# BABESIA ASSOCIATED MULTIORGAN DYSFUNCTION IN DOGS AND ITS AMELIORATIVE MEASURES

## Thesis

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**By**

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# CERTIFICATE- I

This is to certify that the thesis entitled, "**BABESIA ASSOCIATED MULTIORGAN DYSFUNCTION IN DOGS AND ITS AMELIORATIVE MEASURES"** submitted in partial fulfillment of the requirements for the award of the degree of Master of Veterinary Science in the discipline of **Veterinary Medicine** of the faculty of Post-Graduate Studies, Bihar Animal Sciences University, Patna, Bihar is the bonafide research work carried out by **Dr. MENKA KUMARI, Registration No-VM0013/2018-19,** Daughter of Shri. **SURENDRA PRASAD** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

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# LIST OF ABBREVIATIONS AND SYMBOLS

| ABBREVIATIONS | | FULL FORM |
|---|---|---|
| ALT | : | Alanine aminotransferase |
| AST | : | Aspartate aminotransferase |
| ALP | : | Alkaline phosphatase |
| ALB | : | Albumin |
| ANOVA | : | Analysis of variance |
| @ | : | At the rate |
| & | : | And |
| BUN | : | Blood urea nitrogen |
| b.wt | : | body weight |
| Cm | : | Centimetre |
| Cumm | : | Cubic millimeter |
| EDTA | : | Ethylene diamine tetra acetic acid |
| *et al* | : | All others |
| etc | : | Etcetera |
| e.g. | : | Example gratia |
| FIG. | : | Figure |
| $^{o}$F | : | Degree Fahrenheit |
| > | : | Greater than |
| gm | : | Gram |
| g/dl | : | Gram per deciliter |
| g/L | : | Gram per liter |

| | | |
|---|---|---|
| Hb | : | Hemoglobin |
| HCl | : | Hydrogen chloride |
| i.e | : | That is |
| Inj. | : | Injection |
| IU/L | : | International Unit per liter |
| IM | : | Intramuscular |
| IV | : | Intravenous |
| < | : | Less than |
| MODS | : | Multiorgan-dysfunction syndrome |
| mmol | : | Millimole |
| mg | : | Milligram |
| kg | : | Kilogram |
| min | : | minute |
| ml | : | Millilitre |
| Mm | : | Millimeter |
| $M/mm^3$ | : | Million per cubic millimeter |
| % | : | Percentage |
| ± | : | Plus or minus |
| PO | : | Per oral |
| RT | : | Rectal temperature |
| RBC | : | Red blood corpuscle |
| Spp. | : | Species |
| SE | : | Standard error |

| | | |
|---|---|---|
| S.No. | : | Serial Number |
| Sec | : | Second |
| sCr | : | Serum creatinine |
| TEC | : | Total erythrocyte count |
| TLC | : | Total leucocytes count |
| U | : | Unit |
| WBC | : | White blood corpuscles |

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**DEPARTMENT OF VETERINARY CLINICAL MEDICINE**

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Title of thesis **"BABESIA ASSOCIATED MULTIORGAN DYSFUNCTION IN DOGS AND ITS AMELIORATIVE MEASURES"**

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## ABSTRACT

The present work was conducted to study the multiorgan dysfunction in dogs associated with canine babesiosis. Dogs irrespective of age, sex, and breed suffering from fever, anemia, and jaundice were screened for babesia infection. Screening of the babesia-positive infection in dogs was done by blood smear examination. Animals found positive for babesia positive in blood smear examination were subjected for clinical examination and heamato- biochemical examination. A study was done to ascertain multiple organ dysfunction cases. Affected dogs with dysfunction of more than one organ were selected for further study. Twenty-four selected dogs were divided into four groups consisting of six dogs in each group. Dogs naturally infected with babesiosis were grouped irrespective of species in the control group and three treatment groups (i.e., Group -1, 2, 3). Eighteen positive cases of canine babesiosis were selected for therapeutic management with different drugs as well as supportive therapy.

The mean values of rectal temperature; heart rate and respiration rate in dogs with canine babesiosis increased significantly ($p<0.05$) than healthy dogs. The mean values of haematological parameters; HB, TEC decreased significantly ($p<0.05$) but TLC levels increased significantly ($p<0.05$) in dogs with canine babesiosis than healthy control.

Among various biochemical parameters; the levels of ALP, AST, ALT, BUN, CRE were increased significantly ($p<0.05$), but TP and ALB values were decreased significantly ($P<0.05$) in dogs with babesiosis than healthy control dogs.

Among oxidative stress parameters the values of LPO, CAT, SOD increased significantly ($p<0.05$) but GSH values were found to be decreased significantly ($P< 0.05$) in comparison to healthy control.

In USG finding of kidney of some dogs suffering from canine babesiosis is found to be

iso-echoic to spleen, cortex diameter increases and loss of corticomedullary junction; hydronephrosis, hyperechoic cortex.

In USG finding of liver of some dogs suffering from canine babesiosis was hyper-echoic than spleen; roundish boundary suggestive of hepatomegaly

Out of Eighteen dogs that were found to be positive for *Babesia gibsoni*, five dogs were SIRS (Systemic inflammatory response syndrome) positive. The criteria for considering SIRS positive were WBC > 16000(x103/µl), HR>120 bpm and RT > 103.4 ˚F.

In the present study MODS (Multiorgan dysfunction syndrome) were assessed based on the involvement of hematological alteration in all the treated groups of dogs and it was observed that in six (33.3%) babesia affected dogs MODS was evident.

Based on various physiological; hematological and biochemical parameters all the three treatment groups were compared for the efficacy of drugs which was more successful for the treatment of *B.gibsoni* were found to be 50 % and 66.6 % in group-1 and group-2 respectively. In group-3; the recovery percent is maximum i.e 83 %.In comparison, it can be said that Azithral and clindamycin along with supportive therapy is the best module of treatment against *B.gibsoni* infection in dogs.

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# 1 INTRODUCTION

Babesiosis is a disease state caused by the protozoal parasites of the genus Babesia, order Piroplasmida, phylum Apicomplexa. In the dog, Babesia has first described in the 19th century and now four well-recognized species; viz; *B. canis* (Piana and Galli-Valerio; 1885), *B. gibsoni* (Patton; 1910), *B. vogeli* (Reichenow; 1937), and *B. rossi* are identified. Amongst these; *B. canis* and *B. gibsoni* produce comparatively severe disease in exotic breeds of dogs and mild disease in indigenous dogs. The various synonyms of this disease are canine-babesiosis; canine-Piroplasmosis; malignant jaundice; biliary-fever and tick-fever. Initially, Babesia was classified according to its morphology in erythrocytes with the large and small forms being recognized as *B. canis* and *B. gibsoni*; respectively. *B. canis* is endemic in Europe but has been reported sporadically around the world. It is transmitted by Dermacentor spp. and generally causes mild clinical signs which include anorexia; depression; fever; jaundice; anemia and thrombocytopenia. *B. vogeli* is found worldwide and transmitted by *Rhipicephalus sanguineus*. It seldom causes clinical signs. *B. gibsoni* is the most prevalent of small Babesia and endemic in Asia where it is thought to be transmitted by *Haemaphysalis longicornis*. It also occurs sporadically in the rest of the world. *Rhipicephalus-sanguineus* and other species of Rhipicephalus (3 host ticks), *Dermacentor reticulates*; *D. andersoni*, and some species of Haemaphysalis ticks are known to transmit canine babesiosis. Biliary fever is transmitted by blood-sucking ticks under natural conditions. Infection in dogs may also occur by direct transmission via blood transfer from dog bites; blood transfusion or transplacental transmission. Babesia parasites use the tick as a reservoir to reach the mammal's host. It is the second most common blood-borne parasite of mammals after the Trypanosomes. The incubation period averages about two to three weeks. It can be seen as non-pigment-forming pear or singlet-ring-shaped organisms in mammalian erythrocytes. Stage to stage transmission has also been reported by short (1936). Cerebral babesiosis in dogs has been reported by Okan (1978); while Correa (1975) found *B. canis* infection in three days and 6 days old puppies attributable to intrauterine infection. Multiplication of Babesia in vertebrate host occurs in the erythrocyte by budding process or schizogony i.e by asexual reproduction to form two, four, or more trophozoites while the sexual –phase occurs in a variety of ixodid ticks; which transmit the organism transovarially. These trophozoites are liberated from erythrocytes after rupturing of cells and further invade the fresh erythrocytes. This process is repeated till the appearance of parasitaemia which is represented by a series of binary fission followed by an invasion of large no. of circulating erythrocytes. Then sucked up trophozoites by female ticks along with blood; develop in the salivary gland by sexual and carried through the ova to the next generation

of ticks for infecting a fresh host. Piroplasm infects and replicates in the red blood cells; resulting in both direct and immune-mediated haemolytic anaemia; where the red blood cells (RBCs) are broken down through haemolysis (destruction)and haemoglobin is released into the Body. This release of haemoglobin can lead to jaundice and anemia when the body cannot produce enough new red blood cells to replace the ones being destroyed. Immune-mediated haemolytic anemia is likely to be more clinically important than parasite-induced RBC destruction; since the severity of the condition does not depend on the degree of parasitaemia. In tropical and subtropical regions; tick-borne diseases of bacterial, viral and most commonly the haemoprotozoan origin are common features (Irwin & Jefferies; 2004). The tropical climatic condition (hot &humid environment condition) of India favours the growth; development and multiplication of ticks (Jadav *et al*; 2011). As far as the diagnosis of canine babesiosis is concerned, direct microscopic examination of the stained blood smear is the most commonly used method as it is a conclusive, feasible, and cost-effective method. However, blood smears methods do not detect parasites in unapparent or chronic infections (Caccio et al; 2002). As regards, the serological methods, indirect fluorescent antibody test (IFAT) and enzyme-linked immune sorbent assay (ELISA) for *B. gibsoni* parasites, are considered to be highly sensitive, only moderately specific because of antigenic cross-reaction to *B. canis* (Yamne *et al*; 1993). Therefore, the development of a highly specific and sensitive system for the diagnosis of canine babesiosis is still awaited. In this regard, recent advances in molecular biology techniques like polymerase chain reaction (PCR) have made it possible to detect and identify piroplasm with greater sensitivity and specificity than traditional methods (Jefferies et al; 2003). Higher detection of canine babesiosis by (PCR) based assays as compared to microscopy examination has been reported by several authors worldwide indicating the higher sensitivity levels of PCR (Ionita *et al*; 2012). Multiple organ dysfunction Syndrome (MODS) is the simultaneous dysfunction of two or more organ systems. The mortality percentage of dogs suffering from MODS is high. In one study it has been observed that the dogs with two organ involvements, the survival percentage is 45 and three organ involvement 10 percent and more than four organs zero percent survivality (Matijatko et al;2010) Canine babesiosis is responsible for variable clinical manifestation; the virulence and survivality of dogs suffering from babesia vary based on the species of babesia affecting the dogs. This disease is clinically characterized by high fever varying from 40.0˚C to 42˚C (104˚F to 107˚F) for 15-20 days. Rapid-respiration; accelerated –pulse; inappetence; debility and haemolysis with anaemia; hyperbilirubinuria; haemoglobinuria; coagulopathies and organ failure.

Haemolytic anemias, systemic inflammatory response with clinical manifestation of the liver, kidney, pulmonary and cerebral dysfunction were reported by several workers (Jacobson, 2006;

Irwin,2009). In a few cases of acute renal failure, hepatopathy and myopathy were recorded in clinical cases of dogs suffering from canine Babesiosis. The disease is widely prevalent in and around Patna in, Labrador; Doberman's; German shepherd breeds of dogs. This infection in canines causes great loss of pet animals every year. Therefore; proper health coverage of pet animals against this haemoprotozoal disease is of paramount importance. Accurate diagnosis; rational treatment and proper control measures of such a fatal disease require an evolution of precise practical and economical methods to save and protect the susceptible costly pets animals. The Babesia spp. that infects dogs has dissimilar drug susceptibilities and responds differently (Solano-Galleno and Baneth, 2011). A few drugs and drug combinations are used in the treatment of canine babesiosis often without complete parasite elimination leaving treated dogs as a carrier which could relapse with clinical manifestation and multiple organ damage. It is therefore important to treat babesial infections with the most effective anti-protozoal drugs or their combinations. The only method of diagnosis of the disease seems to be the demonstration of pear-shaped protozoa in the erythrocytes of affected animals but by the time the disease has been diagnosed the prognosis of the cases further worsens. Therefore; systematic studies for the early diagnosis and the follow-up rational therapy be undertaken immediately to protect them from their diseases. This objective could be achieved by adopting a better diagnostic technique for immediate diagnosis and specific rational therapy. Very limited studies appeared to have been done on haematological and biochemical changes of blood. Thus, it was thought imperative to investigate these aspects of the disease in the present study to find out a reliable method of diagnosis, treatment, prevention and control of the disease. New formulation for a rational therapy based on specific haematological and biochemical changes of blood would also help much for specific recommendations in field cases. In this way, it is necessary to investigate the new formulation of drugs or a combination of drugs for achieving spectacular success in the treatment of this disease. Therefore, keeping these facts in view this research is planned to evaluate the effect of canine babesiosis on single and multiple organ damage and its therapeutic management with the following objectives.

## OBJECTIVES

1. To study the effect of canine babesiosis on the liver, kidney.
2. To evaluate the RBC dynamics in Babesia-affected dogs.
3. To evolve a package of practice for the management of MODS in dogs caused by canine babesiosis.

# 2 REVIEW OF LITERATURE

## 2.1 Prevalence, transmission and pathogenesis of canine babesiosis

### 2.1.1. Overall prevalence of Babesiosis

(Ristic *et al*., 1982; Ristic,1988) reported babesia species are tick-transmitted intra-erythrocytic protozoan parasites of the phylum Apicomplexa with worldwide distribution.

Patton (1910) reported a first-time small piroplasm infection in canids.

(Boozer and Macintire,2003; Boozer and Macintire,2005 and Taboada and Lobetti,2006) reported multiple species have been documented to infect dogs: *Babesia canis*, *Babesia gibsoni*, *Babesia microti*, *Babesia equi*, *Babesia conradae* (California), and a large unnamed Babesia organism (North Carolina).

(Taboada and Lobetti,2006) reported among subspecies, *B. canis* subsp. canis (moderately pathogenic and *B. canis* subsp. Rossi (severely pathogenic) is limited to Europe and Asia and South Africa, respectively.

(Macintire *et al*.,2003; Taboada and Lobetti,2006; Kraje,2001; Birkenheuer *et al*., 2003) reported *B. canis* subsp. vogeli is the least pathogenic strain and is found in tropical and subtropical areas.

(Birkenheuer *et al*.,2003; Birkenheuer et al.,1999; Irizarry-Rovira et al.,2001; Kocan et al.,2001; Macintire et al.,2002) reported *Babesia gibsoni* (Asian genotype) is an emerging infectious disease with expanding region of endemicity.

(Uilenberg et al., 1989) reported canine babesiosis has been attributed to infection with *Babesia canis*, large babesia species, or Babesia conradae, small Babesia species.

(Kjemtrup *et al*.,2006) found that small babesiosis of dogs occur in South-East Asia, North East Africa, Spain Australia, and the USA

(Conrad *et al*., 1991) found that babesia gibsoni have been reported in India, Korea, Malaysia, Ceylon, and the USA.

(Ayoob and Prittie, 2010) reported particularly in the last decade, an increasing number of Babesia spp. Infections have been described. To date, four Babesia species are documented to

infect dogs in North America: *B. canis*, *B. gibsoni*, *B. conradae*, and a large unnamed Babesia species (North Carolina).

(Sundar *et al*., 2004) reported 0.1% of dogs in Chennai were found positive for *Babesia gibsoni*.

(Chaudhuri,2006) found 9% and 22%of dogs in Utter Pradesh and Assam to be infected with Babesia spp. respectively.

Varshney and Dey (1998) reported the prevalence of babesiosis 0.66% in referral canines at Bareilly in a small-scale study concluding *B. gibsoni* was more prevalent (83.33%) than that by *B. canis* (16.67%).

Saud and Hazarika (2000) reported a 21.68% overall prevalence of canine babesiosis in Guwahati, Assam, India during 1997-1998.

Saud *et al*., (2000) recorded a 21.67% prevalence of babesiosis in dogs in Guwahati, Assam in India.

Bastos *et al*., (2004) carried out a retrospective study of clinical cases of babesiosis in dogs examined at the Veterinary Hospital (Universidade Federal de Minas Gerais) from March 1998 to September 2001. Out of 194, 145 were confirmed to be infected of which 61 dogs were infected with *B. canis* (42%).

Matsuu *et al*., (2004) screened 141 dogs for the incidence of *Babesia gibsoni* infection in Aomori Prefecture, northeastern Japan by using PCR assay and recorded 29.8% (42/141) prevalence of *B. gibsoni* infection.

Miyama *et al*., (2005) carried out an epidemiological survey of dogs suspected of having *B. gibsoni* infection using the Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA). Thirty-five of 115 such dogs (30.4%) were found positive by PCR and/or ELISA.

Samradhni *et al*., (2005) recorded a 64.28% prevalence of babesiosis from suspected 377 dogs for haemoprotista infections.

Ahmed *et al*., (2007) reported the overall prevalence of canine babesiosis in Lahore, Pakistan during a period from January 2004 to December 2005. Overall, 12.49% and 13.97% of these diseases for two ears i.e., 2004-2005 respectively and overall, 81.5% and 18.5% were for **B**. *canis* and *B. gibsoni* respectively for two years.

Ungar *et al*., (2007) screened 7243 cases of dogs referred to Salvador and Metropolitan Region, Bahia, from September 1991 to February 2005 for Babesia spp infection. Blood smear examination revealed overall prevalence was 33.95% for canine babesiosis.

Bashir *et al*., (2009) recorded 2.62% prevalence from 624 blood samples of dogs in Lahore over 12 months from January to December 2006 and recorded that crossbreds were more prone to infection (10.9%) than purebreds.

Kumar *et al*., (2009) screened 4190 peripheral blood smears to find out the prevalence of haemoprotozoans in canines at Madras Veterinary College Teaching Hospital. The peripheral blood smear from the ear tip was collected from dogs exhibiting all or one of the following clinical signs- progressive anaemia, haemoglobunuria, icterus, tick infestation. Pyrexia or enlarged lymph nodes. On blood smear examination 485 (11.6%) blood smears were found to be positive out of 4190 samples. Amongst positive cases, the majority of haemoprotozoand identified were *Babesia gibsoni* (84.9%) and *Babesia canis* (3.9%).

Godara *et al*., (2010) investigated the prevalence of parasitic infections in dogs maintained by urban society in the semiarid Jaipur (Rajasthan) from September 2007 to August 2008. Out of 61, the overall prevalence of haemoprotozoan infection was 16.39%, and Babesia spp. 13.1%.

Selvaraj *et al*., (2010) screened 4896 samples of dogs for the blood parasites. Among them, 426 (8.7%) cases were found to be positive for Babesia infection in which 398 (69%) cases were of *Babesia gibsoni* and (31%) were due to *Babesia canis*.

Abd Rani *et al*., (2011) screened 525 blood smears for the survey of canine tick-borne diseases (TBD) in India by microscopic and PCR from blood samples of dogs obtained from Delhi (n=162) Mumbai (n =101) and Ladakh (n=101) recorded prevalence of babesiosis as *Babesia vogeli* (5.5%) and *Babesia gibsoni* (0.2%). Concurrent infection with more than one TBD pathogen occurred in 39% of cases.

Nalubamba *et al*., (2011) conducted a study on the epidemiology of canine babesiosis in Lusaka, Zambia for 18 months and recorded a 30.18% prevalence of *Babesia canis* infection. Out of 1196 dogs, 361 dogs were positive large-sized Babesia canis infection by microscopic examination of stained blood smears.

Wadhwa *et al*., (2011) recorded a 1.15% incidence of canine babesiosis among 1570 cases brought to College of Veterinary clinics, Palampur, Himachal Pradesh.

Chaudhary (2012) carried out an epidemiological survey of canine babesiosis for 12 month period in Lahore. Out of 6204 dogs, 2.62% were found positive for babesiosis.

Singh *et al*., (2012a) recorded the occurrence of haemoprotozoan infection in 634 dogs for haemoprotozoan parasites presented Small Animal Clinics, GADVASU, Ludhiana, India during the year 2010. Examination of Giemsa-stained peripheral blood smears exhibited 10.21% (47/460) haemoprotozoan comprising of *B. gibsoni* (8.26%) and *B. canis* (0.65%).

Singh *et al*., (2012b) screened 532 blood samples for the prevalence of canine babesiosis at Small Animal clinics, GADVASU, Ludhiana, (Punjab) during a period of one year (January 2011 to December 2011). Overall prevalence was 5.82%) (31/532) comprising of *B. gibsoni* (5.45%) and *B. canis* infections (0.37%) by microscopic examination of Giemsa-stained peripheral blood smears.

Bhattacharjee *et al*., (2013) reported the prevalence of haemoparasites in dogs brought to the Teaching Veterinary Clinical Complex of the College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, and North-East India during January 2009 to December 2010. Out of 2104, 424 dogs were considered suspicious for having haemoparasitic infection based on case history (depression, inappetence, lethargy, and fever, abnormal coloration of stool and urine, and history of tick attachment). Overall prevalence was 57.31% in the hospital population comprising pet (58.03%) and working (54.54%) dogs and 63.64% in the stray dog population. Amongst them, the most predominant haemoprotozoan species were *B. gibsoni* (47.16%) and *B. canis* (1.41%) by blood smear examination.

Razi Jalali *et al*., (2013) carried out the epidemiological survey of Babesia infection in 400 dogs referred to Veterinary Teaching Hospital of Shahid Chmran University of Ahvaz and 5 villages around Ahvaz from November 2008 to March 2010 reporting an overall 3.75% prevalence of *B. canis.*

Shrivastava and Shukla (2013) screened a total of 1500 dogs for the prevalence of canine babesiosis from August 2010 to January 2011 in and around Jabalpur city. The overall prevalence of haemoprotozoa was 10.67% and that of Babesia spp. 6.93% (104 cases).

Bhjattacharjee *et al*., (2014) reported the seroprevalence of vector-borne parasites in naturally exposed dogs of Assam, India by IFAT. Seropositivity against *B. gibsoni* and *B. canis* was found comparatively higher in-hospital dogs (84.0% and 22.0%) than the street dogs (73.3% and 10%).

Konto *et al*., (2014) reported a 12% prevalence of *B. canis* in Maidugiri, North-Eastern Nigeria from December 2009 to November 2011.

Singh *et al*., (2014) studied 214 blood samples from dogs in and around Ludhiana. Punjab (India) is suspected of canine babesiosis. Overall prevalence was 7.47% (16/214) by Microscopic examination of stained thin blood smear for canine babesiosis encompassing 0.93% (2/214) of large Babesia and 6.54% (14/214) of *Babesia gibsoni.*

Shrivastva *et al*., (2014) screened a total of 1680 cases to study the epidemiological pattern of prevalent haemoprotozoa of dogs in Jabalpur (MP). The overall prevalence of haemoprotozoa from November 2012 to October 2013 was 10.60% and the prevalence of Babesia spp. was 1048%.

Das *et al*., (2015) screened 226 numbers of dogs in and around Kolkata from November 2012 to July 2013 for babesiosis. Out of 226m, 72 animals (31.86%) were found clinically and cytological positive comprising 68 and 4 were found infected with a small and large form of Babesia spp. respectively.

Gabrielli *et al*., (2015) recorded a 21.5% prevalence of canine babesiosis in Serbia during the year 2012-2014.

Jadhav *et al*., (2015) studied the epidemiology of canine babesiosis in Gujarat and that was 15.81%.

Kumar *et al*., (2015a) screened a total of 204 canine blood samples for prevalence canine Babesiosis with a history of fever (104°F to 105°F), were collected and examined at Regional Disease Diagnostic Laboratory, Jalandhar (Punjab) during a period of one year (April 2013 to March 2014). Examination of blood smears revealed 8.33% (17/204) of canines were positive for canine babesiosis comprising of *B. gibsoni* 7.84% (16/204) and *B. canis* 0.49% (1/204).

