0.423 ×10^6 /µL and 36.20±4.016 percent respectively. Nandini *et al.* (2016) [11], Sharma *et al.* (2016) [15], An *et al*. (2019) [1], Almendros *et al*. (2020) and Patel *et al*. (2023) [12] reported a significant increase in the mean values of haemoglobin, TEC and PCV on 21st day of treatment with a combination of of Doxycycline @ 5 mg/kg b.wt *per os b.i.d*, Clindamycin @ 25 mg/kg b.wt *per os b.i.d* and Metronidazole @ 15 mg/kg b.wt *per os b.i.d*, for a period of 21 days. It could be attributed to removal of parasites and toxic products as a sequel to red blood cell lysis.

The dogs of this group had mean TLC concentration of 24.12±4.675 × 10^3 /µL which decreased significantly to 12.93±2.23 × 10^3 /µL after therapy. This is in agreement with Nandini *et al.* (2016) [11] and Patel *et al*. (2023) [11]. This could be attributed to the efficacious combination of antibiotic therapy in subsidence of infection and reduction of stress on animal. It stimulates humoral and cellular immunity against *B. gibsoni* infection and results in improvement in clinical condition.

The platelet count in dogs of this group was 58.50±25.06 × 10^3 /µL before therapy. A significant increase in the platelet count to 340.0±107.7 × 10^3 /µL was observed after therapy. Similar findings have been reported by Suzuki *et al.* (2007) [16], Nandini *et al*. (2016) [11] and Swamy *et al.* (2019) [17] and Patel *et al*. (2023) [12]. This could be attributed to the efficacy of combination of antibiotics in decreasing the platelet sequestration and destruction by splenic macrophages.

In dogs of Group I, the mean pre therapeutic ALT concentration was 71.43±6.111 IU/L. After treatment there

was significant decrease in mean concentration of ALT to 34.13±2.035 IU/L. Our findings is in accordance with Sharma *et al*. (2016) [15], Halder and Gupta (2022) [7] and Patel *et al*. (2023) [12] who observed a significant decrease in ALT after treatment.

Dogs of Group I had pre therapeutic mean ALP value of 327.8±22.72 IU/L The post therapeutic value showed a significant decrease with the mean value of 130.1±20.64 IU/L after therapy. This is in agreement with Sharma *et al.* (2016) [15] and Patel *et al.* (2023) [12] who reported that the mean ALP value decreased significantly after treatment with Triple antibiotic therapy.

The pre therapeutic mean creatinine value in dogs of group I was 1.51±0.110 mg/dL which decreased significantly on the 21st day post therapy (0.93±0.088 mg/dL). The dogs in Group I before therapy had significantly increased BUN concentration of 24.63±3.045 mg/dL which decreased significantly to 12.78±1.132 mg/dL post therapy. This is in agreement with Sharma *et al*. (2016) [15] and Patel *et al.* (2023) [12] who observed a significant decrease in creatinine and BUN after treatment. Dehydration can lead to decreased blood flow to the kidneys, causing ischemia and potential damage to the renal parenchyma. Acute injury to the renal parenchyma is reversible if the dehydration is corrected at the earliest.

The pre therapeutic total protein, albumin and globulin in Group I were 5.48±0.212 g/ dL, 1.33±0.066 g/ dL and 4.15±0.194 g/ dL respectively. A non significant increase in the total protein (6.03±0.209 g/ dL) and a significant increase in the albumin value was observed after therapy (2.51±0.074 g/ dL). The Mean globulin level decreased non significantly (3.51±0.195 g/ dL) The present finding is in accordance with Nandini *et al*. (2016) [11] and Patel *et al.* (2023) [12] who also observed an increase in the albumin and total protein value after treatment. Hypoalbuminemia before treatment could be due to protein – losing nephropathy or liver dysfunction. Hypoproteinemia along with hypoalbuminemia, hyperglobulinemia in concomitant TBDs in dogs could be due to chronic inflammatory disease, anorexia or decreased protein intake (Mylonakis *et al.*, 2010) [10].

The mean total bilirubin value was 1.26±0.420 mg/dL which non significantly decreased to 0.38±0.147 mg/dL after treatment. Similar findings have been reported by Nandini *et al.*, 2016 [11]. This decrease could be due to the effect of antibiotics in subsiding the infection and there by reducing hemolysis.

Group II

In this group, all the dogs were treated with combination of Doxycycline, Metronidazole, Clindamycin @ 5 mg/kg b. wt, 15 mg/kg b.wt and 25 mg/kg b.wt per os *b.i.d* respectively for a period of 21 days and Prednisolone @ 2 mg/kg b.wt per os *b.i.d* for 5 days followed by 1 mg/ kg b.wt per os *b.i.d* for next 5 days and 0.5 mg/kg b.wt per os *b.i.d* for another 5 days.