Kumar *et al*., (2015b) screened 432 blood samples collected from dogs presented at veterinary polyclinic Jalandhar, Punjab from April 2014 to December 2014. Blood smears examination revealed that 6.01% (26/432) of canines were positive for canine babesiosis. A higher prevalence of *B. gibsoni* (5.45%) was recorded as compared to *B. canis* infection (0.23%).

Nalubamba *et al*., (2015) carried out retrospective and prospective analysis of clinical records of dogs diagnosed with Babesia infections of the years 2000 to 2013 from practices in Lusaka,

Zambia. Records of 363 dogs with confirmed Babesia infections, in which the highest proportion were mongrels (32.2%).

Rene Martellet *et al*., (2015) screened 140 dogs for babesiosis in southern France from 2010 to 2012 by PCR amplification reporting the prevalence of *B. vogeli* and *B. canis* were 13.6% and 12.9% respectively.

Laia Solano-Gallego *et al.,* (2016) highlighted that canine babesiosis represents a group of diseases and that many species can infect dogs in Europe. Therefore, accurate detection and species recognition are crucial for selecting the most appropriate treatment and determining the most accurate prognosis.

### 2.1.1.2 Breed-wise prevalence of babesiosis

Unger *et al*., (2007) reported higher frequencies of infection were detected in Akita Inu 48.61%, Pitbull 46.91%, Rottweiler 42.23%, Cocker Spaniel 41.93%, not defined breed 41.60% and Boxer 40.47% breeds.

Bashir *et al*., (2009) recorded that crossbreds were more prone to infection (10.9%) than purebreds.

Selvaraj *et al*., (2010) recorded the prevalence of babesiosis in different breeds of dogs in which non-descript dogs were found to be most commonly affected with an incidence of 20% followed by spitz 12%.

Chaudhary. (2012). Recorded crossbreds were more prone to infection than purebreds.

Shrivastave *et al*., (2014) reported 10.60% haemorprotozoa out of 1680 dogs in Jabalpur (Madhya Pradesh). During the study, the maximum prevalence was noticed in the German Shepherd breed, i.e., 15.47% followed by Samoyed, Pug, Non-descript, and Spitz dogs in which prevalence was found to be 15.25%, 12.50%, 10.94%, and 10.13%, respectively. There was significant variation ($P < 0.05$) in the prevalence of babesiosis in various breeds.

Bastos *et al*., (2004) recorded higher frequencies of infection were detected in German shepherd dogs (16.6%), poodles (13.3%), and Rottweilers (11.6%), demonstrating that the disease occurs in dogs of different sizes.

Jadhav *et al*., (2015) recorded a higher incidence of infection in Labradors and Pomeranians breeds of dogs.

### 2.1.1.3. Sex-wise prevalence of babesiosis

Bashir *et al*. (2009) recorded a 2.62% prevalence from 6204 blood samples of dogs in which male dogs were more prone to disease than female dogs (3.39 vs. 1.32%).

Chaudhary (2012) reported that male dogs were more prone to disease than female dogs (3.39 vs. 1.32%).

Singh *et al*., (2012b) recorded prevalence of babesiosis was comparatively higher in females (6.22%) as compared to males (5.57%).

Shrivastave and Shukla (2013) reported 6.93% cases of canine babesiosis, out of 1500 dogs in and around Jabalpur city. Amongst 104 cases positive for Babesia sp., 36.54% were male and 63.46% female, indicating a higher percentage of females among affected animals.

Knoto *et al*., (2014) recorded female dogs were more infected 32 (66.7%) with babesiosis than the male dogs 16 (33.3%).

Shrivastave *et al*., (2014) reported 1060% haemoprotozoa in Jabalpur (Madhya Pradesh). Among them, a sex-wise prevalence study revealed 14.77% prevalence in females as compared with 7.38% in males, Significant difference ($P < 0.05$) was noticed in the presence of both sexes and relative risk analysis revealed that female dogs have two times higher risk of babesiosis as compared to male dogs.

Das *et al*., (2015) recorded a higher incidence of babesiosis in female dogs (53.33%) than male dogs (46.68%).

Kumar *et al*., (2015c) reported a prevalence of 6.01% (n=26) of 432 cases of which, the prevalence was comparatively higher in females (6.42%) than male dogs.

#### 2.1.1.4. Age-wise prevalence of babesiosis

Unger *et al*., (2007) reported the frequencies of Babesia spp. infected dogs by age groups were high for those under twelve months old (42.87%) followed by twelve to forty-eight months old dogs (34.63%) and over forty-eight-month-old dogs (34.38%).

Bashir *et al*., (2009) recorded a 2.62% prevalence from 62.4 blood samples of dogs in which incidence of disease was higher in younger dogs (6.9%) than older age groups.

Selvaraj *et al*., (2010) reported that *Babesia gibsoni* was more prevalent in the canine pediatric population 58% (less than three months) of Chennai.

Nalubamba *et al*., (2011) mentioned that dogs younger than 1 year were more likely to be Babesia positive followed by those between 2 and 5 years old.

Chaudhary (2012) recorded a higher incidence of babesiosis in younger dogs than in older age groups.

Singh *et al*., (2012) recorded the prevalence of *B. gibsoni* in all age groups but *B. canis* was recorded only from the dogs above 1 year of age.

Shrivastave and Shukla (2013) reported 6.93% cases of canine babesiosis from a total of 1500 dogs in and around Jabalpur city. Among the positive cases, 15.38% were under 1 year age, 20.19% of 1-3 year age, and 64.42% of more than 3 years age, This observation reveals infection is more occur in adult than young ones.

Konto *et al*., (2014) conducted a study on 400 dogs. Out of 48 dogs, the ages of 1-6 Months had the highest *B. canis* infection rate of 58.3% while 6-12 and 24-120 months had 12.5% and 29.2% respectively.

Shrivastava *et al*., (2014) screened 1680 dogs among the prevalence of haemoprotozoa was 10.60%. The age-wise prevalence of babesiosis revealed the highest prevalence (13.27%) in the 1-3 years age group, followed by 12.94% prevalence in dogs of 5-7 years age and 12.92% prevalence in 7-9 years age group. However, the lower prevalence was reported in the dogs of, 1 year age (7.32%). The age-wise prevalence showed significant variation ($P < 0.05$) among the groups.

Kumar *et al*., (2015c) reported a prevalence of 6.01% (n=26) of 432 cases of which incidence of disease was higher in >1 year (20/26) than <1 year (6/26) age groups.

### 2.1.1.4. Transmission of babesiosis

(Taboada and Lobetti,2006; Friedhoff,1998) reported the adult female tick is considered most important in vector transmission as transstadial and transovarial (not documented with *B. gibsoni*) infection occur.

(Taboada and Lobetti, 2006; Friedhoff,1998; Igarashi et al.,1988) reported the babesial merozoites results in the production of sporozoites (infective undeveloped cells) within the arthropod salivary glands.

(Fukumoto et al.,2005; Taboada,1998) reported most pets to develop babesiosis infection in neonates as a result of transplacental transmission from the dam, although vector-borne transmission is the natural means.

(Stegeman,2003) reported babesiosis infection transmitted by transfusion from an infected blood donor.

### 2.1.1.5. Pathogenesis /Immune Response

(Homer et al.,2000; Ristic et al.,1982) reported Babesia spp caused disease in the vertebrate hosts through a combination of both direct parasitic induced damage and secondary immune-mediated effects

Wright and Goodger (1988) observed that the pathogenicity of Babesia is determined by many variables including the species and strain of Babesia organisms, and the age and immunologic response of the host. Of this species and strain appear to be most important.

(Homer *et al*.,2000; Ristic,1988) observed as a general rule that the small Babesia organisms are more virulent than large Babesia spp.

Taboada and Lobetti (2006) concluded that the following infection, a variable immune response is generated, dependent upon host factors, infected erythrocytes display parasite antigens on their cell membranes that lead to antibody production, opsonization, and the removal of infected cells by the mononuclear phagocytic cells of hemolymphatic system. Although survivors exhibit no clinical signs, they remain chronic carriers as the immune system is incapable of completely eradicating the infection.

## 2.2 Diagnosis

(Irwin,2010) reported babesiosis should be suspected when a dog is presented to the veterinarian with any of the clinical signs listed earlier or if anaemia or thrombocytopenia is discovered if there is (a history) of tick exposure or history of the animal living in or previous travel to a tick-endemic area, or recent injury from a dog fight should prompt a specific investigation for Babesiosis.

Bourdoiseau (2006) reported in temperate climates, there is a seasonal increase in the incidence of babesiosis during the spring and summer months when the tick vectors are more active and abundant and a decrease in the fall and winter.

(Irwin and Jefferies,2004; Wang et al.,2010) reported in tropical and subtropical climates, the incidence of disease remains unchanged throughout the year.

### 2.2.1 Microscopic examination

Taboada and Lobetti (2006) concluded the definitive diagnosis of Babesia infections requires demonstration of organisms within RBCs. Light microscopic evaluation of blood smears is an excellent diagnostic tool for acute infections with moderate or high parasitaemia. It is often unrewarding, however, in peracute or chronic infections, asymptomatic carriers, and patients with circulatory compromise.

(Abdullahi *et al*.,1990) reported evaluation of smears prepared from capillary blood, such as the ear tip or nail bed enhances the likelihood of organism detection because parasitized erythrocytes tend to sludge in the capillaries.

(Mattia *et al*.,1993) reported evaluation of smears prepared from a concentrated and stained buffy coat 9 Percoll gradient separation) may facilitate diagnosis, as babesia organisms preferentially parasitize reticulocytes over mature RBCs.

### 2.2.2 Clinical signs

(Botros *et al*.,1975; Farwell et al,1982; Groves and Dennis,1972; Birkenheuer et al.,1999; Macintire et al., 2002) reported Infection with *B. gibsoni* (Asian genotype) can result in severe clinical manifestations in some dogs, while others only exhibit mild clinical signs and minimal hematologic or biochemical abnormalities.

Ayoob and Prittie, (2010) observed that canine Babesiosis can be classified as uncomplicated or complicated. An uncomplicated presentation refers to hamolytic anaemia and its accompanying clinical signs (fever, pallor, anorexia, depression, splenomegaly, and tachycardia with hyperdynamic pulse pressures). These signs range from mild to severe and are life-threatening. They referred complicated Babesiosis to clinical manifestations not directly associated with hemolytic anaemia, including cardiovascular, respiratory, hepatic, renal, gastrointestinal, neurologic, and coagulopathic dysfunction.

(Control *et al*., 1991; Freeman et al., 1994) reported the clinical presentation of Babesia spp. infection ranges from peracute to subclinical. Peracute infection is rare and is characterized by severe, extensive tissue damage and a high mortality rate.

(Jacobson and Clark, 1994; Abdullahi., 1990; Irwin and Hutchinson, 1991) reported dogs with acute babesiosis typically present, fever, lethargy, anorexia, splenomegaly, lymphhadenomegaly, thrombocytopenia, and vomiting.

(Taboada and Lobetti, 2006) reported although fatalities are common in puppies with acute disease, adult dogs with acute babesiosis typically survive with appropriate therapy.

(Jacobson and Clark, 1994) reported chronic infection is recognized but its manifestation is poorly characterized.

(Masuda *et al*., Taboada and Lobetti,2006) reported although most subclinically infected dogs never manifest clinical signs, the disease can be precipitated by stress or glucocorticoid therapy).

(Homer *et al*.,2000) reported the most commonly accepted theory is that the immune-mediated hemolytic anaemia is due to Soluble parasite Antigens (SPA) binding to the red blood cell surfaces either through antibody or complement-mediated hemolysis.

(Homer *et al*., 2000) examined in almost all cases, animals can develop a protective immune response after infection and recovery or immunization.

(Homer *et al*., 2000; Ristic et al., 1982) the reported protective immune response does not prevent reinfection but does decrease the degree of parasitaemia, morbidity, and mortality when animals are re-exposed to the parasite.

Abdullahi *et al*., (1990) reported clinical findings in dogs with natural cases of *Babesia canis* infection in Nigeria. They reported that the acute form of the disease was most common. Consistent clinical signs of the acute form included abnormal appetite, lethargy, fever, anaemia, generalized lymphadenopathy, splenopathy, emaciation, and icterus. Anaemia was of the regenerative type in all cases while neutrophilic leukocytosis was mainly observed in hyperacute cases.

Welzl *et al.* (2001) studied systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndromes (MODS) in dogs with complicated babesiosis, and to assess their

impact on the outcome. Ninety-one cases were evaluated retrospectively for SIRS and 56 for MODS. The liver, kidneys, lungs, central nervous system, and musculature were assessed. Eighty-seven percent of cases were SIRS-positive. Fifty-two percent of the cases assessed for organ damage had single-organ damage and 48 % had MODS. The outcome was not significantly affected by either SIRS or MODS, but the involvement of specific organs had a profound effect. Central nervous system involvement resulted in a 57 times greater chance of death and renal involvement in a 5-fold increased risk compared to all other complications. Lung involvement could not be statistically evaluated owing to co-linearity with other organs but was associated with high mortality. Liver and muscle damage was common but did not significantly affect the outcome.

Lobetti and Jacobson (2001) observed that proteinuria, renal tubular casts, and epithelial cells in urine sediment, are commonly observed in both complicated and uncomplicated babesiosis. Renal function and integrity were evaluated using serum urea and creatinine, serum electrolytes (sodium and potassium), fractional clearance of sodium (FcNa) and potassium (FcK), the urine enzyme activity of gamma-glutamyl transpeptidase and alkaline phosphatase, urine protein: creatinine ratio, and urinalysis. All babesiosis groups showed well-concentrated urine elevated serum urea and creatinine. Hyperkalaemia with marginal hyponatraemia was present in a minority of dogs in all groups. Proteinuria was a common finding with mild renal tubular damage in canine babesiosis

Varshney *et al*., (2003) studied 100 clinical cases of canine babesiosis due to *Babesia gibsoni* infection. Cases of babesiosis were scattered throughout the year but 60% were observed from March to June (summer). The disease was acute in pups and a chronic form in adults. Pyrexia was inconsistent and haemoglobinuria, icterus, and ataxia were of rare occurrence. Sonography revealed both hepatic and splenomegaly.

Bastos *et al*., (2004) reported clinical signs associated with 61 dogs infected with *Babesia canis*. The most frequent clinical signs were fever, apathy, anorexia, weight loss, dehydration, abdominal pain, and kidney sensitivity to palpation.

Furlanelloa *et al*., (2005) reported 23 cases of canine babesiosis. The main clinical signs were dehydration (100%), apathy (74%), anorexia or decreased appetite (70%), and fever (68%). The anaemia was present in 74% of the dogs and thrombocytopenia in all dogs.

Ahmed *et al*., (2007) reported clinical signs of canine babesiosis and were characterized by high temperature (from 103°F- 105°F), anaemia, of feed, dehydration, pain on palpation at abdomen, labored breathing. Acute infection was characterized by pyrexia, weakness, mucous membrane pallor, depression, lymphadenopathy, splenomegaly, and general malaise.

Dantas-Torres (2008) mentioned that diagnosis of canine babesiosis is usually based on the presence of suggestive clinical signs (e.g., apathy fever, anorexia, weight loss, pale mucous membranes, and jaundice) and patient history.

Maele *et al*., (2008) reported one case of the cerebral form of babesiosis in 10-year old male Akita Inu referred to the Faculty of Veterinary Medicine with a history of seizures lasting from 3 days, weakness, and anorexia. The clinical signs were elevated temperature (39.8°C), cardiac and respiratory auscultation was normal (heart rate 90 beats/min, respiratory rate 30 breaths/min), mucous membranes were pale pink, and capillary refill time was 2 s. Dehydration was estimated clinically at 5%. Results from a neurological examination were normal, except for dullness. An enlarged spleen was palpated. Urinalysis abnormalities included pyuria, haemoglobinurai, and bilirubinuria. Urine-specific gravity was highly compatible with clinical dehydration.

Varshney *et al*. (2008) studied 102 naturally occurring cases of babesiosis, caused by *B. gibsoni* in dogs at Nandini Veterinary Hospital, Surat The Most common clinical signs noted were anorexia, vomiting, subnormal, normal or increased basal body temperature; emaciation/weight loss; melena; constipation/ diarrhea; tachycardia arrhythmia; tachypnoea/ dyspnoea; haemorrhages (epistaxis); pale/ congested/yellow mucus membranes; nervous deficit; ascites/edema; circling/paresis/ataxia/dullness/depression; increased salivation; splenomegaly and/or hepatomegaly to death) Pyrexia was inconsistent, haemoglobinuria was not seen in any case and jaundice was observed in 5 cases only.

Schoeman (2009) observed that canine and feline babesiosis are diseases characterized by haemolytic anaemia, icterus, and hemoglobinuria. The clinical manifestation of the disease ranges from chronic or subclinical to peracute and fatal, depending on the virulence of the species and the susceptibility of the host.

Cardoso *et al*. (2010) studied 38 dogs having a *B. canis* infection and recorded clinical signs were lethargy (n =24;63%), red urin (n=19;50%), hyperthermia (n=18;47%), anorexia (n=17;45%), pale mucous membranes (n=17; 45%), hypothermia (n=9; 24%), yellow mucous

membranes (n=5; 13%), vomiting (n=4; 11%), abdominal pain (n=3; 8%), ataxia (n=2 5%), uterine discharge (n= 2; 5%), cough (n= 1; 3%), gingival petechiae (n= 1; 3%) and ocular discharge (n=1 3%).

Selvaraj *et al*., (2010) observed that the cases of canine babesiosis having a clinical sign ranged from peracute to subclinical and chronic forms. Acute forms were characterized by fever, lethargy, hemolytic anaemia, lymphadenopathy, splenomegaly, and coagulopathy was found to be the major presenting sign as well as the complicating factor in many cases.

Wadhwa *et al*., (2011) confirmed 18 cases of babesiosis in dogs by blood smear examination Clinical examination revealed elevated rectal temperature (103.90 ± 0.62°F), tachycardia (119.66 ± 13.86/min), and polypnoea (47.83 ± 4.49/min). The most common clinical symptoms observed were inappetence to anorexia, lethargy, recurrent fever, pale mucus membranes, and emesis. Haemoglobinurea was in three *B. canis* positive cases. Bilateral hind limbs edema, ascites, jaundice, and nervous signs were also seen in a few cases, additionally, constipation, diarrhea, melena, salivation, nasal discharge, coughing, and ocular discharge were concurrent signs.

Yadav *et al*., (2011) reported one case of *Babesia gibsoni* infection in 16 months old Labrador bitch presented to Teaching Veterinary Clinical Services Complex, Apollo College of Veterinary Medicine, and Jaipur with a history of anorexia, dullness, weight loss, epistaxis, vomition, constipation, distended abdomen and dribbling urination. Clinical signs were yellowish discoloration of all visible mucus membranes including skin, slight dyspnea, rectal temperature 101.2°F, pulse rate 68/minute, respiratory rate 28/minute, and heavy tick infestation. Ultrasonographic examination revealed hypoechoic images in the liver with enlargement of the liver.

Janus *et al*., (2012) reported one case of a cerebral form of babesiosis in a dachshund pup with a history of inappetence, occasional convulsions, and debility and clinical signs were pale and icteric mucous membranes, rectal temperature was subnormal and Blood smear examination revealed characteristic pyriform shaped *Babesia canis* organism in pairs in more than 50 percent of the erythrocytes.

Andoni *et al*., (2013) studied the clinicopathological in twenty-nine cases of dogs naturally infected with Babesia canis and recorded main clinical signs were dehydration (89.65%),

apathy (58.62%), fever (55.1%), icterus (27.5%), petechiae (10.3%), abdominal pain (41%), anaemia in (79%) and anorexia or decreased appetite (70%).

Daste *et al*., (2013) reported one case of babesiosis in a 5-year-old Scottish terrier who was referred to the emergency department of the Ecole Nationale Veterinaire de Toulouse with clinical signs of dyspnoea and signs suggestive of central neurological disease. Thoracic radiographs showed a diffuse and heavy interstitial/alveolar lung pattern. Cerebral babesiosis and ARDS were confirmed at necropsy. Major pathological findings included erythrocyte aggregation in the lungs, liver, and brain.

Tresamol *et al*., (2013) reported cerebral babesiosis due to *Babesia gibsoni* is a four-year-old male boxer dog with was a history of depression, weakness, ataxia, occasional seizures, and anorexia. On clinical examination, the animal was anemic with pale mucous membranes and was dehydrated. The clinical data were within normal range except for a rise in body temperature (104°F).

Gintaras *et al*., (2014) reported clinical findings in 6 cases of dogs with large Babesia infection. Clinical examination revealed the presence of ticks over the body (6/6), dullness (6/6), variations in the appetite (6/6), rise in rectal temperature (6/6), tachycardia (6/6), tachypnoea (5/6), dyspnoea (5/6), congested mucus membranes with sunken eyeballs (5/6) lymphadenopathy (5/6), pallor (3/6), haemoglobinuria (3/6), tensed abdomen (2/6), yellowish mucus membranes (2/6), yellowish discoloration of the abdomen (2/6), vomitions (2/6), diarrhea (2/6), edema at the legs (1/6) and constipation (1/6). Laboratory urinalysis was done in all the dogs. Abnormal findings were noticed in three dogs included haemoglobinuria, proteinuria, bilirubinuria, and an increased amount of urobilinogen in the urine. Microscopic evaluation of the sediment revealed red blood cells as well as white blood cells, tubular epithelial cells, and crystal formation.

Sarma *et al*., (2014) reported ultrasonographic changes in 10 dogs having babesiosis. Out of ten, hypoechogenicity of the liver was observed in 30% out of which two dogs had gall bladder distension (66.66%). Thirty percent of the dogs showed ascites followed by each 20% of the cases had splenomegaly and hepato-splenomegaly.

Sivajothi *et al*., (2014) reported two different cases of dogs having infection of *B. canis*. One uncomplicated case was having clinical signs were Pyrexia (104.4°F), Tachycardia (122/min), congested mucus membranes, dullness, IN complicated case was having clinical signs were

pyrexia (103.8°F), slight yellowish pale mucus membranes, Tachycardia (132/min) and Tachypnoea (56/min) along with distress, bilateral lymphadenopathy with tensed and slight yellowish discoloration of the abdomen also had decreased urine output, with the passage of reddish colour urine along with constipation and vomitions.

Yogeshpriya *et al*., (2014) reported one case of babesiosis in a three-year-old male Labrador dog. On clinical examination, an animal had a temperature of 103.8°F and icteric conjunctival and oral mucous membrane.

Davitkov *et al*., (2015) screened 60 dogs with clinical findings compatible with babesiosis in Serbia. Infection was diagnosed by microscopic examination and confirmed by PCR. The main clinical signs were apathy, anorexia, fever, brown/red discoloration of urine, pale mucous membranes, icterus, splenomegaly, and vomiting.

Jadhav *et al*., (2015) carried out a study on canine babesiosis and recorded that the maximum number of cases had clinical signs attributable to general state (92.22%) followed by the gastrointestinal system (84.81%), cardiovascular system (60%), nervous system (32.59%) and respiratory system (12.96%). The most common clinical symptoms were fever, anorexia, anaemia, presence of ticks, vomition, diarrhea, dullness, and depression. The chronic form of the disease (48.52%) was more common than the acute (27.78%) and sub-clinical (23.70%). Mortality was positively correlated with cardiac, respiratory, and nervous involvement. Cardiac involvement either as conduction abnormalities or cardiomyopathy was found to be relatively common in *B. gibsoni* infection with electrocardiographic changes in 54.55% of the total cases.

Joice *et al*., (2015) reported different clinical signs in babesiosis in dogs presented in Teaching Veterinary Clinical Complex, College of Veterinary Science & Animal Husbandry, Junagadh Agricultural University, Junagadh for one year and recorded clinical signs of affected animals were anorexia (46%) shivering (23%) inappetence (15.3%) jaundice (0%) inability to bark (7%), blood in the urine (7%), panting (7%), chronic wound (7%) and seizures (7%).

Kumar *et al*., (2015) studied one case of three years old male German shepherd dog weighing 30 kg who was presented at Veterinary Polyclinic Jalandhar with the history of voiding blood mixed urine, vomiting, and icterus with clinical signs was the temperature of 104.6°F and icteric conjunctival and oral mucous membrane. Based upon the blood smear and clinic haematological findings this case was diagnosed as babesiosis.

Nalubamba *et al*., (2015) reported the most common clinical signs in Babesia infection were fever, pallor, lymphadenopathy, anorexia, depression/lethargy, and weight loss.

### 2.2.3. Haematological parameters

Brahma *et al*., (2019) studied the molecular examination of babesiosis and haemato-biochemical changes in canine babesiosis infected dogs. In this study, 8 cases infected with Babesia were confirmed through haematological, biochemical, and multiplex PCR. The most common clinical signs were anorexia, pale or icteric mucous membranes, high rise of temperature, and dark urine colour. The haematological parameters showed a decreased level of RBC, Hb, PCV, Platelets level.

Saud *et al*., (2000) studied haematological changes in 31 affected dogs and recorded a statistically significant ($P<0.01$) decrease in haemoglobin content, packed cell volume, and erythrocyte count which count be attributed to massive red cell destruction and depressed erythropoiesis.

Versheny *et al*. (2003) reported haematological findings in 100 clinical cases of canine babesiosis due to Babesia gibsoni and recorded a wide range of variations in haemoglobin (2-11.8 g/dl), hematocrit (6-39%), total erythrocyte (0.48-4.84 x 106/±µl).

Bastos et al. (2004) reported haematological findings in 61 dogs infected with *Babesia canis*. Haematology revealed 64.3% of dogs showed anaemia, 19.6% had a significant decrease in the PCV (15%), 62.5% had eosinopenia, 64.3% had an increase of neutrophils, and 89.3% presented anisocytosis.

Furlanelloa *et al*., (2005) studied 23 cases of canine babesiosis for haematological changes which revealed anaemia was normocytic and normochromic in all cases, erythrocyte regeneration in three dogs, haemolytic anaemia in 70% dogs, and 30% had non-hemolytic anaemia. Sixty-nine percent of dogs showed leucopenia and 74% neutropenia and leucocytosis due to mature neutrophilia and lymphocytosis.

Samradhni *et al*., (2005) studied naturally infected dogs with babesiosis which showed a statistically significant reduction in haemoglobin, packed cell volume, total erythrocyte count, thrombocytes, lymphocytes, and monocytes with an increase in total leukocyte count and neutrophils.

Niwetpathomwat *et al*., (2006) studied clinicopathological findings in 127 dogs naturally infected with Babesia spp. presented to the department of the Small Animal Teaching Hospital during the period January 2001-December 2003 and recorded hypocytic hypochromic anaemia and thrombocytopenia in most dogs. The total and differential leukocyte counts were not specific.

Ahmed *et al*., (2007) reported the laboratory findings in the case of canine babesiosis were anaemia, thrombocytopenia, hypoalbuminemia, and billirubinurea. Initially, the anaemia was normocytic, regenerative anaemia with reticulocytes.

Zygner *et al*., (2007) reported haematological changes in 248 dogs infected with large Babesia. The most common disorders in affected dogs were thrombocytopenia and anisocytosis. Low erythrocytes values were in 26.2% dogs and low haematocrit in 31.4% dogs, low haemoglobin in 29% of dogs, an increase in MCHC 21% of dogs. 60.5% of dogs presented anisocytosis, 25% poikilocytosis, 23.8% polychromasia, 19.7% hypochromic, and 4.4% erythroblastosis. Thrombocytopenia was detected in 99.5% of dogs, 36.3% of dogs had neutropenia and 21.8% presented a left shift, 14.9% had lymphocytosis ad 7.2% lymphopenia.

Maele *et al*., (2008) reported one case of the cerebral form of babesiosis in 10-year-old male Akita Inu referred to the Faculty of Veterinary Medicine. Haematology reveals anaemia, leukopenia, and thrombocytopenia.

Selvaraj *et al*., (2010) studied haematological abnormalities in babesiosis and recorded decreased values of haemoglobin (8.75 ± 0.15 g/dl) and packed cell volume (22.6 ± 0.18%), increased TLC (20.2 ± 1.12 x 103/cmm). Neutrophils were significantly increased (78%) and lymphocytes reduced (18%).

Zvorc *et al*., (2010) evaluated the haematological changes in 30 dogs naturally infected with large Babesia and recorded erythrocyte number was significantly decreased and as a consequence of erythrocyte decrease, haematocrit was also significantly low. Platelet number and haematocrit were significantly decreased. The most common abnormality in the investigated parameters was thrombocytopenia, which was observed in all cases.

Karunakaran *et al*., (2011) reported hematological changes in *Babesia gibsoni* infection in a 7-year-old German shepherd dog. Haematolgical analysis revealed a haemoglobin value of 3 g%, PCV 20%, and total RBC count 1.1 x 106/cumm.

Shah *et al*., (2011) studied four dogs infected with Babesia presented to the Department of Veterinary clinical services complex, Gadvasu, Ludhiana for haematological changes and recorded normocytic normochromic anaemia and thrombocytopenia. The total and differential leukocyte counts were not specific.

Wadhwa *et al*. (2011) reported haematological findings in 18 dogs positive for babesiosis. Haematology revealed significant decrease in Hb – 5.80 ± 0.30g/dl and Haematocrit – 20.80 ± 0.30% values and non-significant increase in TLC- 10.82 ± 2.11x103/ml, Neutrophils- 68.60 ± 6.72%, Lymphocytes- 23.00 ± 4.58%, Monocytes- 2.66 ± 1.20% and Eosinophils – 2.33 ± 1.33% values in infected dogs.

Yadav *et al*., (2011) reported haematological and biochemical changes in one case of *Babesia gibsoni* infection. Haematological findings were Hb – 9g/dl, PCV- 26%, TEC- 5.83x106/mm3, MCV- 44.59ft, MCH- 15.44pg, MCHC-34.62%, TLC- 11.75x103/mm3, DLC (%): N-72, L-27, M-1, E-0, B-0.

Andoni *et al*., (2012) reported haematological changes in six dogs which were positive for *B. canis* on blood smear examination. Haematology revealed hypocytic hypochromic anaemia and 20% of the cases had a packed cell volume (PCV) less than 24%, thrombocytopenia in all cases, and platelets counts were lower than 50x103 cell/µl indicating Babesia infection in dogs caused anaemia and thrombocytopenia.

Bhojne *et al*., (2013) reported haematological changes in two cases of babesiosis in dogs revealed haemoglobin was 4.2 gm% and 6.8 gm%.

Gintaras *et al*., (2014) screened 300 dogs at the Veterinary Academy of Lithuanian University of Health Science (LUHS) and Dr. L. Kriauceliunas Small Animal Clinic for babesiosis during 2003-2012. Among 300 dogs, 186 dogs were having haematological abnormalities and 114 dogs were with normal haematological findings which shows that babesiosis can be characterized by marked thrombocytopenia, neutrophilic leukogram profile change to lymphocytic-plasmocytic, and monocytosis. Also, there was often a tendency to anaemia and leukopenia.

Reddy *et al*., (2014) reported haematological findings in six cases of babesiosis in dogs and recorded a statistically significant reduction in RBC, Hb concentration, PCV percentage, and platelet count were recorded among infected dogs. A significant decrease in WBC count and neutropenia along with lymphocytosis and monocytosis was noted.

Shrivastava *et al*., (2014) reported 18 dogs that are positive for babesiosis on blood smear examination. Haematology reveals TEC, Hb, and PCV were significantly lower, indicating anaemia in affected animals. The TLC increased significantly with the significant increase in neutrophils and reduction in a lymphocyte in affected animals. Profound thrombocytopenia was also observed in affected animals.

Sivajothi *et al*., (2014) recorded haematological changes in one uncomplicated case of *B. canis* infection. Haemotology revealed anaemia (8.8 g/dl), TEC (4.9x106/cumm), leukocytosis (9600/cumm) with neutrophilia (6912/cumm), lymphocytosis (2496/cumm) and eosinophilia (192/cumm).

Sivajothi *et al*., (2014) recorded haematological changes in one complicated case of *B. canis* infection. Haemotology revealed leucocytosis (10640/cumm), Neutrophils (7236/cumm), Lymphocytes (3192/cumm), and Eosinophils (212/cumm) and decreased platelet count of (82,000/µl).

Vishnurahav *et al*., (2014) studied haematological changes in six dogs that were positive for *Babesia gibsoni* on blood smear examination. Haematology revealed TEC, Hb, and PCV were reduced. There were lymphocytosis and Thrombocytopenia.

Yogeshpriya *et al*., (2014) reported one case of babesiosis. Haemogram revealed leukocytosis (22,000/cumm) with neutrophilia. Haemoglobin and volume of packed cells were 5% and 16%, respectively.

Davitkov *et al*., (2015) reported 60 cases of Babesia infection in dogs in Serbia. The main clinicopathological findings were slight to severe thrombocytopenia and mild to very severe normocytic normochromic anaemia.

Kumar *et al*. (2015) studied one case of babesiosis in three years old male German shepherd. Haemogram revealed leukocytosis (22,000/cumm) with neutrophilia. Haemoglobin and volume of packed cells were 5.5 g% and 18%, respectively.

Nalubamba *et al*., (2015) reported laboratory findings in 363 cases of Babesia infection in dogs. The most consistent haematological abnormalities were anaemia (96.4%), nucleated erythrocytes (42.2%), and hypochromasia (34.7%) cases.

## 2.2.4. Serological parameters

Brahma *et al*., (2019) studied the molecular examination of babesiosis and haemato-biochemical changes in canine babesiosis infected dogs. In this study, 8 cases infected with Babesia were confirmed through haematological, biochemical, and multiplex PCR. The most common clinical signs were anorexia, pale or icteric mucous membranes, high rise of temperature, and dark urine colour. The biochemical parameters showed an increased level of WBC, ALT, ALP, Total bilirubin, BUN, and creatinine value.

Saud and Hazarika (2000) studied biochemical changes induced by canine babesiosis and the author recorded significant increases in blood glucose, blood urea nitrogen, conjugated bilirubin, serum alkaline phosphatase, serum glutamic pyruvic transaminase, and serum glutamic oxalacetic transaminase and a significant decrease in total serum iron levels.

Varshney *et al*., (2003) reported serological findings in 100 clinical cases of canine babesiosis due to Babesia gibsoni and recorded serum alkaline phosphatase (28 to 400 U/L). ALT (40 to 460 U/L) and AST (50 to 520 U/L) activity values also varied from case to case.

Scally *et al*., (2004) carried out retrospective screening of approximately 7000 recorded cases screened at the Section of Clinical Pathology, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, the University of Pretoria to know urea and creatinine values are reliable indicators of azotaemia in canine babesiosis or not. They conclude that serum urea and serum creatinine do not behave predictably over a range of azotaemia in canine babesiosis, as they do in non-babesiosis patients. They therefore may not reflect the presence of azotaemia and possibly renal disease accurately in some babesiosis patients.

Zygner *et al*., (2007) reported biochemical abnormalities observed in serum of 202 dogs infected with large Babesia in Warsaw (Poland) and recorded the elevated activity of ALT, AST and ALP was detected accordingly in: 64.9, 92.6, and 31.7% of dogs. Elevated creatinine concentration and BUN were detected accordingly in 30.7 and 62.4% of dogs. Decrease of TP, albumin, BUN, and hypoglycemia was detected accordingly in: 19.8, 32.7, 1.5, and 18.3% of dogs concluding an increase of activity of transaminases and ALP, elevated creatinine concentration, hypoalbuminemia, and hypoglycaemia. These abnormalities resulted from hepatopathy, renal failure, and fasting.

Shah *et al*., (2011) reported biochemical changes in 4 dogs positive for Babesia infection presented to the Department of Veterinary Clinical Services Complex, GADVASU, Ludhiana from August 2008 to April 2009 and recorded values of Blood Urea Nitrogen (BUN), creatinine, total protein, albumin, bilirubin, Aspartate amino Transferase (AST), Alanine amino Transferase (ALT), Alkaline Phosphatase (ALP) were varied for each dog.

Wadhwa *et al*., (2011) studied biochemical changes in 18 dogs positive for babesiosis there were significant changes in Total protein – 7.93±0.81g/dl, AST-80.33±8.76 U/l, ALT-86.75±7.86 U/l values, and non-significant changes in Blood urea nitrogen – 13.30±3.20mg/dl and creatinine – 2.20±0.90 mg/dl in an infected dog.

Yadav *et al*., (2011) reported biochemical changes in one case of *Babesia gibsoni* infection. Serological finding was TP – 4.8 gm/dl, Total bilirubin – 10.327 mg/dl, AST- 79.405 IU/L and ALT- 135.887 IU/L.

Andoni *et al*., (2013) studied serological changes in 29 cases of *B. canis* infection in dogs and recorded mean ± SD values of Alanine amino Transferase (ALT), Aspartate amino Transferase (AST), Bilirubin was 71.94 ± 40.51, 37.73 ± 13.14, 0.75 ± 0.3 respectively. Azotemia was present in 24.1% of dogs and Urea was elevated with normal creatinine in eight dogs 27.5% cases.

Reddy *et al*. (2014) reported serological changes in six cases of babesiosis in dogs and recorded a reduction in total protein, serum albumin, glucose levels. Increased BUN, creatinine, SGPT levels were noticed.

Shrivastave et al., (2014) observed 18 dogs that are positive for babesiosis on blood smear examination. Biochemical analysis revealed levels of ALT, ALP, and total direct and indirect bilirubin were significantly higher in the affected animal. These changes may be attributed to haemolysis and cellular damage to the hepatic cells. The blood glucose, BUN, and Creatinine level were decreasing significantly in the affected animal.

Sivajothi *et al*., (2014) recorded serum biochemical changes in one complicated case of *B. canis* infection. Serum biochemical parameters revealed decreased total protein (6.0 g/dl), serum albumin (2.2g/dl). Increased BUN (28 mg/dl), creatinine (1.8 mg/dl) SGPT (224 IU/I) levels and urine analysis revealed positive hays test and presence of RBC in the sediment of urine.

Vishurahav *et al*., (2014) recorded serological changes in six dogs that were positive for Babesia gibsoni on blood smear examination which were hypoproteinemia, hyperglobulinemia, and increase in total and direct bilirubin.

Avinash *et al.* (2017) revealed that the mean values of haemoglobin and total erythrocyte counts in dogs with babesiosis decreased significantly ($p<0.01$) in comparison to healthy dogs. Among differential leukocyte count, mean values of neutrophils and eosinophils increased while lymphocytes decreased ($p<0.01$) in dogs with babesiosis in comparison to healthy dogs. Serum biochemistry revealed an increased ($p<0.01$) value of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and globulin as well as a decrease in albumin levels ($p<0.05$) in dogs with babesiosis as compared to healthy dogs.

### 2.2.5 Diagnosis using different test

Ahmed *et al*., (2007) used the Diff-Quik and Giemsa stain for the diagnosis of Babesia spp. infection. The stained smear demonstrates 2.4 X 5.0 mm, pyriform shaped, an intra-erythrocytic parasite which was usually paired (*B. canis* or 1.0 mm X 3.2 mm pleomorphic, single to multiple, intra-erythrocytic organism (*B. gibsoni*).

Maele *et al*., (2008) confirmed one case of *B. canis* infection by blood smear examination having a large (2.4 µm to 5.0 µm), pyriform shaped organisms within the erythrocyte.

Cardoso *et al*., (2010) diagnosed 41 cases of dogs having a *B. canis* infection by microscopic examination of peripheral blood smear evaluation which showed intra-erythrocytic piroplasms morphologically compatible with B. canis (3-5 µm long and mainly occurring in pairs or single ring shapes).

Ilie *et al*., (2010) screened 103 dogs suspicious of canine babesiosis in the Banet area. Out of 103, 16 (15.5%) were positive for Babesia spp. Infections and in which 11 cases (68.8%) large Babesia species by microscopic examination appear blue colored, pear-shaped, present in the center of red cells were identified and in 5 cases small Babesia species rod or cocci shaped, small and blue colored were found.

Peter (2010) studied parasites of the closely related genus and found that Theileria and Babesia are also capable of infecting dogs. For accurate diagnosis DNA-based, molecular techniques are useful for differential diagnosis.

Andoni *et al*., (2013) confirmed 29 cases of *B. canis* infection by microscopic examination having a pear-shaped inside infected erythrocytes with Wright-Giemsa stain.

Aysul *et al*., (2013) screened two dogs suspected of canine babesiosis having clinical signs of pyrexia, weakness, mucous membrane pallor, and depression living in Aydm, Turkey, in February 2009. Microscopic examination of Giemsa-stained peripheral blood smear reveals small (1-3µm in diameter, ring, oval, or comma-shaped) piroplasms suggestive of *Babesia gibsoni* which were confirmed by PCR.

Bhojne *et al*., (2013) diagnosed two cases of babesiosis in dogs by peripheral blood smears from ear tip stained with Leishman stain which revealed small, single, signet ring-shaped trophozoites of the parasites in more than 40 percent of erythrocytes confirmed as babesiosis.

Razi Jalali *et al*., (2013) screened 400 dogs referred to Veterinary Teaching Hospital of Shahid Chamran University of Ahvaz and nearby four villages which were confirmed positive for intraerythrocytic piroplasms in binary quaternary form of *B. canis* based on blood smear examination with Giemsa stain.

Bhattacharjee *et al*., (2014) prepared thin blood smears from blood samples of dogs and stained them with Giemsa for microscopic examination for haemoparasites.

Konto *et al*. (2014) confirmed 48 cases of dogs in Maidugiri, North-Eastern Nigeria as a positive for babesiosis based on blood smear stained with Giemsa stain.

Sivajothi *et al*., (2014) confirmed two cases of *B. canis* infection by examining stained blood smears having typical intra-erythrocytic paired pear-shaped piroplasms.

Sarma *et al.* (2014) found the ultrasonographic changes of liver and spleen in 101 positive cases of tick-borne intracellular haemoparasitic diseases in dogs. An abdominal survey of ultrasonography revealed hypoechogenicity of the liver, gall bladder distension, splenomegaly, hepato-splenomegaly, and ascites in various tick-borne intracellular diseases viz. ehrlichiosis, babesiosis, anaplasmosis, hepatozoonosis, and in mixed infection. Correlations of USG findings with the biochemical parameters help diagnose organ damage.

Das *et al*., (2015) examined thin blood smears of 226 dogs using Giemsa stain resulting in 72 positive cases. Cytological studies revealed four large-sized 2.4 x 5 mm bodies of Babesia spp. within the red blood cells and of the positive cases 68 numbers were small approximately 1 x 2.5 mm in size pleomorphic form of Babesia spp. without pyriform shape present in the center

having signet, rod, or cocci shape and a single organism per RBC was common but multiple forms were also observed.

Kumar *et al*., (2015a) screened a total of 204 canine blood samples for the prevalence of Canine Babesiosis with a history of fever by examination of blood smears. To make a thin blood film, a drop of blood was placed on a clean glass slide drawn into a smear air-dried, fixed in methanol, stained with Giemsa stain, and examined under a light microscope by using the oil immersion objective (100X). In the positive case, intra-erythrocytic forms (piroplasms) were observed. A blood smear revealed 8.33% (17/204) of canines were positive for canine babesiosis comprising of *B. gibsoni* 7.84% and *B. canis* 0.49%.

Mahalingaiah *et al.* (2017) found that blood smear examination and PCR a confirmatory gold standard test for canine Babesiosis. They examined 102 blood samples of dogs and screened that 40 (39.2%) and 66 (64.7%) were positive by blood microscopy and PCR, respectively. The overall prevalence of canine babesiosis was 31(30.3%) and 50(49.0%) for B. canis and B. gibsoni, respectively. Mixed infections both with *B. canis* and *B. gibsoni* were detected in 25 (24.50 %) samples. A higher incidence of canine babesiosis was seen in the age group above 1-2 years (23%), breed-wise in Labrador Retrievers (26.0%) and gender-wise in male dogs (57.5%).

Petra *et al.* (2018) diagnosed canine babesiosis by microscopy, serological and molecular methods. They mentioned that accurate detection and species recognition are important for the selection of the appropriate therapy, monitoring, and prediction of the outcome of the disease.

Wang *et al.* (2019) observed the presence of piroplasms with nested PCR and gene sequencing targeting the 18S rRNA gene. Based on blast analysis of the 18S rRNA gene sequences, results revealed that seven dogs (5.4%) were infected with *Babesia canis canis.* The sequences were compared with those in GenBank, and alignments showed that all B. *canis canis* isolates belonged to genotype B.

Brahma *et al.* (2019) studied the molecular examination of babesiosis and haemato-biochemical changes in canine babesiosis-infected dogs. In this study, 8 cases infected with *Babesia* were confirmed through haematological, biochemical, and multiplex PCR. The most common clinical signs were anorexia, pale or icteric mucous membranes, high rise of temperature, and dark urine colour. The haematological and biochemical parameters showed a

decreased level of RBC, Hb, PCV, Platelet's level, and increase the level of WBC, ALT, ALP, Total bilirubin, BUN, and creatinine value.

### 2.2.6 Organ Involvements in Babesiosis

(Makinde and Bobade, 1994) reported Parasitic activity directly damages the erythrocyte cell membrane, resulting in increased osmotic fragility and subsequent intravenous haemolysis.

(Adachi *et al*., 1994; Adachi and Makimura, 1992) reported elevated concentrations of antierythrocytic membrane antibodies and erythrocyte bound immunoglobulin G have been documented in dogs infected with *B. gibsoni.*

(Taboada and Lobetti, 2006) reported diagnosis requires demonstration of saline agglutination of RBCs or spherocytosis or both.

(Farewell *et al*., 1982) reported a positive Coomb's test is not considered a reliable tool for the diagnosis of IMHA in babesiosis as 84% of canine patients infected with *B. canis* or *B. gibsoni* have positive Coombs tests.

(Morita *et al*, 1996; Lobetti and Reyers 1996; Otsuka et al., 2002) reported oxidative stress can cause lipid peroxidation and erythrocytic injury, with resultant methemoglobinaemia. Methemoglobinuria, as well as elevated methemoglobin to total hemoglobin ratios, have been documented in Babesia-infected dogs.

(Otsuka *et al*.2001; Otsuka et al., 2002) reported increased macrophage production of superoxide and other reactive oxygen species has been demonstrated in *B. gibsoni* infected dogs.

(Adachi *et al*.1994; Morita et al, 1996) reported studies of experimentally induced *B. gibsoni* infection suggest that free radical-initiated oxidative stress to the RBC is necessary for antierythrocytic membrane antibody production.

(Murase *et al*.1996, Otsuka, 2002) reported erythrocytic oxidation may enhance susceptibility to macrophase–mediated bone marrow phagocytosis.

(Taboada and Lobetti,2006) reported lipid peroxidation of erythrocytes also decreases erythrocytes also decrease RBC membrane pliability, resulting in the slowed passage and further damage to the erythrocyte as it traverses capillary beds. The capillary sludging of

erythrocytes, in combination with soluble parasite proteases, activate the kallikrein system leading to the production of fibrinogen-like protein.

(Uilenberg *et al*.,1989; Jacobson and Lobetti,1996; Lobetti and Reyers,1996) reported protein induces RBC aggregation and promotes vascular stasis, which leads to ischemia, thrombosis, and end-organ damage. The CNS, kidney, and muscle appear to be the organs most affected by the resultant tissue hypoxia.

(Welzl *et al*.,2001; Jacobson,2006; Uilenberg et al.,1989; Jacobson and Clark,1994; Jacobson and Lobetti,1996; Lobetti et al.,1996; Jacobson,1994; Leisewitz et al.,2001; Jacobson 2006; Mahr et al.,2000; Keller et al.,2004; Mathe et al.,2006) reported multiple Organ Dysfunction Syndrome (MODS) can occur subsequent to infection with the more pathogenic Babesia species, particularly *B. canis* subsp. rossi. Documented complications include red biliary syndrome, thrombocytopenia, Disseminated Intravascular Coagulation (DIC), Acute Renal Failure (ARF), hepatopathy, rhabdomyolysis, noncardiogenic pulmonary edema.CNS dysfunction, pancreatitis, systemic hypotension, cardiac dysfunction, hypoglycemia, hypoxemia, and metabolic acidiosis with hyperlactatemia.

(Jacobson and clark, 1994; Jacobson and Lobetti, 1996) reported although severe anaemia and resultant cellular hypoxia contribute to these complications, it is suspected that systemic inflammatory mediators (cytokines and reactive oxygen species) generated by host tissues and inflammatory mediators (cytokines and reactive oxygen species) generated by host tissues and inflammatory cells are largely responsible for end-organ damage.