The dogs in Group II had significantly decreased mean concentration of Hb, TEC and PCV of 6.48±0.406 g/dL, 2.72±0.266 × 10^6 / µL, and 19.38±1.971 percent respectively. After treatment, there was significant increase in mean concentration of Hb, TEC and PCV to 12.98±0.839, 5.86±0.334 and 40.78±2.550 respectively. The results of the present study is in accordance with Ashwini *et al*. (2017) [2] and Lachungpa *et al.* (2020) [8] who observed a significant increase in the mean values of haemoglobin, TEC and PCV after treatment. This could be attributed to reduction in the parasitic load by the combination of antibiotics and the effect of Prednisolone for suppressing the auto antibodies targeting the erythrocyte antigens.

The dogs of this group had mean WBC concentration of 23.65±6.744 × 10^3 /µL which decreased significantly to 13.87±1.687 × 10^3 /µL after therapy. This is in agreement with Ashwini *et al*. (2017) [2] and Lachungpa *et al.* (2020) [8]. This could be attributed to the efficacious combination of antibiotic therapy and Prednisolone in subsidence of infection and reduction of stress on animal. Glucocorticoids decrease the number of lymphocytes by redistributing T-lymphocytes and B- lymphocytes in circulatory pool (Fauci, 1975) [3]. Hence few lymphocytes are exposed to antigen, which decreases the activation division of these cells. Thus affecting the counts and antibody production as only few cells are available for antibody production.

The platelet count in dogs of this group was 83±14.68 × 10^3 /µL before therapy. A significant increase in the platelet count to 337.8±98.67 × 10^3 /µL was observed after therapy. Similar findings have been reported by Ashwini *et al*. (2017) [2] and Lachungpa *et al.* (2017) [22]. This could be attributed to the addition of Prednisolone to the combination of triple antibiotic therapy. Glucocorticoids upregulate megakaryocyte gene expression associated with cytoskeleton reorganization. Upregulation of guanine deaminase is largely responsible for glucocorticoid stimulation of thrombopoiesis (Grodzielski and cidlowski, 2023) [6]. Glucocorticoids reduce the rate of platelet destruction if IMHA is associated with concurrent IMTP.

In dogs of Group II, the mean ALT concentration was 74.32±5.610 and ALP value was 418.4±73.50 IU/L. After treatment there was significant decrease in mean concentration of ALT and ALP to 31.33±1.797 and 223.2±79.95 IU/L respectively. This was in accordance with Lachungpa *et al.* (2020) [8]. The decrease in mean ALT and ALP value after treatment could be attributed to the reversal of hypoxia induced Liver damage.

The pre therapeutic mean creatinine value in dogs of group II was 1.86±0.152 mg/dL which decreased significantly on the 21st day post therapy (0.88±0.083 mg/dL). This is in accordance with Lachungpa *et al.* (2020) [8] who observed non significant decrease in the creatinine value post treatment, whereas Ashwini *et al.* (2017) [2] observed non significant difference in creatinine levels post therapy.

The dogs in Group II before therapy had BUN concentration of 30.63±2.065 mg/dL which decreased significantly to 14.52±0.542 mg/dL post therapy. Similar findings have been reported by Lachungpa *et al.* (2020) [8] who observed a non significant decrease in BUN post therapy and Ashwini *et al*. (2017) [2] reported non significant difference post therapy.

The pre therapeutic total protein, albumin and globulin in Group II were 5.96±0.210 g/ dL, 1.85±0.170 g/ dL and 4.11±0.284 g/ dL respectively. A non significant increase in the total protein (6.41±0.558 g/ dL) and a significant increase in the mean albumin (3.26±0.345g/ dL) levels was observed after therapy while the Mean globulin decreased (3.15±0.269 g/ dL) non significantly after treatment. The findings of our study is in accordance with Ashwini *et al*. (2017) [2] and Lachungpa *et al.* (2020) [8], who observed non significant increase in the total protein and albumin.

The mean total bilirubin value was 1.26±0.424 mg/dL which non significantly reduced to 0.36±0.066 mg/dL after treatment. Similar findings have been reported by Ashwini *et al.* (2017) [2] and Lachungpa *et al.* (2020) [8] who also observed non significant reduction in the total bilirubin value. This reduction could be attributed to the effect of antibiotics in subsiding the infection and the effect of prednisolone for suppressing the auto antibodies targeting the erythrocyte antigens, therby reducing the hemolysis.