(Welzl *et al*.,2001) reported a retrospective study of canine babesiosis in South Africa documented the development of systemic inflammatory response syndrome in 87% of infected dogs. Of these 52% had single-organ damage and 48% had evidence of multiple-organ dysfunction.

(Taboada and Lobetti, 2006) reported there will be a paradoxical phenomenon of hemoconcentration in conjunction with severe intravascular hemolysis, commonly referred to as a red biliary syndrome.

Jacobson (2006) reported systemic inflammation leads to top vasculitis, increased capillary permeability, and the extravasion of intravascular fluids. Concurrent hypoalbuminaemia contributes to the fluid shift, and hemoconcentration ensures. This phenomenon typically

occurs in conjunction with other complications and is associated with a significantly higher mortality rate than seen with anaemia alone.

(Taboada and Lobetti, 2006; Tuttke et al, 2003) reported thrombocytopenia can occur in combination with other hematologic abnormalities or as a singular intity, and maybe transient or persistent.

(Taboada and Lobetti, 2006) reported that it is commonly seen in both *B. canis* subsp. rossi and *B. gibsoni* infection is the most consistently reported hemostatic abnormality.

(Welzl et al.,2001; Jacobson,2006; Mahr et al.,2000) reported ARF is uncommon, although it is a devastating consequence of complicated Babesia infection, and has been associated with up to the 5-fold increased risk of death.

(Wozniak *et al*., 1997) reported the pathogenesis of ARF is multifactorial. Postulated causes of renal hypoxia include anaemia, capillary sludging, and systemic hypotension with compensatory renal vasoconstriction. Immune-mediated development of membranoproliferative glomerulonephritis may also contribute to renal damage; multifocal deposits of immunoglobulin M have been demonstrated in glomeruli of infected patients via immunohistopathogenic testing.

(Scally, *et al* 2006) reported BUN measurement is affected by intravascular hemolysis, and is, therefore, an unreliable marker of renal dysfunction in the patient population. Serum creatinine concentration is unaffected by hemolysis and remains a useful diagnostic tool.

(Lobetti and Jacobson, 2001; Bonfanti, 2004) reported the degree of proteinuria appears to correlate with the severity of systemic disease rather than the degree of renal damage.

(Wozniak *et al*.,1997) reported Icterus and elevated hepatic enzymes occur frequently. Although the associated hemolytic anaemia contributes to hypoerbilirubinemia, it is not the sole cause. Centrilobular hepatitis is the most commonly documented and severe histopathologic change in babesial infection and is most likely the result of hypoxia liver damage.

(Wozniak *et al*., 1997) reported kupffer cell hypertrophy and increased numbers of CD31 lymphocytes and macrophages have been demonstrated, suggesting that immune-mediated inflammation likely plays a contributing role.

(Welzl *et al*., 2001) reported although histopathogenic and laboratory evidence of hepatic damage is common, hepatopathy is not reported to significantly affect the outcome.

(Jacobson, 2006; Welzl *et al*., 2001) reported Rhabdomyolysis can complicate babesiosis. It is characterized by muscle pain, tremors, pigmenturia, and elevated concentrations of myoglobin, creatinine kinase, and alanine aminotransferase.

Jacobson and Clark (1994) observed that Acute Respiratory distress Syndrome (ARDS) is a common and important complication of the more pathogenic strains of Babesia spp. Clinical features include tachypnoea, dyspnoea, a moist cough, serosanguineous frothy respiratory secretions, and hypoxemia. A systemic inflammatory response syndrome (SIRS), secondary to the production of inflammatory cytokines and reactive oxygen species, likely plays an important role in the pathogenesis of this complication.

(Wozniak *et al*., 1997) reported inflamed pulmonary arteries multifocal deposits of immunoglobulins M antibodies within the walla have been demonstrated in dogs with *B. gibsoni.*

(Welzl *et al*., 2001; Jacobson,2006; Mahr et al.,2000) reported the development of ARDS is a catastrophic complication and is associated with a marked elevation in mortality.

(Okan,1978; Jacobson and clark,1994) reported both cerebral and cerebellar signs are uncommonly in dogs infected with *B. canis* subsp. rossi, and include loss of consciousness, stupor, coma, tremors, seizures, paddling, nystagmus, anisocoria, central blindness, ataxia, tetra-and paraparesis, aggression, and vocalization.

(Jacobson,2006) reported peracute to acute onset is common. Postulated pathogenesis includes endothelial cell damage and necrosis followed by segmental microvascular necrosis with perivascular edema and hemorrhage.

(Welzl *et al*.,2001; Jacobson 2006; Mahr *et al*.,2000) reported although clinical signs resolve in some patients following antibabesial therapy, the development of neurologic signs is associated with a high mortality rate and a 57-fold increased risk of death.

(Jacobson,2006; Mahr *et al*.,2006) reported acute pancreatitis is a newly described but common occurrence in canine Babesiosis.

(Mahr *et al*.,2000) reported clinical signs include inappetence, vomition, diarrhoea, and abdominal pain. Proposed mechanisms of injury include ischemia-reperfusion, altered blood flow and oxygen delivered due to hypotensive shock, anaemia, and hemoconcentration, altered lipid metabolism, and pro-inflammatory cytokine production.

(Breitschwerdt *et al*., 1983) reported pancreatitis more commonly occurs in patients with MODS (80% suffer concurrent complications) and is associated with a 185 increase in mortality.

(freeman *et al*.,1994; Jacobson *et al*.,2000; Lobetti and Jacobson,2001) reported hypotensive shock commonly accompanies canine babesiosis, and can occur at any point in the disease process.

(Taboada and Lobetti, 2006) reported proposed mechanisms to include kallikrien activation with resultant vasodilation (relative hypovolemia), production of vasoactive proteins, increased capillary permeability, decreased intravascular volume, and myocardial depression.

(Jacobson *et al*., 2000) reported the incidence of hypotension increases with disease severity but does not differentiate survivors from nonsurvivors.

(Lobetti,2005; Lobetti *et al*.,2002) reported cardiac dysfunction is a rare occurrence in canine babesiosis. Cardiac troponin I levels are increased in infected dogs and correlate with histologic cardiac changes, clinical severity, and survival. Reported ECG abnormalities to include heart block, ventricular premature contractions, and prolonged QRS and ST-segment changes. These ECG findings are not associated with disease severity, histopathologic changes, cardiac troponin levels, or outcome except ventricular premature contractions with are significantly associated with cardiac troponin I concentration.

(Lobetti *et al*., 2002) reported left ventricular failure with volume overload and hypotension has been reported, and associated histopathologic changes include hemorrhage, necrosis, inflammation, and the presence of fibrin microthrombi in the myocardium.

Keller *et al*. (2004) reported hypoglycemia is a relatively common complication of the more pathogenic strains of Babesia spp.

(Keller *et al*.,2004; Rees and Schoeman,2008; Nel *et al*.,2004; Jacobson and Lobetti,2005) the reported incidence of 20% Hypoglycemia has been significantly correlated with mortality.

(Keller *et al*.,2004) reported risk factors include a collapsed state, severe anaemia, icterus, vomiting, and immaturity (less than 6 months of age). Collapsed stage and young age are the most strongly correlated variables with an increased occurrence of hypoglycemia of 17.8 and 2.8 times, respectively.

(Keller *et al*.,2004; Rees and Schoeman,2008) reported hypoglycemia must be differentiated from cerebral babesiosis as cerebral disease carries a far poorer prognosis. Speculated causes of hypoglycemia include increased glucose consumption secondary to anaerobic glycolysis, hypermetabolism and enhanced cellular uptake of glucose, depletion of hepatic glycogen stores, and hepatic dysfunction with impaired gluconeogenesis hyperinsulinemia was once a speculated cause; a recent study, however, failed to demonstrate any significant alteration in insulin concentrations.

(Leisewitz *et al*.,2001) reported a study documenting concurrent metabolic acidiosis with respiratory alkalosis in 91% of dogs infected with *B. canis* subsp. rossi. Eighty-two percent of patients in this study were hyperlactatemic, suggestive of lactic acid production secondary to tissue hypoxia as the primary mechanism for the observed metabolic acidiosis.

(Jacobson, 2000; Jacobson and Lobetti, 2005; Nel *et al*., 2004) reported concentrations of lactate and pyruvate were significantly higher in nonsurvivors and can be used as an indicator of disease severity.

Leisewitz *et al*. (2001) proposed that respiratory alkalosis is likely due to hypoxemia-induced hyperventilation. In addition, hypoalbuminemic alkalosis was present in all dogs.

## 2.3. Therapeutic management and prevention of Babesiosis in dogs

### 2.3.1. Drugs used to treat babesiosis

(Birkenheuer *et al*,1999; Farwell *et al*,1992., Fowler *et al*,1972 Groves and Dennis,1972; Groves and Vanniasingham,1970; Itoh et al.,1988., Ruff *et al*.,1973; Stegeman et al.,2003; Takahashi,1984; Yamane et al.,1993) reported no drugs have been demonstrated to clear *B. gibsoni* (Asian genotype) infections from dogs including diminazene aceturate and imidocarb diproprionate.

(Birkenheuer, 2009) reported the clinical signs associated with piroplasmosis typically begin to improve within I week of beginning specific therapy. If patients are not responding to

specific therapy, secondary treatments should be administered, or alternative diagnoses should be considered.

### 2.3.1.1. Diminazine aceturate

Varshney *et al*., (2003) reported diminazene aceturate @ 3.5mg/kg b.w.t. intramuscularly on 2 consecutive days or 7.5 mg/kg b.wt. A single dose was found clinically effective.

Vial and Gorenflot (2006) suggested that a single intramuscular injection of diminazene aceturate at a dose of 5 mg/kg is effective in canine babesiosis.

Lin and Huang (2010) reported that Doxycycline-Enrofloxacin-Metronidazole combination with diminazene diaceturate was found superior to only Doxycycline-Enrofloxacin-Metronidazole combination to treat naturally occurring canine babesiosis caused by *Babesia gibsoni* though no significant overall efficacy was seen in management efficacy between the two protocols.

Selvaraj *et al*., (2010) recorded a success rate of 94% in babesiosis by diminazene aceturate @ dose rate of 5 mg/kg body weight.

Wadhwa *et al*., (2011) treated 18 cases of babesiosis in dogs with two doses of diminazene aceturate @5 mg/kg b wt i/m at 48 hr interval, fluid therapy (dextrose saline @ 40 ml/kg b wt. daily till oral consumption began), Inj. imferon (iron dextran 50 mg/ml) 2 ml i/m on alternate days (3-4 injections), liver tonic (Inj. Belamyl @ 1 ml i/m on alternate day for 3-4 occasion) and syp. Polybion 1-2 tsp b.i.d orally for one week. After treatment recovery was achieved in 5-7 days.

Janus *et al*., (2012) treated one case of the cerebral form of babesiosis due to *B. canis* with Berenil (Diminazene aceturate) 0.25 ml intramuscularly. Inj. dextrose 25% solution 25 ml intravenously and Inj. imferon 1 ml intramuscularly. The condition of the animal improved by the third day and was cured by the 10th day.

Torbika *et al*., (2013) stated that treatment with diminazene aceturate at the dose rate of 3.5 mg/kg BW in case of *Babesia canis* infection resulted in significant regression of clinical symptoms like high body temperature within 24 hours, hemoglobinuria, haematological values (Hb, RBC, PCV) were gain normal within 7 days.

Reddy *et al*., (2014) treated six cases of dogs infected with Babesia spp. Dogs were treated with inj. Diminazene aceturate (Berenil RTU, Intervet) @ 5 mg/kg body weight IM. Symptomatic therapy along with fluids was given based on the requirement of the individual case.

Sivajothi *et al*., (2014) reported that diminazene aceturate at dos rate of 5 mg. kg BW was found effective in uncomplicated cases of canine babesiosis than complicated ones.

Yogeshpriya *et al*., (2014) treated one case of babesiosis in a three-year-old male Labrador dog weighing 30 kg presented to University Veterinary Hospital, Mannuthy with a history of voiding blood mixed urine, vomiting, and icterus. The case was treated with diminazene aceturate 1.5 ml i.m. and hematinics. The dog showed a good response and an uneventful recovery.

Joice *et al*., (2015) effectively managed *B. canis* infection with Inj. Diminazene aceturate at the dose of 3.5 mg/kg body weight.

Koster *et al*., (2015) stated that *B. canis* infection can be successfully treated with Diminazene aceturate (3.5 mg/kg subcutaneously or intramuscularly).

Reddy *et al.* (2016) examined the dogs revealed ticks over the body, congested conjunctival mucus membranes, dullness, fever, tachycardia, tachypnoea in all the dogs. Some of the dogs had icterus, lymphadenopathy, and haemoglobinuria. Laboratory examination of the clinical samples revealed a reduction in haemoglobin concentration, erythrocyte count, platelet count, serum total protein, serum albumin, and glucose levels. Increased serum SGPT, creatinine, BUN levels were recorded. Dogs were treated with inj. Diminazene aceturate (Berenil RTU) @ 5 mg/kg body weight, single dose along with supportive and symptomatic therapy in individual cases.

### 2.3.1.2. Clindamycine + Diminazine aceturate

Wulansari *et al*., (2003) reported the efficacy of clindamycin in experimentally infected with Babesia gibsoni in five beagle dogs. Dogs were administered clindamycin at 25 mg/kg body weight, per os, q 12 hr from 7 days to 21 days post-infection (PI). On the acute stage of infection, clindamycin treatment resolved anaemia and other clinical findings. There was no significant difference between treated and untreated dogs either in parasitemia levels or Babesial IgG antibody levels. However, morphological changes that indicated degeneration in

the majority of parasites were observed. The antibody levels of treated dogs were significantly higher than those of untreated dogs. These results suggested that clindamycin might not eliminate rapidly parasites from peripheral blood, but damage parasites, which might stimulate efficiently humoral and cellular immunity against Babesia infection and result in an improvement of clinical conditions.

Suzuki *et al*., (2007) reported the effectiveness of combination therapy using clindamycin, metronidazole, and doxycycline against *babesia gibsoni* Infection. The combination therapy successfully eliminated B. gibsoni in peripheral blood in 3 of 4 dogs however the remaining dog showed obvious uncontrolled relapse after a temporary recovery.

Varshney *et al*., (2008) treated 10 cases of *B. gibsoni* infection in dogs with clindamycin @ 25 mg/kg i.v. twice daily till dehydration, blood pressure, and arrhythmias became normal followed by a single injection of diminazene aceturate @ 5.0 mg/kg in once)

Selvaraj *et al*., (2010) successfully treated cases of babesiosis in non-responsive diminazene aceturate by clindamycin @ dose rate of 25 mg/kg body weight orally for every 12 hours for 14 days.

Jadav *et al*., (2011) stated that clindamycin @ 25 mg/kg orally every 12 hours for 1-3 weeks can be effective for the treatment of babesiosis in dogs.

### 2.3.1.3. Imidocarb Dipropionate

Euzeby *et al*., (1980) cured clinical *B. canis* infection with imidocarb diapropionate in 24 hours and no relapse was observed up to 11 months.

Ogunkoya *et al*., (1981) found 94 percent success in the treatment of natural *B. canis* infection with imidocarb dipropionate.

Adeyanju and Aliu (1982) found 95.5 percent success in the treatment of *B. canis* infection with imidocarb dipropionate .3.3 percent cases were detected positive after 6 weeks of this treatment.

Awaz *et al*., (1984) found berenil and imidocarb very effective in experimental *B. canis* infection.

Gad Baneth (2018) studied that Canine babesiosis is a tick-borne disease caused by several Babesia spp. which has different susceptibility to anti-protozoal drugs. The large form of

Babesia spp is susceptible to Imidocarb and Diaminazine aceturate while the smaller form is resistant to these drugs which can be treated by a combination of novel drugs.

### 2.3.1.4. Prevention

(Martinod *et al*., 1985) reported frequent visual inspection of skin and hair coat is an effective means of tick control as a minimum of 2-3 days of tick engorgement is necessary for parasite transmission.

(Dryden and Payne, 2004; Dryden *et al*., 2006; Estrada-Pena and Ascher, 1999; Hunter, 1997; Cruthers *et al*.,2003; Epe *et al*., 2003; Elfassy *et al*.,2001; Last *et al*.,2007) reported visual inspection should be combined with topical acaricide therapy to prevent tick infestation. topical therapies of proven benefit include amitraz-impregnated collars, fipronil and imidacloprid-permethrin applied once monthly, all resulting in the prevention of tick attachment or tick death within 24-48 hours.

(Ayoob and prittie, 2010) reported frequent blood smear evaluation and PCR testing of blood donors are recommended to prevent the transmission of infected blood.

### 2.3.1.5. Oxidative Stress parameters

Todorova *et al* (2005) studied 56 clinically healthy dogs of matched age and sex. The reference values of principal parameters of oxidative stress—malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT)—were determined in the blood samples of dogs. It was found that MDA plasma concentrations in male dogs were higher than those in females. The SOD activity did not show any sex differences in dogs.

Nohl *et al*., (1996) reported free radicals and other reactive oxygen species (ROS) have been implicated as playing an important role in tissue damage in a variety of pathological processes.

Halliwell (1994) reported overproduction of ROS in diverse pathological conditions leads to oxidative damage to macromolecules, resulting in enhanced lipid peroxidation, DNA strand breaks, and protein damage.

Kumar *et al*., (2006) reported 21 dogs suffering from severe chronic ehrlichiosis alone (n = 14) or concurrent infection of *E. canis* and *B. gibsoni* (n = 7). The mean (±SD) values of LPO in healthy and infected dogs are Significantly ($p < 0.05$) lower and higher erythrocytic LPO concentrations were recorded in dogs with concurrent infections. There was no significant

difference ($p > 0.05$) in the LPO concentration between dogs positive for *E. canis* alone and healthy dogs. . The higher concentrations of LPO in erythrocytes from dogs with concurrent ehrlichiosis and babesiosis can probably be ascribed to the multiplication of *B. gibsoni.*

Clemens and Waller (1987) reported higher polyunsaturated fatty acid (PUFA) levels, continual exposure to high concentrations of oxygen, or the presence of iron, a powerful metal catalyst, render erythrocytes highly susceptible to peroxidative damage.

Muduuli *et al*., (1982) Thus, higher production of peroxyl radicals and consequent elevated LPO concentration renders the erythrocytes more fragile and prone to lysis in cases of concurrent babesiosis.

Kumar *et al.,* (2018) reported that the value of MDA (3.53±0.43 nmol MDA/mg of Hb) was significantly higher whereas, SOD (35.99±1.96 U/mg of Hb) was significantly lower in apparently healthy geriatric dogs as compared to the healthy adult dogs (1.11±0.26 nmol MDA/mg of Hb and 51.68±3.49 U/mg of Hb, respectively).

Crnogaj *et al.*, (2017) studied a sample of 40 dogs suffering from babesiosis and detected a more pronounced decrease in antioxidant biomarkers (SOD, GPx, and catalase) in dogs with moderate anaemia compared to those with mild anaemia.

Crnogaj *et al., (*2015) reported oxidative stress could have a possible causative role in the clinical severity of the disease. Concentrations of MDA on the first day were significantly increased in dogs with uncomplicated ($P<0.001$) and complicated babesiosis ($P<0.001$) compared to controls. On the seventh day, the concentration of MDA was significantly higher in the group of dogs with complicated babesiosis ($P = 0.003$) than in the uncomplicated group. Furthermore, dogs in the multiple organ dysfunction syndrome group had significantly increased MDA concentrations compared to dogs in the uncomplicated ($P = 0.006$) and single complication groups ($P = 0.025$). There was a significant positive correlation between MDA concentration and the outcome of the disease (Tau-b 0.309; $P = 0.005$). The present study concludes that, on the seventh day, increased lipid peroxidation was still present in the affected group of dogs.

Teodorowski *et al*.,(2021) study aimed to demonstrate a link between uncomplicated Babesia canis infection in 15 naturally occurring cases of canine babesiosis dogs.The study was based on The levels of the erythrocytic antioxidant enzymes - SOD and CAT - were significantly higher in the infected dogs than in cytologically negative dogs.Oxidative stress can be posited

as one of the mechanisms leading to anaemia in dogs with babesiosis, and therefore antioxidant biomarkers could be used as indicators of disease severity and prognostic markers.

Crnogaj *et al*., (2010) reported the presence of oxidative stress by examining serummalondialdehyde (MDA), an end product of lipid peroxidation. Levels of serum MDAwere significantly higher in sick dogs (36.90 μmol/l ± 13.95) than healthy animals (8.13 μmol/l ± 1.78. The study demonstrated the involvement of oxidative damage in dogs naturally infected with B. canis.

Murase *et al.*, (1996) reported oxidative damage to canine erythrocytes infected *Babesia gibsoni* was investigated. *B. gibsoni* was cultured together with erythrocytes from normal dogs. When parasitaemia reached a peak level, concentration malonaldehyde (MDA), an end product of lipid peroxidation, in erythrocytes, was significantly higher than at cultivation Day 0. These results suggested that oxidative damage to erythrocytes was induced by the multiplication of *B. gibsoni,* and that non-parasitized erythrocytes were also exposed to oxidative stress during the infection by *B. gibsoni.*

Otsuka *et al*., (2001) studied to clarify the mechanism underlying the oxidative process in erythrocytes infected with *Babesia gibsoni*. The results indicate that superoxide anions are increased in erythrocytes parasitized with *B. gibson*, and suggest that oxidative damage, due to lipid peroxidation, might be caused in host erythrocytes by the parasite.

Chaudhuri *et al*., (2008) demonstrated increased activity of erythrocytic antioxidant enzymes CAT, SOD, and LPO. Levels of enzymes were significantly ($P < 0.01$) higher in sick dogs than those of cytologically negative dogs.

Moral *et al*. (1997) indicated that SOD, catalase, and glutathione peroxidase are major enzymes, es present in RBC to counteract toxic effects of ROS such as superoxide radicals and hydrogen peroxide.

Clemens and Waller (1987) reported that RBC membrane phospholipids are highly susceptible to peroxidation and accumulation of phospholipids hydroperoxides in RBC occurs due to injury to erythrocytes membrane.

(Jose *et al*., 1999) reported that in the body $H_2O_2$ is detoxified by Catalase and glutathione peroxidase (GSH-Ps). Although GSH-Ps shares substrate $H_2O_2$ with Catalase, it alone can react effectively with lipid and other organic hydroperoxides being the major source of protection against low levels of oxidative stress.

# 3 MATERIALS AND METHODS

## Materials:

### 1. Equipment

20degree Celcius Deep freezer (Denmark), Spectrophotometer (Systronics, India), Water bath (B.R Biochem, India), Centrifuge machine (Remi, India), Weighting balance (Mettler Toledo, India), Binocular microscope (Magnus, India).

## Methods:

### 1. Workplace

The present study on canine babesiosis associated with multiple organ dysfunctions in dogs is carried out in the department of veterinary medicine from the cases of veterinary clinical complex B.V.C, Patna. The cases referred from the adjoining hospital of the Bihar government were also be included.

### 2. Selection of animal

Dogs irrespective of age, sex, and breed suffering from fever, anemia, and jaundice was screened for babesiosis infection. Screening of the babesia-positive infection in dogs was done by blood smear examination. Animals found positive for babesia positive in blood smear examination was subjected to clinical examination and haemato- biochemical examination from the selection of multiple organ dysfunction cases. Affected dogs with dysfunction of more than one organ were selected for their studies.

**Blood Smear examination** Blood was collected from the dogs for Giemsa-stained, thin blood smear examination to detect Babesia species in RBC.

## Staining with Giemsa solution:

## Method:

1. The smears was fixed in methanol for 5 min and stained by Giemsa stain (Coles, 1986).

2. The dilute sample was applied for 45 minutes.

3. Blood smears were then rinsed with distilled water and air-dried.

## Examination of stained smears:

The blood smears were examined under 100x oil emersions field (OIFs), Results were interpreted as babesiosis when piroplasm Babesia or developing stages were found in as least one cell of erythrocytes.

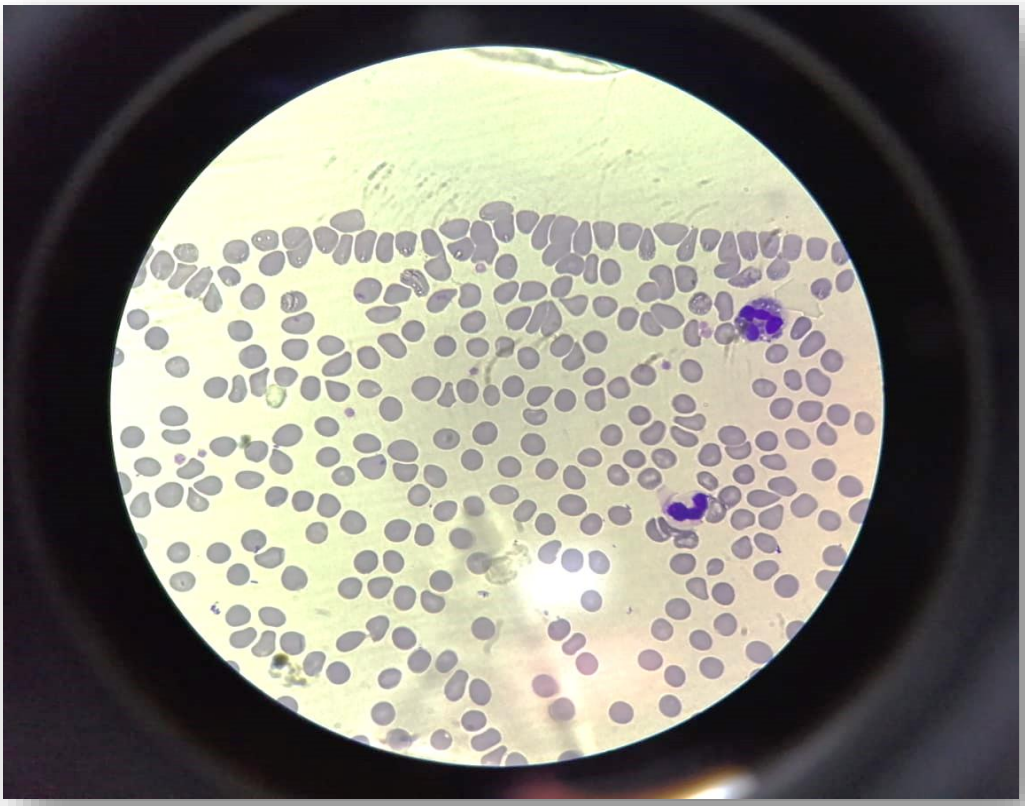

**Fig: -3.1 Peripheral blood smear showing *B. gibsoni* organism**

## 3. Clinical examination:

Clinical examination of affected cases was carried out to evaluate the history taken from the owner, body weight, rectal temperature(˚F), respiratory rate(1min), pulse rate (per min), were recorded on 0 days,$7^{th}$ day, and $14^{th}$ days period.

## 4.Ultrasonography Examination:

Ultrasonography examination of the liver, kidney, and abdomen will be done on 0, 7, and 14th days to evaluate the abnormalities in the texture, shape, and size of the organ and the shape occupying lesion in the abdominal cavity. This will be done as per the method described by Dominique and Marc (2008).


**Fig: -3.2 Ultrasonography of Babesia positive dog**

## 1. Laboratory examination:

Examination of Giemsa-stained peripheral blood smears for presence of intraerythrocytic piroplasm of Babesia spp. For microscopic examination; analysis of haematological parameters from obtained blood samples and analysis of serological parameters were carried out in the Department of Veterinary Biochemistry and Diagnostic Laboratory of Bihar veterinary college, Patna.

## 1. Collection of Blood:

Blood and serum samples from healthy dogs as control and positive*B.gibsoni* dog were collected for different laboratory examinations and haemato-biochemical changes using standard kits. After confirmation by blood smear test, six ml blood was withdrawn from the cephalic vein on $0,7^{th},14^{th}$ days from babesia positive dogs. Out of which 3ml was collected in a tube containing anticoagulant EDTA vacutainer for haematological analysis and 3ml in sterile

plain plastic vacutainer which was centrifuged at 3000rpm for 15 minutes to separate serum and stored at -20˚C for further investigation.


**Fig: -3.3 Blood collection**

## ESTIMATION OF HAEMATOLOGICAL PARAMETERS:

Haematological analysis was performed from whole blood collected in an anticoagulant containing vial within 24 hours after collection. Following haematological parameters were estimated

**1. TEC**

**2. Hb%**

**3. TLC**


**Fig: -3.4 Counting of TEC and TLC**

## Estimation of Haemoglobin (Hb):

Haemoglobin concentration was estimated by the cyanmethemoglobin method by Drabkin's solution.

## Procedure:

1. In a clean and dry test tube, 5 ml of Drabkin's solution was taken then adding 20µl of EDTA blood and then leave for 10 min.
2. After 10 min absorbance readings were taken at 540 nm in the spectrophotometer and the volume was expressed in gm /dl.

$$\text{Haemoglobin(gm/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 15.06$$

## Total Erythrocyte Count:

TEC was estimated manually by the haemocytometers (Neubauer's chamber). as described by Schalm et al. (1986). Anticoagulated blood was drawn up to 0.1 marks of the RBC pipette followed by RBC diluting fluid up to 101 marks. Then the pipette was rotated between the fingers for a few seconds to facilitate proper mixing of the contents, following a few minutes. The counting chamber was charged after discarding the first erythrocytes were counted in the exact position of the Neubauer chamber. The values were then expressed as millions per cubic millimeters ($10^6$/cumm).

## Total Leucocyte Count (TLC):

Anticoagulated blood was drawn up to the WBC pipettes' 0.5 marks followed by WBC diluting fluid up to 11 marks. The pipette was then rotated between the fingers for several seconds to facilitate proper mixing of the contents. After a few minutes, the counting chambers were charged after discarding the first few drops of the diluting samples. Once the cell settled down, the leucocytes were counted in five large squares. The values were then expressed in terms of thousand per cubic millimeters ($10^3$/cm). This was also estimated by haemocytometers as described by Schalm *et al.* (1986)

## ESTIMATION OF SERUM BIOCHEMICAL PARAMETERS: -

It was done on the 0,7th and 14th days of treatment with the help of a biochemical test. Serum was harvested from collected blood after centrifugation at 25, 00 rpm for 10 minutes. Serum was used for estimation of biochemical parameters like Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Serum creatinine, Total Plasma Protein (TPP), Albumin, Blood Urea Nitrogen (BUN) were evaluated by using an autobio-chemical analyzer using standard kits manufactured by coral clinical systems.

1. **LFT (Liver Function test): -** Total protein, Albumin, ALT, ALP.
1. **KFT (Kidney Function Test): -** BUN, Creatinine
1. **Muscle Function**: - AST

## Total Serum Protein (Tsp):

Estimation of Serum total protein was done by the Biuret method as described by Webster (1977) using the commercial kit coral clinical system as per manufacturer's protocol and was expressed in gm/dl.

## Serum Albumin:

Estimation of serum albumin was done by Bromocresol Green (BCG) method as described by Doumas *et al*. (1971) by using the commercial kit coral clinical system as per manufacturer's protocol and was expressed in gm/l.

## Blood Urea Nitrogen (BUN):

Estimation of Serum Urea was done by GLDH kinetic method using the commercial lit coral clinical system as per manufacturer's protocol and was expressed in mg/dl.

BUN (mg/dl) = concentration of serum urea × 0.467

## Serum Creatinine (Scr):

Estimation of Serum creatinine was done by modified Jaffe's kinetic method described by (Brower 1980) by using the commercial kit coral clinical system as per manufacturer's protocol and was expressed in mg/dl.

## Serum Aspartate Amino Transferase (ALT)/Serum glutamic pyruvate transaminases (SGPT):

The ALT level in serum was estimated by the IFCC method of Reitman and Frankel (1957) by commercial kit coral clinical system as per manufacturer's protocol was expressed in IU/L.

## Serum Aspartate Amino Transferase (AST) /Serum glutamic oxaloacetic transaminases (SGOT):

The level of AST in serum was estimated by the IFCC method of Reitman and Frankel (1957) by commercial kit coral clinical system as per manufacturer's protocol was expressed in IU/L.

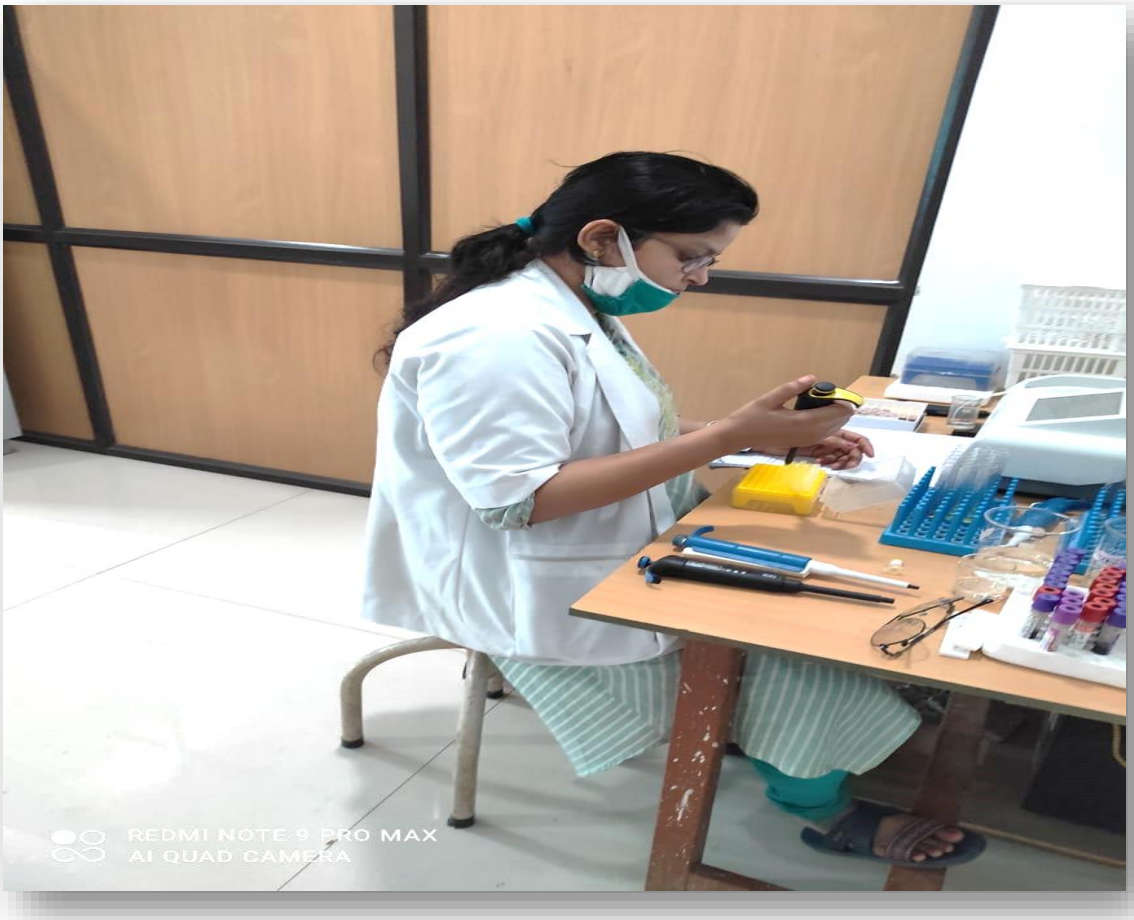


**Fig: 3.5 Serological parameters estimation on biochemical analyser**

## 3. Experimental design:

24 selected dogs were further divided into four groups. Each group was consist of six animals. The detail of the therapeutic study is given below.

## Therapeutic management of positive cases:

Eighteen positive cases of canine babesiosis were selected for therapeutic management with different drugs as well as supportive therapy. Dogs naturally infected with babesiosis were grouped irrespective of species in the control group and three treatment groups (i.e., Group -1, 2, 3) shown in Table-1.

## Treatment protocol for babesiosis positive dogs

| Control Group | Group -1 | Group-II | Group-III |
|---|---|---|---|
| No Medication | Imidocarb dipropionate @6.6mg/kg s/c repeat in 2 weeks.<br><br>Supportive:<br>1. DNS @ 10-20 ml/kg b.wt.<br>2. RL @ 10-20 ml/kg b.wt.<br>3.N-acetylcystine@70mg/kg b.wt.<br>4. Frusemide @2mg/kgb.wt.<br>5.Metron@20mg/k.bwt | Clindamycin<br>+<br>Diaminazine aceturate<br>Clindamycin @ 30mg/kg orally for 10days.<br><br>Diaminazine aceturate @ 3.5mg/kg b.wt.<br><br>Supportive<br>1. DNS @ 10-20 ml/kg b.wt.<br>2. RL @ 10-20 ml /kg b.wt.<br>3.Nacetylcystine@70 mg/kg b. wt<br>4. Frusemide @ 2mg/kg b. wt.<br>5.Metron@20mg/k.bwt | Clindamycin<br>+<br>Azithromycin<br>Clindamycin @30 mg/b.wt.<br>Azithromycin@10mg/kg b. wt.<br>Orally for 10days.<br><br>Supportive<br>1. DNS @ 10-20 ml/kg b.wt.<br>2. RL @ 10-20 ml /kg b.wt.<br>3. N-acetylcysteine@70 mg/kg b.wt.<br>4. Frusemide @ 2 mg/kgb.wt.<br>5.Metron@20mg/k.bwt |

## Group 1:

The affected dogs in this group were given treated with Imidocarb dipropionate @6.6mg/kg s/c repeat in 2 weeks on the first day after diagnosis and sample collection.

## Group 2:

The affected group in this group given treatment with Clindamycin @ 30mg/kg orally O.D for 10days and Diaminazine aceturate @ 3.5mg/kg b.wt. I/M repeat after 24 hours on the first day after diagnosis and sample collection.

## Group 3:

The affected dogs in this group were given treatment with Clindamycin @30 mg/b.wt. Azithromycin@10mg/kg b. wt. Orally OD for 10days.

## 10. Oxidative stress parameter by blood haemolysate antioxidant estimation:

Blood samples were collected with anticoagulant and were centrifuged at 2000 rpm for 15 min. Then plasma and buffy coat were removed and then separated from RBCs.Now this erythrocyte pellet was diluted by using cold water to 1:10 dilution to haemolyse erythrocyte and make RBCs haemolysate. This haemolysate was stored at -20 till further used for estimation of the following antioxidant enzyme.

1ml blood (15ml tube)

⇩

Centrifuge (3000 rpm for 30 minutes)

⇩

Remove plasma (Supernatant)

⇩

RBC Pellet (1:10 dilution) by chilled D.W

⇩

Freeze for further use (-20$^{o}$C)

CAT, SOD, MDA, GSH will be estimated on 0, 7 and 14$^{th}$ days of treatment.

- **Catalase activity (CAT)**

Activities of Catalase enzymes were estimated spectrophotometrically as per the method given by Cohen *et al*. (1970). The reaction commences with the addition of 50 µl of haemolysate sample to 2.95 ml of phosphate buffer- $H_2O_2$ solution give an absorbance of 0.5-0.6 at 240 nm. The decrease in absorbance of 0.05 at 240 nm corresponds to the disappearance of 3.45 µmoles of $H_2O_2$, the units of Catalase activity per ml of sample was calculated as below.

0.05 Change in absorbance = 3.45 µmoles of $H_2O_2$ disappeared

'A' change in absorbance in 50 µl sample = 3.45 X A/ 0.05

So, 'A' change in absorbance in 1 ml sample = 3.45 X A/0.05 X 0.05)

= 1380 A

Catalase per gm Hb = $\frac{1380A \times 30}{Hb}$

## 1. Reagents for estimation of Catalase activity

a. Phosphate buffer solution pH 7.4

| | |
|---|---|
| Disodium Hydrogen Phosphate | 0.68gm/100ml D.W |
| Potassium dihydrogen phosphate | 0.89gm/100ml D.W |

Add the former to the latter in the ratio of 61:39

b. PBS –Hydrogen peroxide solution

| | |
|---|---|
| Hydrogen peroxide | 100µl |
| PBS to make | 100 ml |

- **Superoxide dismutase (SOD)**

Erythrocytic superoxide activity was measured using the method as described by Madesh and Balasubramanian (1997) with some modifications. It involves generation of superoxide by pyrogallol autoxidation and the inhibition of superoxide-dependent reduction of tetrazolium dye MTT [3-4-5 dimethyl thiazol 2-xl) 2,5 diphenyl tetrazolium bromide] to its formazan, measured at 570nm.In this method the total volume of 1515µl consisted of 650 µl PBS,10 µl

haemolysate,30 µl MTT and 75 µl of freshly prepared 100 mM pyrogallol solution to be added at the end. Sample was replaced with PBS in blank. After an incubation period of 5 minutes, 750 µl DMSO was added and absorbance was taken at 570 nm.

$$SOD(U) = \frac{\text{mg of Hb in 10 µl Haemolysate X 50 X DF}}{Y\%}$$

$$Y = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

OD of test = U-B

OD of Control = C-B

$$\text{SOD per gm Hb} = 2 \times 100 \times \frac{\text{OD of test}}{\text{OD of control}} \times \frac{\text{DF}}{\text{gm Hb}}$$

## Reagents for estimation of SOD activity

| | |
|---|---|
| a. 1.25 mM MTT solution | 2.58 mg MTT |
| Distilled water to make | 5 ml |
| b. 100 mM pyragallol solution | 6.3 mg Pyrogallol |
| Distilled water to make | 100 ml |

- **Lipid peroxidation (LPO):**

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thoibarbutric acid (TBA) as per modified method of Stock and Dormandy (1971) as described by Jain (1986).0.2ml of blood haemolysate sample was suspended in 0.8ml of phosphate buffer saline (PBS) followed by addition of 0.5 ml of Tri chloro acetic acid.Mixture was vortexed and incubated for 2 hours at 37˚C.Now suspention was centrifuged at 2000 rpm for 15 minutes .1 ml of each supernatant was transferred into another tube and to this 0.075 ml EDTA and 0.025 ml of thiobarbitutric acid was added.The mixture was heated in boiling water bath for 15 minutes and then allows cooling at room temperature. Absorbance was measured at 535 nm.

Calculation was done using the molar extinction coefficient (EC) of MDA-TBA complex at 535 nm, i.e.,$1.56 \times 10^5$ $mmol^{-1}cm^{-3}$. The amount of LPO was expressed as nano mol of MDA formed per ml of Whole blood by employing following formula.

MDA produced (nmol/mg of Hb) =

$$\frac{\text{OD of test}}{\text{EC}} \times \frac{1}{\text{Volume of supernatant added x Hb}} \times \text{dilution factor}$$

## Reagents for lipid peroxidation

a. Trichloroacetic Acid (TCA) 30% solution

| | |
|---|---|
| Trichloroacetic acid | 30 g |
| Distilled water to make | 100 ml |

b.1% Thiobarbituric acid(TBA) solution

| | |
|---|---|
| Thiobarbituric acid | 1 g |
| 0.05 N NaOH to make | 100 ml |

c. EDTA solution (0.01M)

| | |
|---|---|
| EDTA | 3.722 g |

- **Reduced glutathione (GSH)**

Reduced glutathione level in blood was estimated using the method given by Prins and Loos (1969). Haemolysate sample (0.2ml) was added to 4ml of $H_2SO_4$ and mixed carefully. After 10 min of standing at room temperature, 0.5ml of tungustate solution was added to clear the brown haemolysate. The tube was stoppered and the mixture was shaken vigoursly for 5 min. The stopper was removed and suspention was allowed to stand for 5 min in order to avoid crust formation on top of supernatant. The suspention was then centrifuged for 15 min at 2000 rpm at room temperature. After centrifugation 2ml of supernatant was added to 2.5 ml of Tris – buffer ,0.2 ml of DTNB reagent was added and mixed well.

## Reagents for glutathione estimation

a. $H_2SO_4$-0.08 N in distilled water

b. Tungustate solution

| | |
|---|---|
| Sodium tungustate | 0.3M |
| EDTA | 0.1M |

c Tris buffer (1M) pH8

| | |
|---|---|
| tris-hydroxymethly aminomethane | 1M |

d. DTNB reagent

| | |
|---|---|
| Sodium chloride | 0.14 M |
| Disodium Hydrogen Phosphate | 0.009M |
| Sodium dihydrogen phosphate | 0.000013 M |
| DTNB | 40mg |
| Distilled water to make | 100ml |

Within a minute, absorbance was measured at 412 nm against blank in which 2ml of distilled water was substituted for supernatant.

GSH levels was conveniently calculated by using the extinction co-efficient (EC) 13100$M^{-1}$ $cm^{-1}$ and results are expressed as mM GSH per ml of serum (Mm/ml), using the following formula.

$$\text{GSH (mM/gHb)} = \frac{\text{OD of test}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture}}{\text{Volume of supernatant added}} \times \text{dilution factor}$$

**Fig: -3.6 Measurement of oxidative stress parameters**

## Statistical analysis:

Statistical analysis was performed by using software (SPSS) as described by Snedecor and Cochran (1994).

- 24 positive dogs for babesiosis was screened for multiple organ dysfunctions (MODS) and will be treated differently in four clinical groups. The criteria multiple organ dysfunction are as follows: -

(a)Systemic inflammatory Response Syndrome (SIRS)

SIRS was done as per method described by Hauptman *et al.,* 1997.

SIRS-Positive:

WBC <6000 or >16000or>3% band cells

With

-Rectal temperature <38.1>39.2$^{O}$C

-HR> 120/bpm

(b) Cut-off points for organ damage

**i. Liver**

ALT>80U/L

ALP>380U/L

GLDH>16U/L

Bile acids>30µmol/L

**ii. Kidney**

Creatinine >150µmol/L

**iii. Lungs**

$SPO_2$<60µmol/L

**iv. Muscles**

Creatinine Kinase >300U/L

# 4 RESULTS AND DISCUSSION

| Table 4.1 Mean ± SE values of haemoglobin (gm/dl) of healthy & Babesia positive dogs in different treatment groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $12.53 \pm 0.18^{ax}$ | $6.52 \pm 0.07^{bx}$ | $9.11 \pm 0.19^{cdx}$ | $9.19 \pm 0.040^{dx}$ |
| $7^{th}$ day | $13.02 \pm 0.22^{axy}$ | $7.15 \pm 0.02^{by}$ | $9.87 \pm 0.20^{cdy}$ | $10.55 \pm 0.09^{dyz}$ |
| $14^{th}$day | $13.40 \pm 0.31^{ay}$ | $8.11 \pm 0.03^{bz}$ | $10.96 \pm 0.055^{cz}$ | $12.47 \pm 0.13^{az}$ |

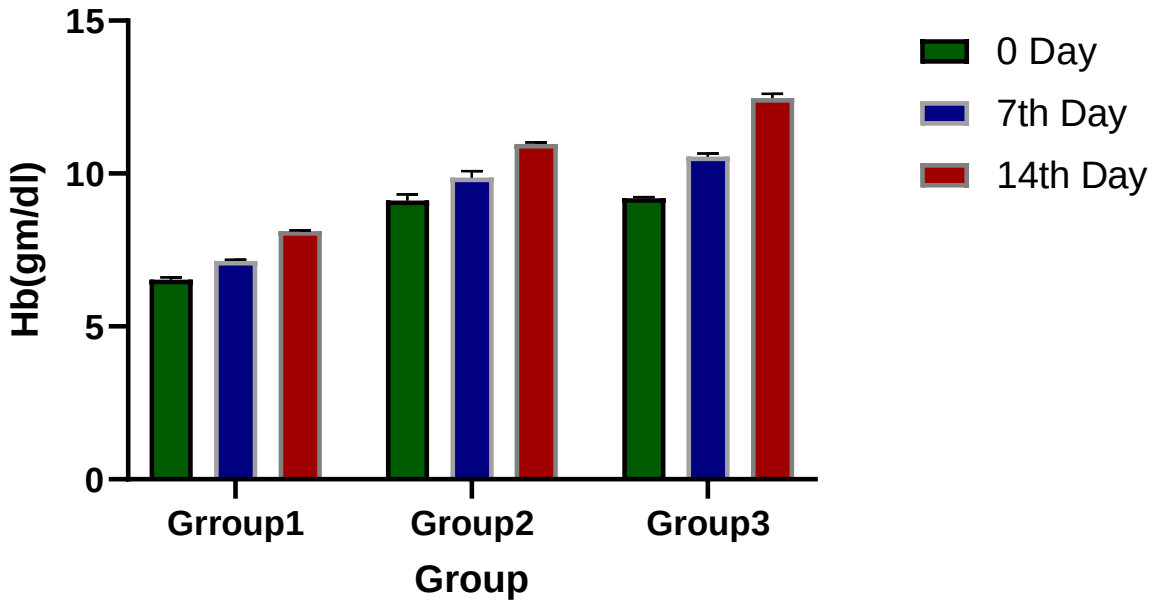


**Fig.4.1.1 Bar diagram of Hb level in different treatment groups of Babesia positive dog.**

## Results: -

In present study the Hb concentration was measured in pre treatment and post treatment of healthy and different treatment group. Variation in Hb value in different group and days of on 0,7,14 days were found as mentioned below.