Group III

In this group, all the dogs were treated with combination of Doxycycline, Clindamycin and Metronidazole @ 5 mg/kg b.wt ,15 mg/kg b.wt, 25 mg/kg b.wt and combination of Azathioprine @ 2mg/kg b.wt *per os s.i.d* for 5 days followed by 1 mg/kg *per os s.i.d* for next 5 days and 0.5 mg/kg *per os s.i.d* for another 5 days and Prednisolone @ 1.5 mg/kg b.wt *per os b.i.d* for a period of 5 days followed by 1 mg/kg b.wt *per os b.i.d* for next 5 days followed by 0.5 mg/kg b.wt *per os b.i.d* for another 5 days.

The dogs in Group III had significantly decreased mean concentration of Hb, TEC and PCV of 4.95±0.409 g/dl, 1.75±0.159 × 10^6 / µL, and 16.27±1.213 percent respectively. After treatment, there was significant increase in mean concentration of Hb, TEC and PCV to 11.08±1.459, 4.83±0.658 and 34.08±3.984 respectively. The results of the present study is in accordance with Piek *et al.* (2008) [13], Lachungpa *et al.* (2020) [8], Franco *et al.* (2021) [4] who observed a significant increase in the mean values of haemoglobin, TEC and PCV after treatment. This could be attributed to reducing the parasitic load by the combination of antibiotics and the effect of prednisolone and azathioprine due to reduced erythrophagocytosis of opsonised RBC or decreased production of antibodies by reducing lymphocytes in the circulation pool.

The dogs of this group had mean WBC concentration of 24.63±5.531 × 10^3 /µL which decreased significantly to 11.12±1.176 × 10^3 /µL after therapy. This is in agreement with Lachungpa *et al.* (2020) [8], Franco *et al.* (2021) [4]. This could be attributed to the efficacious combination of antibiotic therapy in subsiding the infection, along with Prednisolone and Azathioprine which primarily suppress lymphocyte activation and proliferation, reducing antibody production (Ghazlat, 2009) [5].

The platelet count in dogs of this group was 58.50±24.76 ^{b} × 10^3 /µL before therapy. A significant increase in the platelet count to 232.3±33.53 × 10^3 /µL was observed after therapy. Similar findings have been reported by Piek *et al.* (2008) [13], Lachungpa *et al.* (2017) [22]. This could be attributed to the addition of Prednisolone and Azathioprine to the combination of triple antibiotic therapy.

In dogs of Group III, the mean ALT and ALP concentration was 67.40±5.552 IU/L and 273.9±41.26 IU/L respectively. After treatment there was a significant increase in mean concentration of ALT and ALP to 128.4±4.639 and 551.9±33.81 IU/L respectively. A significant increase in Mean ALT levels and ALP levels was observed after treatment. The findings of the present study is in accordance with Lachungpa *et al.* (2020) []. These changes could be attributed to Azathioprine induced hepatopathy (Wallisch and Trepanier, 2015) [21].

The pre therapeutic mean creatinine value in dogs of group II was 1.73±0.098 mg/dL which decreased significantly on the 21st day post therapy (0.88±0.113 mg/dL). This is in accordance with Lachungpa *et al.* (2020) [8] who observed non significant decrease in the creatinine value post treatment.

The dogs in Group III before therapy had BUN concentration of 36.58±1.307 mg/dL which decreased significantly to 17.58±0.969 mg/dL post therapy. This is in agreement with Lachungpa *et al.* (2020) [8] who observed a significant decrease in BUN post therapy.

The pre therapeutic total protein, albumin and globulin in Group III were 5.65±0.306 g/ dL, 2.18±0.147 mg/dL, 1.51±0.132 g/ dL and 4.13±0.265 g/ dL respectively. A non significant decrease in the total protein (5.31±0.407 g/ dL) and a significant decrease in the albumin 1.51±0.132 g/ dL was observed. There was a mild decrease in Mean globulin level (3.80±0.395 mg/dL) which was not statistically significant. The findings of our study is in accordance with Lachungpa *et al.* (2020) [8], who observed non significant difference after treatment.

The mean total bilirubin value was 1.115±0.1948 mg/dL which non significantly reduced to 0.500±0.894 mg/dL after treatment. Similar findings have been reported by Lachungpa *et al.* (2020) [8] who also observed non significant reduction in the total bilirubin value. This reduction could be attributed to the effect of antibiotics in subsiding the infection and the effect of Prednisolone and Azathioprine for suppressing the auto antibodies targeting the erythrocyte antigens, therby reducing the hemolysis.

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