The value of Hb in group-1 on 0,7,14 days were found 6.52 ± 0.07, 7.15 ± 0.02 and 8.11 ± 0.03 respectively. The value of Hb in group-2 on different time interval on 0, 7,14 days were found 9.11± 0.19, 9.87 ± 0.20, and 10.96 ± 0.055 respectively. The value of Hb in group-3 on different time interval on 0, 7,14 days were found 9.19± 0.040, 10.55 ±0.09 and 12.47± 0.13 respectively. The value of Hb in healthy control was significantly higher ($p< 0.05$) than all the treatment groups.

Mean±SE value of Hb in all treatment group were significantly lower($p<0.05$) than the healthy control group on zero day of the treatment. On day zero the value of Hb between group-1 and group-2 were significantly different. The Hb concentration in group-2 were significantly higher ($p<0.05$) than group-1. However, group-3 values were almost same and non significant.

Similarly on day $7^{th}$ the values of Hb were significantly lower than the control group in all treatment group. On day $7^{th}$ the value of Hb between group-1 and group-2 were significantly different. The Hb concentration in group-2 were significantly higher than group-1; however, group-3 values were significantly higher than group-1 and group-2.

Again on $14^{th}$ days the value of Hb in group-1 and group-2 were significant in comparision to control group and group-3 was non significant in comparision to control group. On the $14^{th}$ day of treatment, the values in group-2 were significantly higher than group-1ans significantly lower than group-3.

In treatment group-1, group-2 and group-3; Mean± SE value of Hb on different days of treatment were compared and it has been found that Hb values significantly increases from 0 to 7 and7-14 days interval in all the three groups.

The value of Hb in clinically affected dogs with Babesia is found to be lower than the healthy control is due to disruption of RBC due to presence of piroplasm inside the RBC which results in the Haemolysis and release of Hb from ruptured RBC. Decreased Hb levels could be due to epistaxis, petechial hemorrhages and intravascular haemolysis or due to severe anaemia.

Decrease in Hb levels were in agreement with reports of Furlanello *et al*. (2005) Niwetpathomwat et al. (2006), Shah et al. (2011), Wadhwa et al. (2011), Andoni et al. (2012), Andoni et al. (2013), Reddy et al. (2014) and Nalubamba et al. (2015), Avinash et al (2017); and my research finding is agreement of those findings.

| Table 4.2 Mean ±SE values of TLC (×10$^3$/µl) of healthy & Babesia positive dogs in different treatment groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | 12.14 ± 0.27$^{ax}$ | 13.51 ± 0.17$^{bx}$ | 14.92 ± 0 .25$^{cx}$ | 16.27 ± 0.18$^{dx}$ |
| 7$^{th}$ Day | 11.79 ± 0.22$^{ay}$ | 13.03 ± 0.26$^{bcy}$ | 13.97 ± 0.15$^{cx}$ | 15.12 ± 0.04$^{dy}$ |
| 14$^{th}$ Day | 11.50 ± 0.21$^{az}$ | 12.587 ± 0.16$^{bxy}$ | 11.42 ± 0.07$^{ay}$ | 11.84 ± 0.44$^{abz}$ |

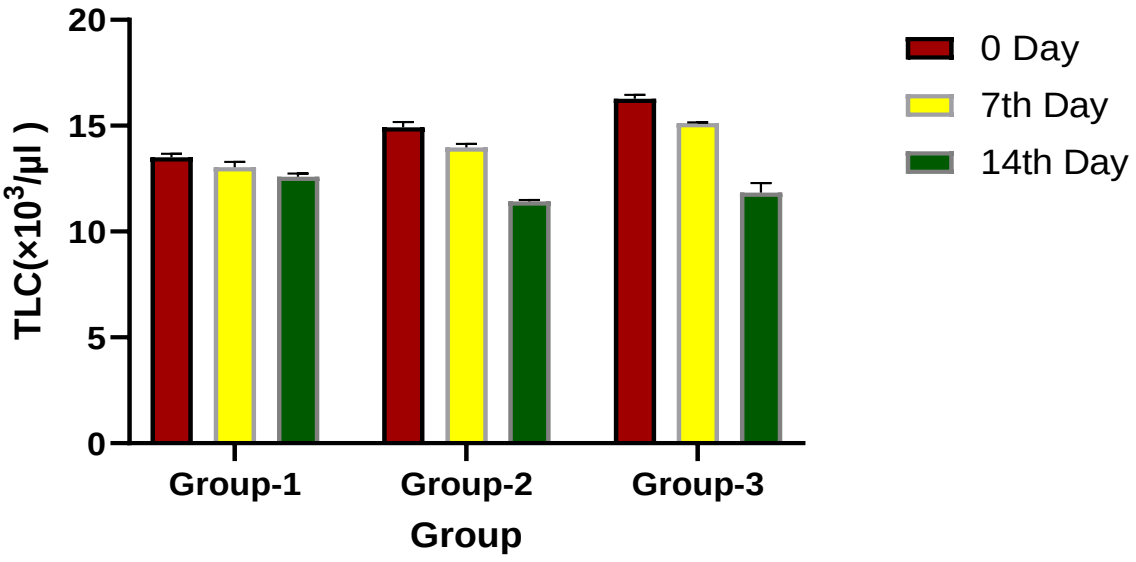


**Fig.4.2.1 Bar diagram of TLC level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study TLC, the level was measured in pre-treatment and post-treatment in healthy and different treatment groups. Variation in TLC value in different groups and days of treatment on 0,7,14 days were found as below.

The value of TLC in group-1 on different time interval on 0,7,14 days were found 13.51 ± 0.17, 13.03 ± 0.26 and 12.587 ± 0.16 respectively.

The value of TLC in group-2 on different time interval on 0, 7, 14 days were found 14.92 ± 0 .25, 13.97 ± 0.15 and 11.42 ± 0.07 respectively.

The value of TLC in group-3 on different time interval on 0, 7, 14 days were found 16.27 ± 0.18, 15.12 ± 0.04 and 11.84 ± 0.44 respectively.

The value of TLC in Healthy control was significantly lower ($p>0.05$) than all the treatment groups.

The mean ± SE value of TLC in all treatment group were significantly higher($p>0.05$) than the healthy control group on day zero of the treatment.

Similarly on day 7 again the values of TLC were significantly higher than the control group in all the treatment groups.

Again, on day 14th the values of TLC in treatment group-2 and group-3 were non-significant in comparison to the control group. However, it was significantly higher ($p>0.05$) in the group -1as compared to the control group.

In treatment group-1 Mean ± SE value of TLC on different days of treatment were compared and it has been found that the TLC values on day 14th were similar as day zero and day 7 and non-significant, but on day 7th it was significantly higher($p>0.05$) than the day zero and day 14th.

Similarly in treatment group -2 the variation in TLC values on different days of treatment 0,7,14 was compared and it was found that on Day 0,7, the values were almost identical and non-significant ($p>0.05$). However, the values on days -14 significantly reduced than the zero and 7th days values($p<0.05$).

Again, on treatment group-3 the variation in TLC values on different days of treatment 0,7,14 was compared and it was found that on days 0 and7 the value was almost identical and non-significant ($p>0.05$). However, the values on the 14th day were significantly reduced to the values on the 7th day of treatment($p<0.05$).

The value of TLC in clinically affected dogs with Babesia infection found to be higher than the healthy control is due to systemic inflammatory response syndrome. Activation of inflammatory cascade due to sepsis in Babesia-associated MODS patients (Liss,2012).

The increase in TLC levels was in agreement with reports of, Batos et al (2004), Selvaraj et al., (2010), Shrivastava et al., (2014), and my research is also the collaboration of this findings. However, Wadhwa et al (2011) and Reddy et al. (2014) reported the presence of significant leucopenia in dogs with Babesiosis which is opposite to my finding.

| Table 4.3 Mean ± SE values of TEC (×$10^6$/µl) of healthy & Babesia positive dogs in different treatment groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $6.61 \pm 0.20^{ax}$ | $4.985 \pm 0.11^{bx}$ | $3.478 \pm 0.09^{cx}$ | $4.190 \pm 0.12^{dx}$ |
| $7^{th}$ Day | $6.68 \pm 0.21^{ax}$ | $4.660 \pm 0.07^{bdx}$ | $3.860 \pm 0.06^{cy}$ | $4.600 \pm 0.06^{dx}$ |
| 14th Day | $7.18 \pm 0.21^{ay}$ | $5.837 \pm 0.04^{by}$ | $4.347 \pm 0.03^{cz}$ | $5.270 \pm 0.14^{dy}$ |

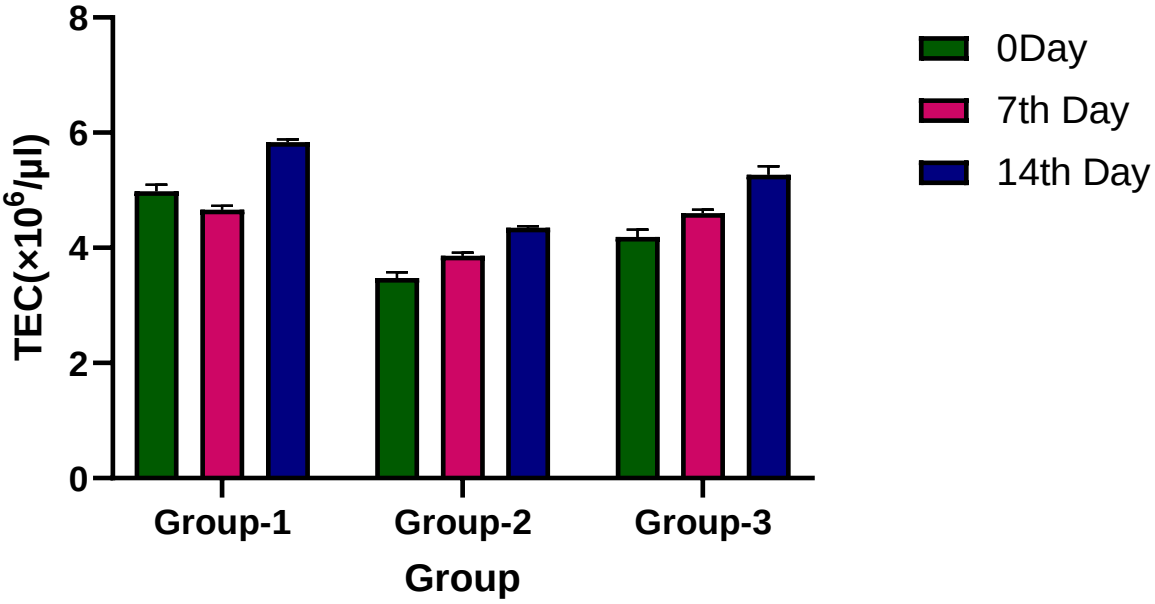


**Fig.4.3.1 Bar diagram of TEC level in different treatment groups of Babesia positive dog.**

## Results:

In the present study, the TEC level was measured in pre-treatment and post-treatment in the healthy and different treatment groups. Variation in TEC value in different groups and days of interval on 0,7,14 days were found respectively. The value of TEC in group-1 on different time interval on 0,7,14 days were found 4.985 ± 0.11, 4.660 ± 0.07 and 5.837 ± 0.04 respectively. The value of TEC in group-2 on different time interval on 0, 7, 14 days were found 3.478 ± 0.09, 3.860 ± 0.06 and 4.347 ± 0.03 respectively.

The value of TEC in group-3 on different time interval on 0, 7, 14 days were found 4.190 ± 0.12, 4.600 ± 0.06 and 5.270 ±0.14 respectively.

The value of TEC in Healthy control was significantly higher ($p< 0.05$) than all the treatment groups. Mean± SE value of TEC in all treatment groups was significantly lower than the the healthy control group on day zero of the treatment.

On day zero the value of TEC in group-1, group-2, and group-3 was significantly different.

On zero-day, the value of TEC was significantly highest in group-1 and lowest in group-2.

Similarly, on Day 7th the value of TEC was significantly lower than the control group in all treatment groups. On day 7th the value of TEC between group-1 and group-2 was significantly different. The value of TEC in group-2 was significantly lower than group-1.

However, the value of TEC in group-3 was significantly higher than group-2 and almost similar to group-1. Again, on the 4th day, the value of TEC in all the three treatment groups was

significantly lower than the healthy control group. On day 14th days of treatment, the values of TEC were significantly highest in group-1 and lowest in group-2.

In treatment, group-1 Mean ± SE values of TEC on different days of treatment were compared and it has been found that TEC values on the 7th day were similar to day zero and non-significant. But on day 14th it was significantly higher than day zero and day 7th.

The value of TEC in clinically affected dogs with Babesia infection found to be lower than the healthy control is due to disruption of RBC from the presence of piroplasm inside the RBC which results in the Haemolysis and release of Hb from ruptured RBC into the reticuloendothelial system and other phagocytic system leads to the development of reduction in TEC concentration in Blood. Decreased TEC levels could be due to epistaxis; petechial haemorrhages and extravascular haemolysis or due to severe anemia (Ettinger andFeldman,2006).

Decrease in TEC levels were in agreement with reports of Niwethomwat et al., (2006) Andoni et al. (2012), Reddy et al. (2014), Nalubamba *et al*. (2015), Avinash *et al*. (2017) and my research is also the collaboration of these findings.

| Table 4.4 Mean ± SE values of SGPT(ALT)(IU/L) of Healthy & Babesia positive dogs in different treatment groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $36.43 \pm 1.57^{ax}$ | $63.98 \pm 0.32^{bx}$ | $71.36 \pm 1.77^{cx}$ | $137.35 \pm 0.50^{dx}$ |
| $7^{th}$ Day | $36.90 \pm 1.29^{ax}$ | $62.71 \pm 0.53^{bx}$ | $67.81 \pm 1.24^{cy}$ | $130.56 \pm 0.86^{dy}$ |
| 14th day | $34.86 \pm 0.86^{ax}$ | $56.11 \pm 0.20^{by}$ | $60.75 \pm 0.32^{cz}$ | $120.33 \pm 0.52^{dz}$ |

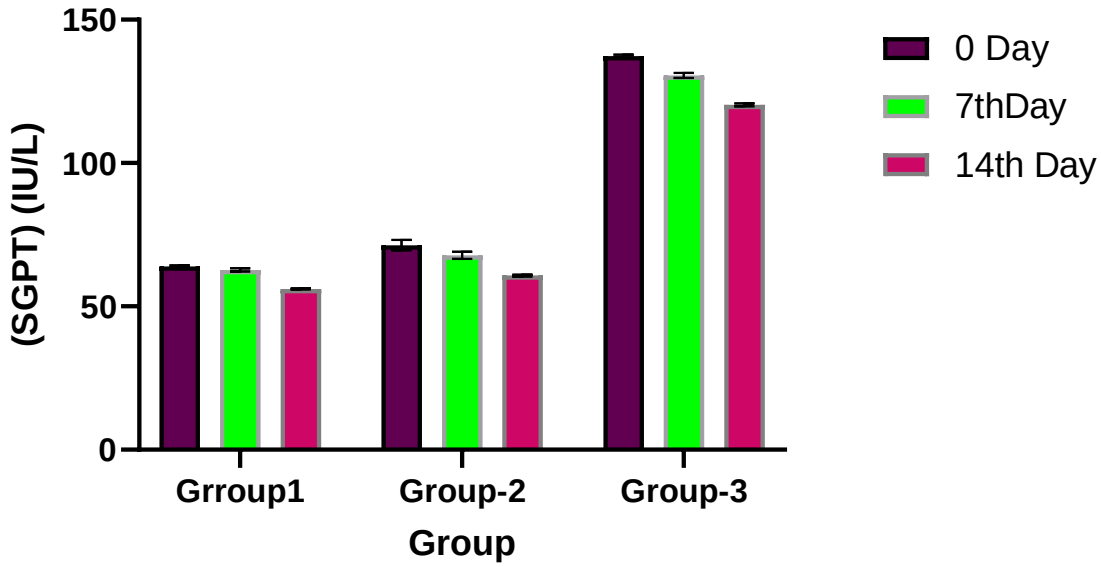


**Fig.4.4.1 Bar diagram of SGPT level in different treatment groups of Babesia positive dog.**

## Results: -

In present study the ALT level was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in ALT value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of ALT in group-1 on different time interval on 0,7,14 days were found 63.98 ± 0.32 , 62.71± 0.53 and 56.11 ± 0.20 respectively.

The value of ALT in group-2 on different time interval on 0, 7, 14 days were found 71.36 ± 1.77, 67.81± 1.24 and 60.75± 0.32 respectively.

The value of ALT in group-3 on different time interval on 0, 7, 14 days were found 137.35 ± 0.50, 130.56 ± 0.86 and 120.33 ± 0.52 respectively.

The value of ALT in Healthy control was significantly lower ($p< 0.05$) than all the treatment groups.

Mean ±SE value of ALT in all treatment groups was significantly higher than the healthy control group on day zero of the treatment.

Similarly on day 7 again the values of ALT were significantly higher than the control groupin all the treatment groups.

Again, on day 14th, the values of ALT were significantly higher than the control group in all treatment group.

In treatment group-1; the Mean ±SE value of ALT on different days of treatment were compared and it has been found that ALT values on day 7th were similar to day zero and non significant ($p>0.05$) in comparison to day zero. But on day 14th the value of ALT was significantly lower than day zero and day 7th.

Similarly in treatment group-2 the variation in ALT values on different days of treatment 0,7,14 was compared and it was found that ALT values significantly decrease from 0 to 7 and 7 to 14 days of interval ($p<0.05$).

Again, in treatment group-3 the variation in ALT values on different days of treatment 0,7,14 was compared and it was found ALT values significantly decreases from day zero to day 7th and day 7th to day 14th ($p<0.05$).

The value of ALT in clinically affected dogs with Babesia infection found to be higher than the healthy control is due to the escape of these enzymes from damaged hepatic parenchyma cells with necrosis or altered membrane permeability indicating hepatic dysfunction (Wadhwa et al.,2011).

The increase in ALT levels was in agreement with reports of Furlanello *et al*. (2005), Wadhwa *et al*. (2011), and Reddy *et al*. (2014) and my research is also the collaboration of these findings.

| Table 4.5 Mean ± SE values of SGOT (AST)(IU/L) of healthy & Babesia positive dogs in different treatment groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $38.66 \pm 0.86^{ax}$ | $63.11 \pm 0.15^{bx}$ | $69.08 \pm 0.23^{cx}$ | $97.49 \pm 0.03^{dx}$ |
| $7^{th}$ Day | $36.91 \pm 0.78^{ay}$ | $60.06 \pm 0.43^{by}$ | $63.08 \pm 0.19^{cy}$ | $97.49 \pm 0.05^{dy}$ |
| $14^{th}$ day | $35.68 \pm 0.71^{az}$ | $54.56 \pm 0.14^{bz}$ | $65.12 \pm 0.20^{cz}$ | $89.87 \pm 0.10^{dz}$ |

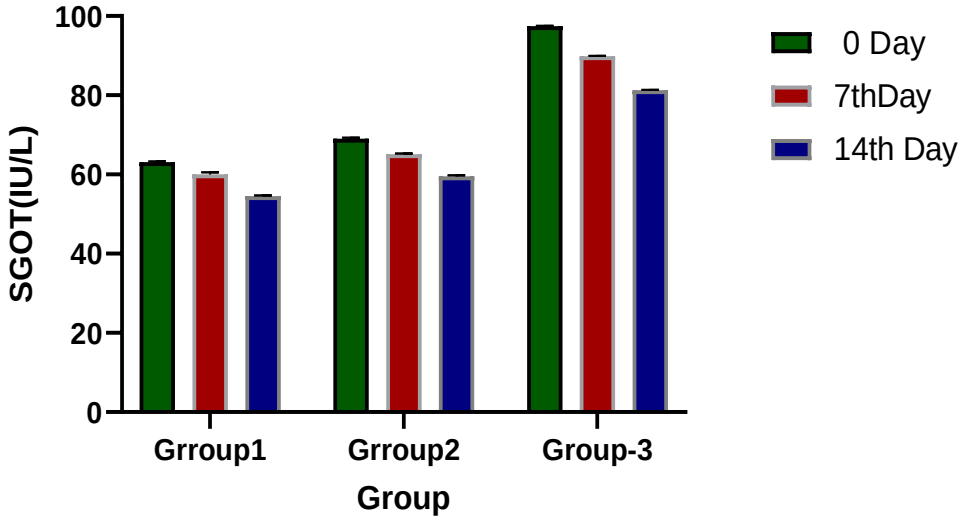


**Fig.4.5.1 Bar diagram of SGOT level in different treatment groups of Babesia positive dog.**

## Results: -

In present study the AST level was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in AST value in different groups and days of dogs on 0,7,14 days were found respectively.

The value of AST in group-1 on different time interval on 0,7,14 days were found 63.11 ± 0.15, 60.06 ± 0.43 and 54.56 ± 0.14 respectively.

The value of AST in group-2 on different time interval on 0, 7, 14 days were found 69.08 ± 0.23, 63.08 ± 0.19 and 65.12 ± 0.20 respectively.

The value of AST in group-3 on different time interval on 0, 7, 14 days were found 97.49 ± 0.03, 97.49 ± 0.05 and 89.87 ± 0.10 respectively.

The value of AST in Healthy control was significantly higher ($p< 0.05$) than all the treatment groups.

Mean ±SE value of AST in all treatment groups were significantly higher than the healthy the control group on day zero of treatment.

Similarly on day 7 and day 14th; again the values of AST were significantly higher than the control group in all the treatment groups.

In treatment group-1; the Mean ±SE value of AST on different days of treatment were compared and it has been found that AST values significantly decreases from 0 to 7 and 7 to 14days interval in all the three groups.

The value of AST in clinically affected dogs with Babesia infection found to be higher than the healthy control is due to the escape of these enzymes from damaged hepatic parenchyma cells with necrosis or altered membrane permeability indicating hepatic dysfunction (Wadhwa et al.,2011).

The increase in AST levels was in agreement with reports of Furlanello et al. (2005), and Wadhwa et al. (2011) and my research is also the collaboration of these findings.

**Table 4.6 Mean ±SE values of BUN (mg/dl) of healthy & Babesia positive dogs in different treatment groups at various time interval.**

| Days | Control Group | Group-1 | Group-2 | Group-3 |
|---|---|---|---|---|
| 0 Day | 13.82 ± 0.48$^{ax}$ | 29.96 ± 0.01$^{bx}$ | 31.52 ± 0.03$^{cx}$ | 26.15 ± 0.01$^{dx}$ |
| 7$^{th}$ Day | 13.44 ± 0.32$^{ax}$ | 29.63 ± 0.17$^{bx}$ | 30.80 ± 0.03$^{cy}$ | 25.17± 0.01$^{dy}$ |
| 14$^{th}$ Day | 13.33 ± 0.24$^{ax}$ | 28.38 ± 0.02$^{by}$ | 29.81 ± 0.01$^{cz}$ | 23.31 ± 0.03$^{dz}$ |

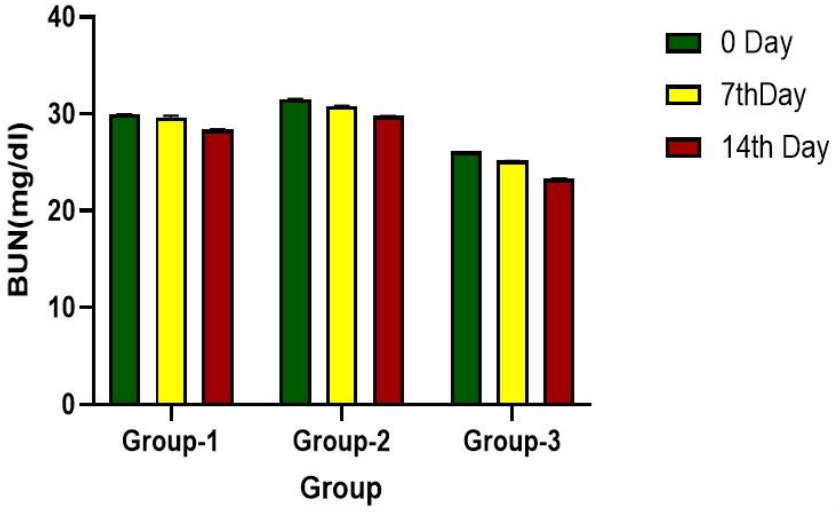


**Fig.4.6.1 Bar diagram of BUN level in different treatment groups of Babesia positive dog.**

## Results: -

In present study the BUN level was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in BUN value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of BUN in group-1 on different time interval on 0,7,14 days were found 29.96 ± 0.01, 29.63 ± 0.17, 28.38 ± 0.02 and respectively.

The value of BUN in group-2 on different time interval on 0, 7, 14 days were found 31.52 ± 0.03, 30.80 ± 0.03 and 29.81 ± 0.01 respectively.

The value of BUN in group-3 on different time interval on 0, 7, 14 days were found 26.15 ± 0.01, 25.17± 0.01, 23.31 ± 0.03 and respectively.

The value of BUN in Healthy control was significantly higher ($p< 0.05$) than all the treatment groups.

Mean ±SE value of BUN in all treatment group were significantly higher than the healthy control on day zero, day 7th, and day 14th of treatment.

In treatment group-1; the Mean ±SE value of BUN on different days of treatment was compared and it has been found that BUN values on day 7th were similar to day zero and non-significant. But on day 14th it was significantly higher than day zero and day 7th.

Similarly in group-2 and group-3 the variation in BUN values on different days of treatment 0,7,14 was compared and it has been found that BUN values significantly decrease from 0 to 7 and 7 to 14 days of interval.

The value of BUN in clinically affected dogs with Babesia infection found to be higher than the healthy control is due to sequential failure of the renal system in dogs with babesiosis might be responsible for retention of nitrogenous waste products in the body (Karlsson *et al*.2012).

The increase in BUN levels was in agreement with reports of Reddy et al. (2014), Sivajothi *et al.*, (2014) and my research is also the collaboration of these findings.

| Table 4.7 Mean ±SE values of CRE (mg/dl) of healthy & Babesia positive dogs in different treatment groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $0.95 \pm 0.007^{ax}$ | $0.92 \pm 0.009^{acx}$ | $0.99 \pm 0.014^{adx}$ | $2.82 \pm 0.050^{bx}$ |
| $7^{th}$ Day | $0.96 \pm 0.018^{ax}$ | $0.87 \pm 0.009^{byz}$ | $0.94 \pm 0.007^{ay}$ | $2.33 \pm 0.007^{cy}$ |
| $14^{th}$ Day | $0.95 \pm 0.011^{ax}$ | $0.85 \pm 0.012^{bcz}$ | $0.87 \pm 0.010^{cz}$ | $1.96 \pm 0.013^{dz}$ |

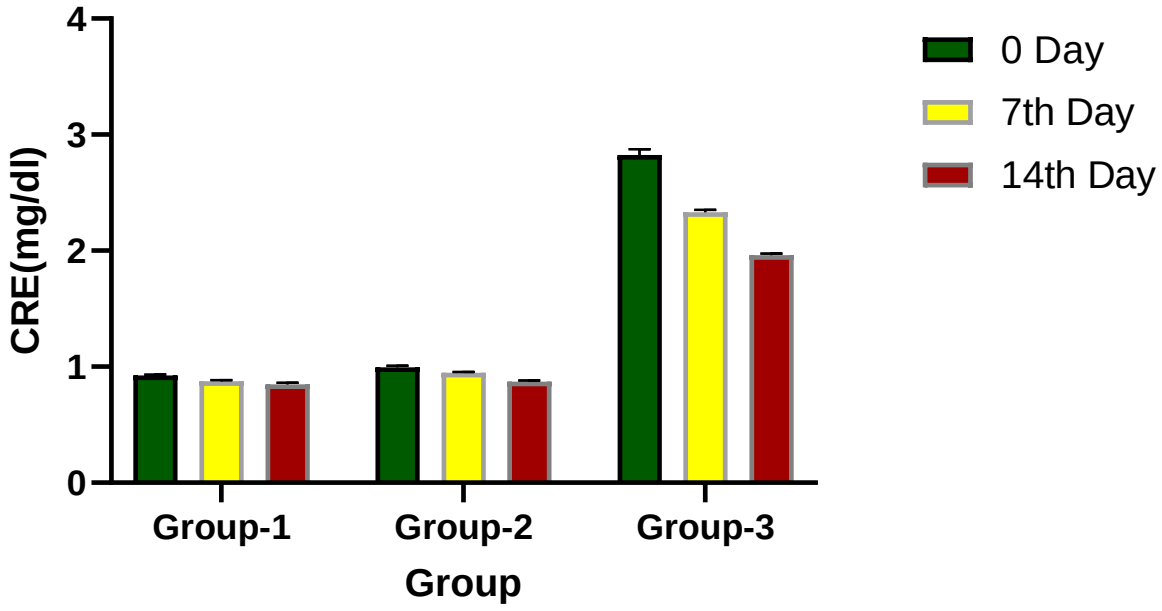


**Fig.4.7.1 Bar diagram of CRE level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the creatinine level was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in creatinine value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of creatinine in group-1 on different time interval on 0,7,14 days were found 0.92 ± 0.009, 0.87± 0.009, and 0.85 ± 0.012 respectively.

The value of creatinine in group-2 on different time interval on 0, 7, 14 days were found 0.99± 0.014, 0.94 ± 0.007 and 0.87 ± 0.010 respectively.

The value of creatinine in group-3 on different time interval on 0, 7, 14 days were found 2.82 ± 0.050, 2.33± 0.007, and 1.96 ± 0.013 respectively.

On day zero Mean ±SE values of creatinine in treatment group-1 and group-2 were similar the as healthy control group and non significantly. But in group-2 the creatinine value was significantly higher as compared to the control group on day zero.

Similarly on day 7 again the values of BUN in group-1 and group-3 were significantly different from the control group. In group-1 it is a significantly lower value as compared to healthy control and in group-3 it is a significantly higher value as compared to healthy control.

In group-2 value of creatinine similar to healthy control and non-significant.

Again on day 14th, the values of creatinine in all treatment groups were significantly higher than healthy control($p<0.05$).

In treatment group-1; the Mean ±SE value of creatinine on different days of treatment was compared and it has been found that creatinine values on day 7th and day14th significantly decrease as compared to day zero. But the creatinine values on day 14th are similar as compared to day 7th and non-significant ($p>0.05$).

Similarly in treatment group-2 and group-3 the variation in creatinine on different days of treatment 0,7,14 was compared and it was found that the values of creatinine significantly decrease from day zero to day 7 and day 7 to day 14th.

Sequential failure of the renal system in dogs with canine babesiosis might be responsible for the retention of nitrogenous waste products in the body (Karlson et al.,2012).

The difference between values of creatinine in babesia-positive dogs and healthy dogs was statistically non-significant. This was in agreement with Wadhwa et al. (2011).

However, Andoni et al. (2013), Reddy et al. (2014), and vishnurahav et al. (2014) reported a significant increase in creatinine in babesia-positive dogs than healthy dogs.

| Table 4.8 Mean ±SE values of TP (gm/dl) of healthy & Babesia positive dogs In different treatment groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | 5.81 ± 0.109$^{ax}$ | 5.11 ± 0 .017$^{bx}$ | 6.11 ± 0.021$^{ax}$ | 4.95 ± 0.017$^{cx}$ |
| 7$^{th}$ Day | 5.76± 0.060$^{ax}$ | 5.13± 0.012$^{by}$ | 6.11 ± 0.027$^{cy}$ | 5.00 ± 0.016$^{dy}$ |
| 14$^{th}$ Day | 5.71 ± 0.053$^{ax}$ | 5.56 ± 0.034$^{az}$ | 6.93 ± 0.018$^{bz}$ | 5.93 ± 0.012$^{cz}$ |

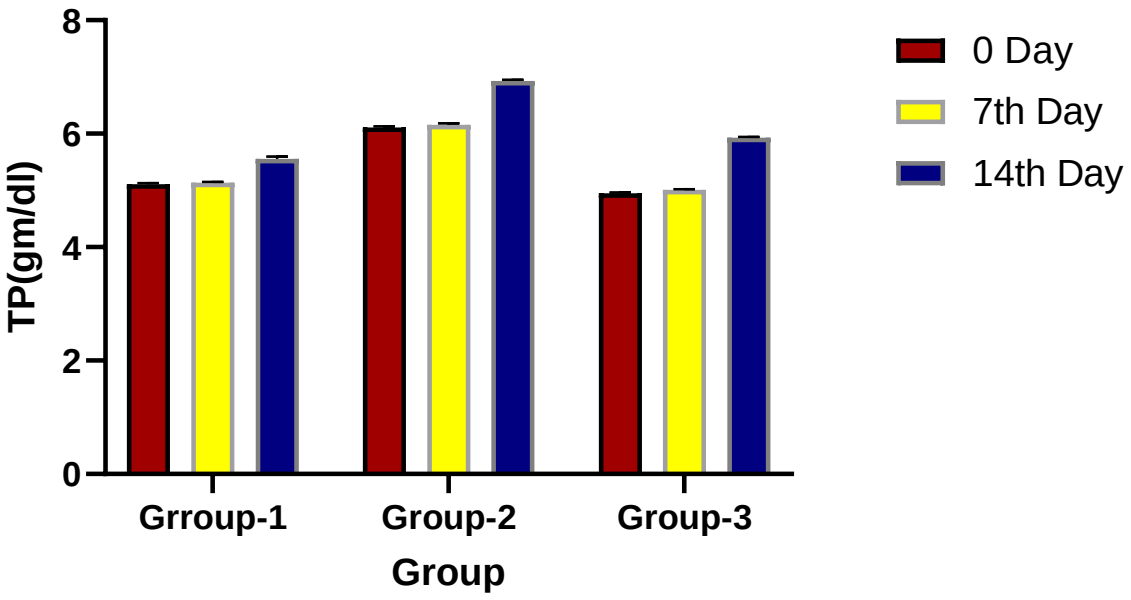


**Fig.4.8.1 Bar diagram of TP level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the total protein level was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in total protein value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of total protein in group-1 on different time interval on 0,7,14 days were found 5.11 ± 0 .017, 5.13 ± 0.012 and 5.56 ± 0.034 respectively.

The value of total protein in group-2 on different time interval on 0, 7, 14 days were found 6.11 ± 0.021, 6.11 ± 0.027 and 6.93 ± 0.018 respectively.

The value of total protein in group-3 on different time interval on 0, 7, 14 days were found 4.95 ± 0.017, 5.00 ± 0.016 and 5.93 ± 0.012 respectively.

On day zero; the Mean ±SE values of TP in treatment group-1 and group-3 were significantly lower in comparison to healthy control on the zero-day of treatment. But TP value in group-2 was found to be similar to the control group and non-significant.

Similarly on day 7th again the values of TP were significantly different from the control group in all the treatment groups. The values of total protein on day 7th in group-1 and group-3 were significantly lower than in the healthy control group, but in group-2 it was significantly higher than healthy control.

Again, on day 14th, the values of TP in treatment group-1 were non-significant in comparison to the control group. However, it was significantly higher in group-3 and significantly lower in group-2 than healthy control.

In treatment group-1, group-2, and group-3, TP values on different days of treatment were compared and it has been found that TP values significantly increase from 0 to 7 and 7 to 14 days of interval in all the three groups.

Low protein levels can be attributed to low hepatic perfusion and reduced protein synthesis (Maynard et al., 1997).

Reddy et al. (2014), Vishurahav et al., (2014) reported a decreased total protein value in Babesia-positive dogs in comparison to healthy control.

| Table 4.9 Mean ±SE values of ALB (gm/dl) of healthy &Babesiosis positive dogs in different treatment groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $3.38\pm 0.04^{ax}$ | $2.29 \pm 0.04^{bcx}$ | $2.37\pm 0.03^{cx}$ | $1.83 \pm 0.01^{dx}$ |
| $7^{th}$ Day | $3.23 \pm 0.08^{ax}$ | $2.45 \pm 0.05^{bdy}$ | $2.72\pm 0.04^{cy}$ | $1.83\pm 0.03^{dy}$ |
| $14^{th}$Day | $3.12\pm 0.06^{ax}$ | $2.54 \pm 0.05^{bz}$ | $4.05\pm 0.02^{cz}$ | $3.67\pm 0.03^{dz}$ |

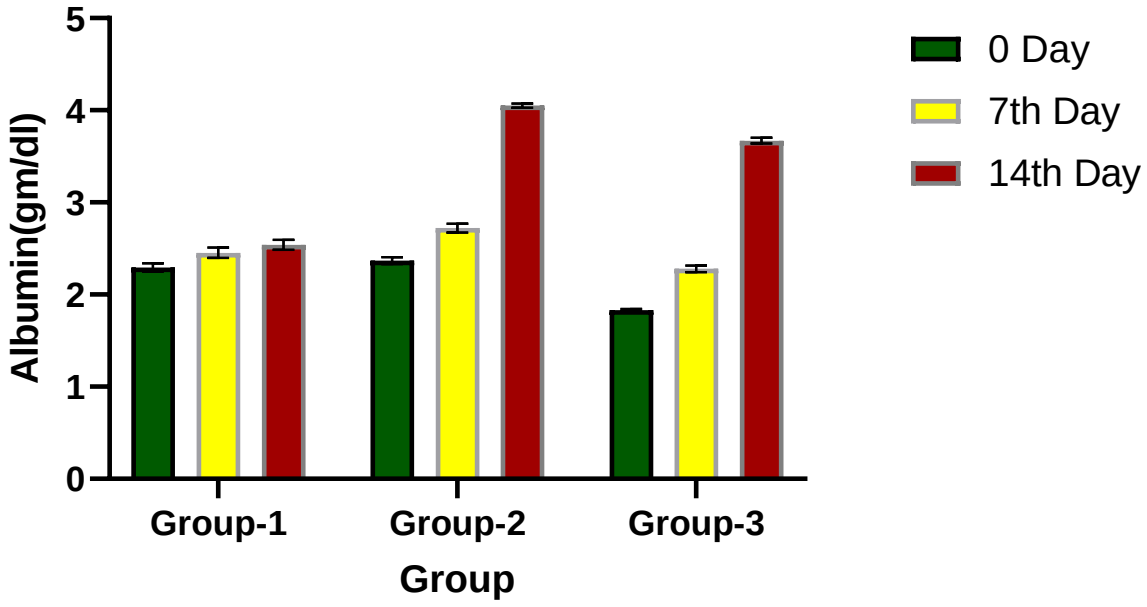


**Fig.4.9.1 Bar diagram of ALB level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the albumin level was measured in pre-treatment and post-treatment in healthy and different treatment groups.

Variation in albumin value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of albumin in group-1 on different time interval on 0,7,14 days were found 2.29 ± 0.04, 2.45 ± 0.05 and 2.54 ± 0.05 respectively.

The value of albumin in group-2 on different time interval on 0, 7, 14 days were found 2.37 ± 0.03, 2.72 ± 0.04, 4.05 ± 0.02 and respectively.

The value of albumin in group-3 on different time interval on 0, 7, 14 days were found 1.83 ± 0.01, 1.83 ± 0.03 and 3.67 ± 0.03 respectively.

Mean ± SE value of albumin in all treatment groups were significantly lower from health control group.

Similarly on day 7 again the values of albumin were significantly lower than the healthy the control group in all the three treatment groups.

Again, on day 14th, the values of albumin in treatment group-1 were significantly lower, and in treatment group-2 and group-3 were significantly higher than the healthy control.

In treatment group-1, group-2 and group-3; Mean ± SE values of albumin on different days of treatment were compared and it has been found that albumin values significantly increase from 0 to 7 and 7 to 14 days interval in all the three groups.

Low albumin levels can be attributed to low hepatic perfusion and reduced protein synthesis (Maynard et al., 1997).

Reddy et al. (2014), Vishurahav et al., (2014) reported a decrease in Albumin value in Babesia-positive dogs in comparison to healthy control.

| Table 4.10 Mean ±SE values of ALP (U/L) of healthy & Babesia positive dogs in different treatment groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $66.88 \pm 0.35^{ax}$ | $229.43 \pm 3.76^{bx}$ | $184.26 \pm 0.50^{cx}$ | $264.96 \pm 0.49^{dx}$ |
| 7th Day | $66.30 \pm 0.53^{ay}$ | $216.23 \pm 4.31^{by}$ | $160.93 \pm 1.62^{cy}$ | $234.38 \pm 1.62^{dy}$ |
| 14th day | $65.88 \pm 0.41^{ax}$ | $193.23 \pm 0.87^{bz}$ | $115.64 \pm 0.42^{cz}$ | $168.82 \pm 0.39^{dz}$ |

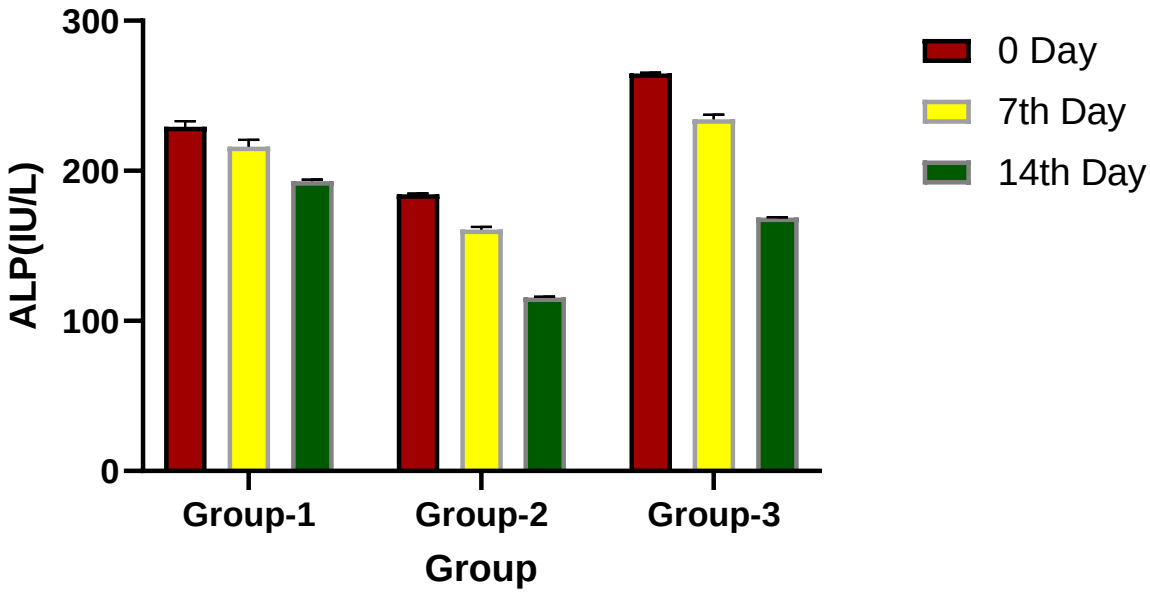


**Fig.4.10.1 Bar diagram of ALP level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the alkaline phosphatase level was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in alkaline phosphatase value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of alkaline phosphatase in group-1 on different time intervals on 0,7,14 days were found 229.43 ± 3.76, 216.23 ± 4.31 and 193.23 ± 0.87 respectively.

The value of alkaline phosphatase in group-2 on different time interval on 0, 7, 14 days were found 184.26 ± 0.50, 160.93 ± 1.62 and 115.64 ± 0.42 respectively.

The value of alkaline phosphatase in group-3 on different time intervals on 0, 7, 14 days were found 264.96± 0.49, 234.38± 1.62 and 168.82 ± 0.39 respectively.

Mean ± SE value of alkaline phosphatase in all treatment groups were significantly higher than the healthy control group on day zero, day 7th, and day 14th.

In treatment group-1, group-2, group-3 alkaline phosphatase on different days of treatment were compared and it has been found that alkaline phosphatase values significantly decrease from 0 to 7 and 7th to 14 days of interval in all the three groups.

An increase in the level of ALP was may be due to damage or abnormal function of the biliary system (Crnogaj et al; 2010).

The level of ALP increased significantly ($p< 0.05$) in dogs with babesiosis than healthy dogs. This finding was in agreement with Furlanello et al. (2005) and Shah et al (2011).

**Table 4.11 Mean ±SE values of Respiratory rate (Breaths/min) of healthy & Babesia positive dogs in different treatment groups at various time interval.**

| Days | Control Group | Group-1 | Group-2 | Group-3 |
|---|---|---|---|---|
| 0 Day | $21.50 \pm 2.09^{ax}$ | $29.833 \pm 1.01^{bx}$ | $31.33 \pm 1.14^{cbx}$ | $32.83 \pm 1.01^{acx}$ |
| 7th Day | $24.00 \pm 1.86^{ax}$ | $28.83 \pm 1.30^{ax}$ | $29.00 \pm 0.73^{ax}$ | $27.83 \pm 1.85^{ay}$ |
| 14th Day | $26.33 \pm 0.76^{ax}$ | $27.66 \pm 0.88^{abx}$ | $26.33 \pm 0.61^{aby}$ | $23.16 \pm 0.70^{az}$ |

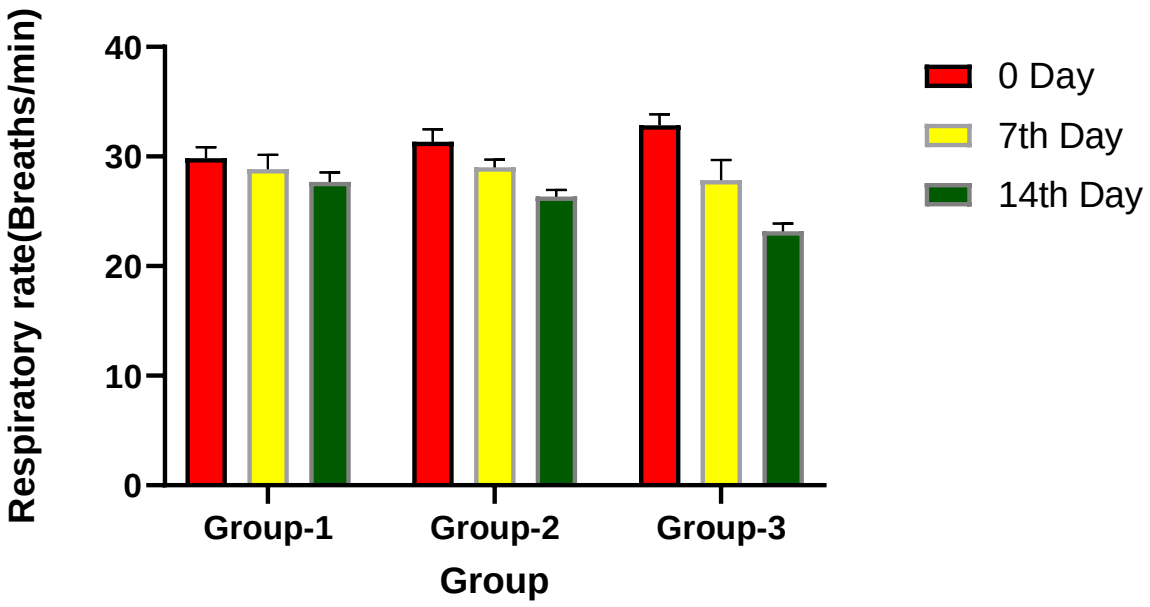


**Fig.4.11.1 Bar diagram of RR level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the respiratory rate value was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in respiratory rate values in different groups and days of treatment on 0,7,14 days were found respectively.

The value of respiratory rate in group-1 on different time intervals on 0,7,14 days was found 29.833 ± 1.01, 28.83 ± 1.30 and 27.66 ± 0.88 respectively.

The value of respiratory rate in group-2 on different time interval on 0, 7, 14 days were found 31.33 ± 1.14, 29.00 ± 0.73 and 26.33± 0.61 respectively.

The value of respiratory rate in group-3 on different time interval on 0, 7, 14 days were found 32.83 ± 1.01, 27.83± 1.85 and 23.16 ± 0.70 respectively.

On day zero Mean ± SE value of respiratory rate in treatment group-1 and group-2 were significantly higher than healthy control. But in group-2 the value of respiratory rate is non-significant in comparison to healthy control.

Similarly on day 7th and day 14th the value of respiratory rate was found to be non-significant in comparison to healthy control.

In treatment group-1; Mean ± SE value of respiratory rate on different days of treatment were compared and it has been found that respiratory rate values on day 7th and day 14th were non significant in comparison to day zero.

Similarly in treatment group-2 the variation in respiratory rate values on different days of treatment; 0,7,14 was compared and it was found that the values on day 0 and day 7 were non-significant. However, the values on day 14th significantly reduced in comparison to day zero and day 14th.Again in treatment group-3 the variation in respiratory rate values on different days of treatment 0,7,14 was compared and it was found that the values of respiratory rate significantly decrease from 0 to 7 and 7 to 14th day.

In the present study, it has been found that parameters of respiratory rate were found to be elevated in comparison to the control group. This might be due to reduced RBC number in the blood which leads to less oxygen exchange from the environment and to maintain the optimum oxygen level in the tissue, respiratory rate increases.

The level of the respiratory rate increased in dogs with babesiosis than in healthy dogs. This finding was Ahmad et al., (2007), Varshney *et al.*, (2008), Wadhwa *et al*., (2011).

| Table 4.12 Mean ± SE values of Rectal temperature (˚F) of healthy & Babesia positive dogs in different treatment groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | 101.76 ± 0.15$^{ax}$ | 103.4 ± 0.14$^{bx}$ | 104.28 ± 0.21$^{cdx}$ | 105.0 ± 0.22$^{dx}$ |
| 7$^{th}$ Day | 101.08± 0.22$^{ax}$ | 103.08± 0.13$^{by}$ | 103.86 ± 0.24$^{cdx}$ | 103.06 ± 0.26$^{acy}$ |
| 14$^{th}$ day | 101.55 ± 0.24$^{ax}$ | 102.66 ± 0.08$^{bz}$ | 101.96± 0.20$^{aby}$ | 101.06 ± 0.37$^{abz}$ |

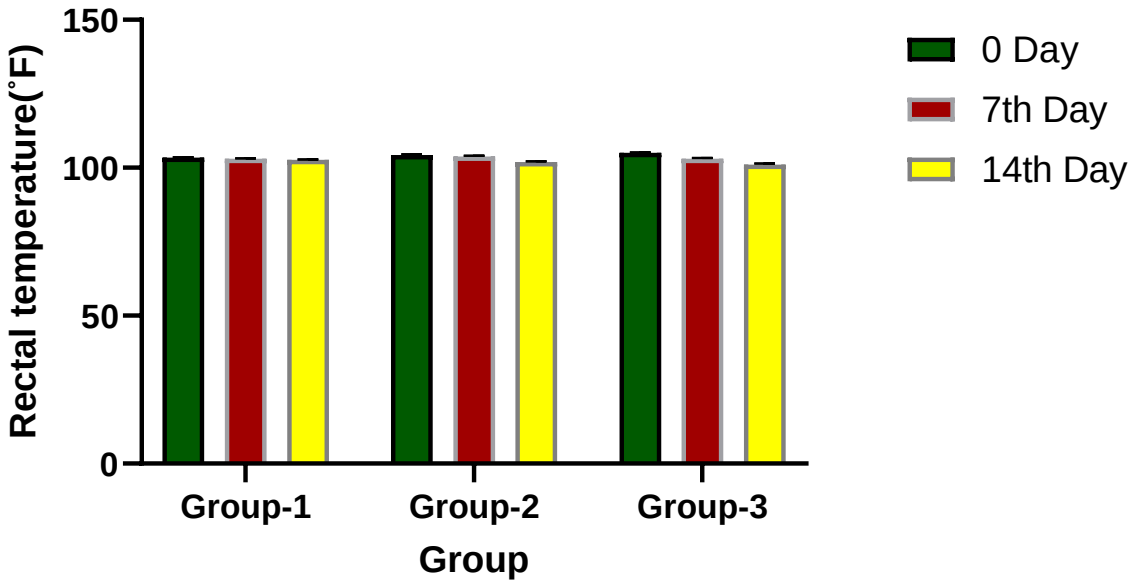


**Fig.4.12.1 Bar diagram of Rectal tempeature level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the rectal temperature value was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in rectal temperature value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of rectal temperature in group-1 on different time interval on 0,7,14 days were found 103.4 ± 0.14, 103.08± 0.13, and 102.66 ± 0.08 respectively.

The value of rectal temperature in group-2 on different time interval on 0, 7, 14 days were found 104.28 ± 0.21, 103.86 ± 0.24 and 101.96± 0.20 respectively.

The value of rectal temperature in group-3 on different time interval on 0, 7, 14 days were found 105.0 ± 0.22, 103.06 ± 0.26 and 101.06 ± 0.37 respectively.

Mean ± SE value of rectal temperature in all treatment groups were significantly higher than the healthy control group on day zero of the treatment.

Similarly on day 7 again the values of rectal temperature were significantly higher in group-1 and group-2 and non-significant in group-3 in comparison to healthy control.

Again, on the day, 14ththe value of rectal temperature was found to be significantly increased in group-1 and non-significant in group-2 and group-3 in comparison to healthy control.

In treatment group-1 and group-2; Mean ± SE values of rectal temperature on different days of treatment were compared and it has been found that the values on day zero and day 7th were non-significant. However, the value on day 14th significantly reduced in comparison to day zero and day 14th.

Similarly in group-3 the values of rectal temperature on different days of treatment on different days of treatment; 0,7,14 was compared and it has been found that the values of rectal temperature significantly decrease from 0 to 7 and 7 to 14th day.

In the present study, it has been found that parameters of rectal temperature were found to be elevated in comparison to the control group. This might be due to an inflammatory reaction.

The level of rectal temperature increased in dogs with babesiosis than in healthy dogs. This finding was Tresamol et al., (2013), Davitkov *et al*., (2015), Kumar et al., (2015), Nalubambaet al., (2015).

| Table 4.13 Mean ± SE values of Pulse rate (Beats/min) of healthy & Babesia positive dogs in different groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $104.16 \pm 2.97^{ax}$ | $124.66 \pm 1.14^{bx}$ | $133.83 \pm 0.79^{cdx}$ | $130.66 \pm 1.33^{dx}$ |
| $7^{th}$ Day | $106.16 \pm 2.79^{ax}$ | $123.00 \pm 1.03^{bcy}$ | $124.16 \pm 2.12^{cx}$ | $116.33 \pm 4.24^{bcy}$ |
| $14^{th}$ Day | $104.00 \pm 2.89^{ax}$ | $117.16 \pm 1.40^{bcz}$ | $117.66 \pm 2.34^{cy}$ | $116.3 \pm 1.45^{az}$ |

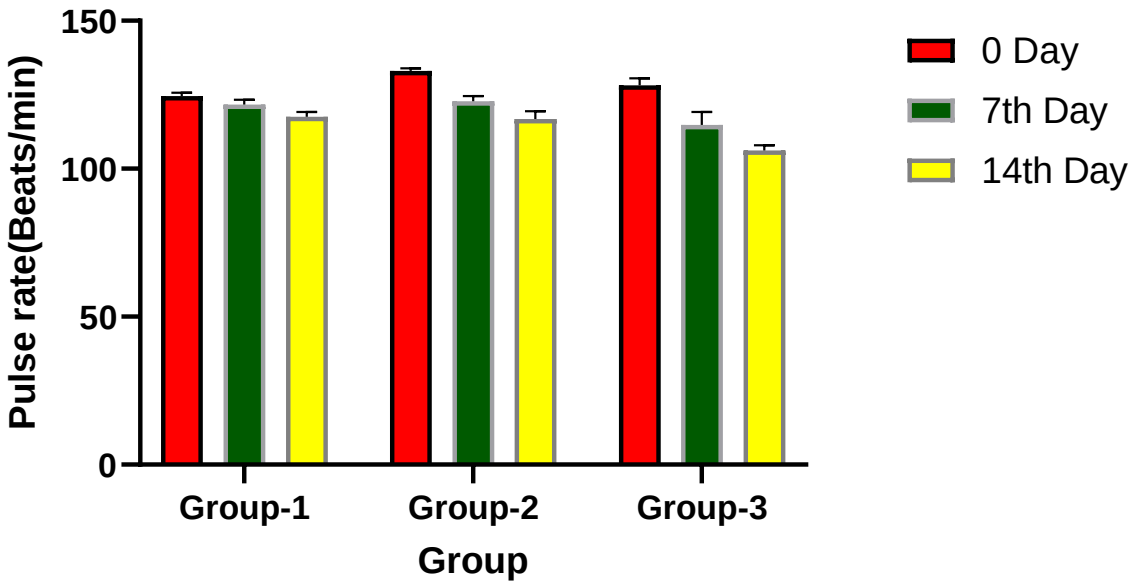


**Fig.4.13.1 Bar diagram of PR level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the pulse rate value was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in pulse rate values in different groups and days of treatment on 0,7,14 days were found respectively.

The value of pulse rate in group-1 on different time intervals on 0,7,14 days was found 124.66 ± 1.14, 123.00 ± 1.03 and 117.16 ± 1.40 respectively.

The value of pulse rate in group-2 on different time interval on 0, 7, 14 days were found 133.83± 0.79, 124.16 ± 2.12 and 117.66 ± 2.34 respectively.

The value of pulse rate in group-3 on different time interval on 0, 7, 14 days were found 130.66 ± 1.33, 116.33 ± 4.24 and 116.3 ± 1.45 respectively.

Mean ± SE values of pulse rate in all treatment groups were significantly higher than the healthy control group on day zero of treatment.

Similarly on day 7, the values of pulse rate were significantly higher than the control group in all treatment groups.

Again on day 14th the values of pulse rate in treatment group-1 and group-2 were significantly higher than in the control group. However, in group-3 the value was similar and non-significant in comparison to the control group.

In treatment group-1 and group-3 Mean ± SE values on different days of treatment were compared and it has been found that pulse rate values significantly decrease from day zero to day 7th and day 7th to day 14th.

Similarly in treatment group-2 the variation in pulse rate values on different days of treatment 0,7,14 was compared and it was found that on day 0 and day 7th the values were non-significant ($p > 0.05$). However, the values on day-14 significantly reduced than the zero days and 7th-day values ($p < 0.05$).

In the present study, it has been found that the parameters of pulse rate were found to be elevated in comparison to the control group; which might be due to anaemia, in consequence, that the heart has pumped more and more blood to maintain normal oxygen and nutritional support to tissue.

Level of Pulse rate increased in dogs with babesiosis than healthy dogs. This finding was Varshney *et al*., (2008)., Wadhwa *et al*., (2011), Jadhav et al., (2015).

| Table 4.14 Mean ± SE values of SOD (U/gm of Hb) of healthy & Babesia positive dogs in different groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $4577.16 \pm 1.41^{ax}$ | $4870.26 \pm 2.64^{bx}$ | $5860.37 \pm 10.56^{cx}$ | $6863.71 \pm 4.02^{dx}$ |
| $7^{th}$ Day | $3734.56 \pm 0.82^{ayz}$ | $4365.40 \pm 8.274^{by}$ | $4768.77 \pm 15.88^{cy}$ | $5369.60 \pm 2.69^{dy}$ |
| $14^{th}$ Day | $3735.95 \pm 1.12^{az}$ | $3539.59 \pm 2.50^{bz}$ | $3878.94 \pm 1.35^{cz}$ | $4029.97 \pm 34.95^{dz}$ |

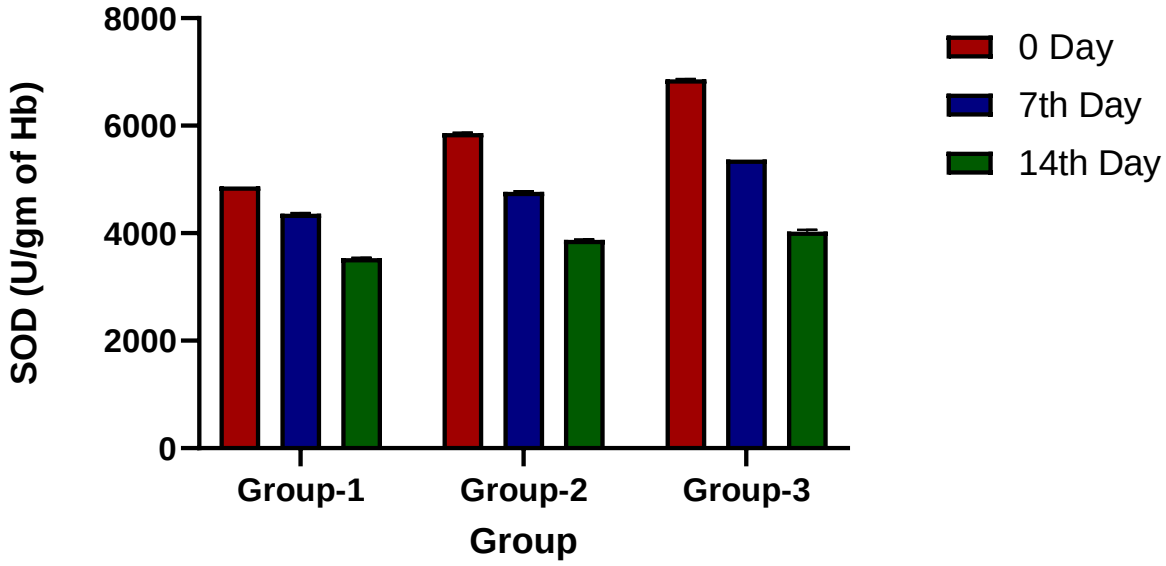


**Fig.4.14.1 Bar diagram of SOD level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the SOD level was measured in pre-treatment and post-treatment in the healthy and different treatment groups. Variation in SOD value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of SOD in group-1 on different time interval on 0,7,14 days were found 4870.26 ± 2.64, 4365.40± 8.274, and 3539.59± 2.50 respectively.

The value of SOD in group-2 on different time interval on 0, 7, 14 days were found 5860.37 ± 10.56, 4768.77± 15.88, and 3878.94± 1.35 respectively.

The value of SOD in group-3 on different time interval on 0, 7, 14 days were found 6863.71 ± 4.02, 5369.60 ± 2.69, and 4029.97± 34.95 respectively.

Mean ± SE values of SOD in all treatment groups were significantly higher than the healthy the control group on day zero, day 7th, and day 14th of treatment.

In treatment group-1; group-2; group-3 the Mean ± SE values of SOD on different days of treatment was compared and it has been found that SOD values significantly decrease from day 0 to day 7th and day 7th to day 14th.

SOD is the major enzyme present in RBC to counteract toxic effects of ROS such as superoxide radicals and hydrogen peroxide; Moral *et al*. (1997). Synthesis of SOD

by body's is self-correcting mechanism to counter oxidative damage due to parasitaemia and multiplication of parasites inside the cells; hence SOD value increased in Babesia positive dogs.

Teodowski et al(2021); Otsukaet al (2001); Chaudhari et al(2007) reported SOD activity in Babesia gibsoni infected dogs were significantly ($p<0.01$) higher than the cytologically negative dogs.

Crnogaj et al (2017) found SOD value decreases in Babesia infected dogs.

| Table 4.15 Mean ±SE values of LPO (n moles/mg Hb) of healthy & Babesia positive dogs in different groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $1.78\pm 0.016^{ax}$ | $2.58\pm 0.046^{bx}$ | $3.68\pm 0.054^{cx}$ | $5.73\pm 0.046^{dx}$ |
| $7^{th}$ Day | $1.75\pm 0.04^{acx}$ | $1.83\pm 0.046^{ay}$ | $2.63\pm 0.12^{by}$ | $3.38\pm 0.067^{cy}$ |
| $14^{th}$ Day | $1.41\pm 0.011^{acy}$ | $1.43\pm 0.038^{az}$ | $1.79\pm 0.039^{bz}$ | $2.23\pm 0.048^{cz}$ |

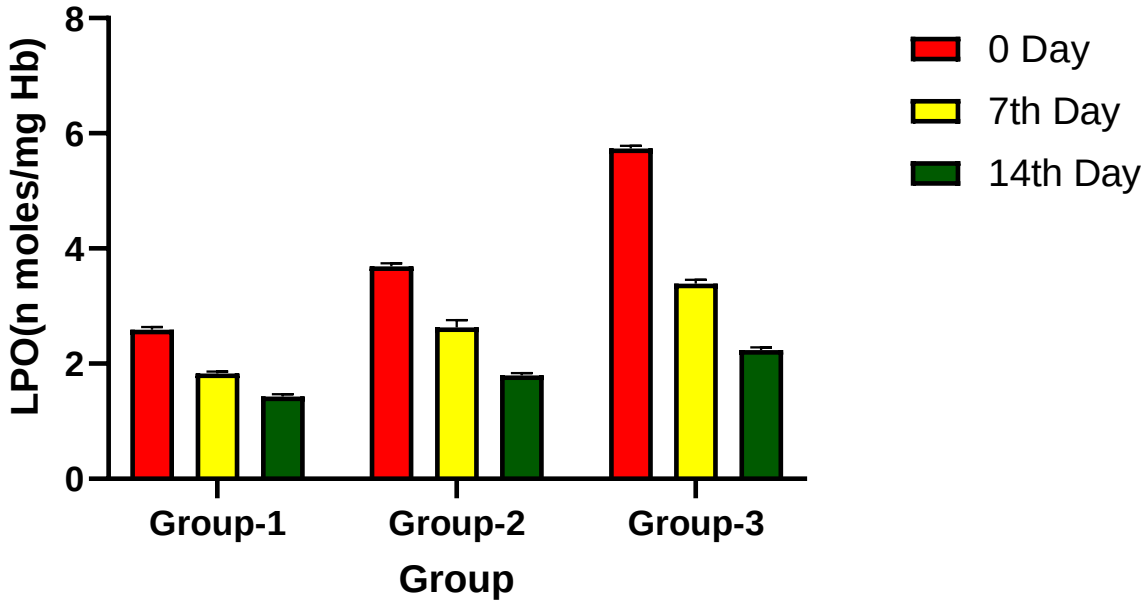


**Fig.4.15.1 Bar diagram of LPO level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the LPO level was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in LPO value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of LPO in group-1 on different time interval on 0,7,14 days were found 2.58 ± 0.046, 1.83 ± 0.046 and 1.43 ± 0.038 respectively.

The value of LPO in group-2 on different time interval on 0, 7, 14 days were found 3.68± 0.054, 2.63± 0.12 and 1.79± 0.039 respectively.

The value of LPO in group-3 on different time interval on 0, 7, 14 days were found 5.73± 0.046, 3.38± 0.067 and 2.23± 0.048 respectively.

Mean ± SE value of LPO in all treatment groups were significantly higher than the healthythe control group on day zero of treatment.

Similarly on day 7th and day 14th the values of LPO were non-significant than healthy control in group-1 and significantly higher in group-2 and group-3.

In treatment group-1, group-2, and group-3 Mean ± SE values of LPO on different days of treatment was compared and it has been found that LPO values significantly decreases in all the three groups from day zero to day 7th and from day 7th to day 14th.

Exacerbated systemic inflammation and reduced metabolic usage of oxygen cause increased production of oxygen free radicals in patients Higher production of peroxyl radicals and consequent elevated LPO concentration renders the erythrocytes more fragile and prone to lysis in cases of concurrent babesiosis; Muduuli et al., (1982). Increased LPO levels in babesia-positive dogs indicated oxidative damage in the erythrocytes of these animals.

Chaudhari et al (2007); Murase et al (1996); Kumar et al (2006); Crnogaj et al (2010); Crnogaj *et al* (2017) found increased levels of SOD levels in Babesia positive dogs.

| Table 4.16 Mean ±SE values of GSH (µmol/gm Hb) of healthy & Babesia positive dogs in different groups at various time interval. | | | | |
|---|---|---|---|---|
| **Days** | **Control Group** | **Group-1** | **Group-2** | **Group-3** |
| 0 Day | $1.38 \pm .011^{ax}$ | $0.57 \pm .009^{bx}$ | $0.52 \pm .009^{cx}$ | $0.48 \pm .009^{dx}$ |
| $7^{th}$ Day | $1.21 \pm .082^{ay}$ | $0.83 \pm .008^{bcy}$ | $0.84 \pm .010^{cy}$ | $0.91 \pm .011^{bcy}$ |
| $14^{th}$ Day | $1.20 \pm .02^{ax}$ | $0.98 \pm .013^{bz}$ | $1.04 \pm .012^{cdz}$ | $1.10 \pm .021^{dz}$ |

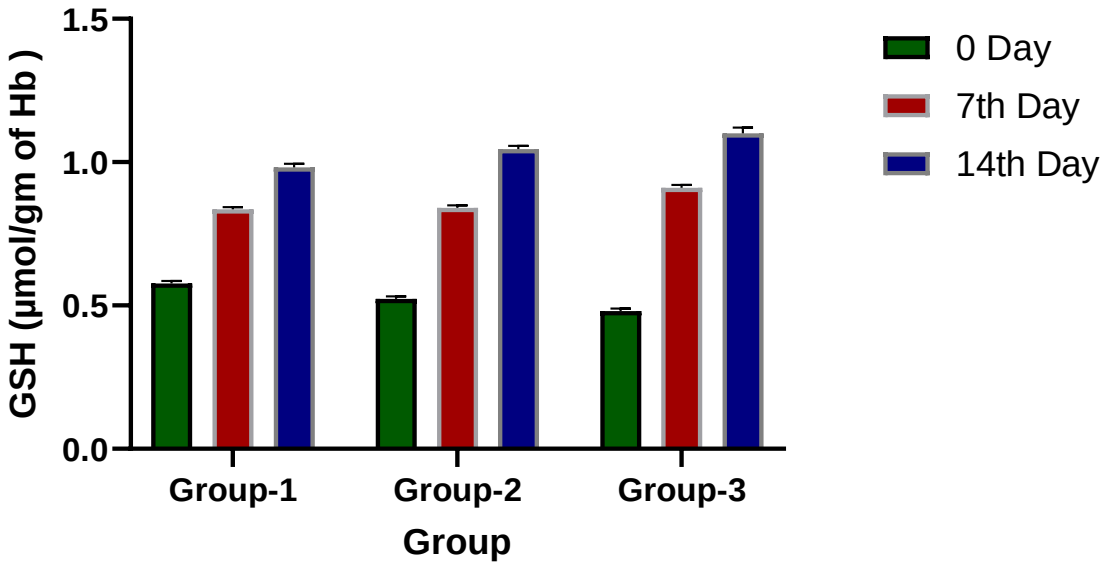


**Fig.4.16.1 Bar diagram of GSH level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the GSH level was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in GSH value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of GSH in group-1 on different time interval on 0,7,14 days were found 0.57 ± .009, 0.83 ± 0.008 and 0.98 ± 0.013 respectively.

The value of GSH in group-2 on different time interval on 0, 7, 14 days were found 0.52 ± 0.009, 0.84 ± 0.010 and 1.04 ± 0.012 respectively.

The value of GSH in group-3 on different time interval on 0, 7, 14 days were found 0.48 ± 0.009, 0.91 ± 0 .011 and 1.10 ± 0 .021 respectively.

Mean ± SE values of GSH in all treatment groups were significantly lower than healthy control on day zero, day 7th, and day 14th of the treatment.

In treatment group-1, group-2, and group-3 Mean ± SE values of GSH on different days of treatment were compared and it has been found that GSH values significantly increase from 0 to 7th and 7th to 14 days of interval in all the three groups of treatment.

Reduced GSH levels in the patient might be due to the utilization of GSH in neutralizing the free radicals production in Babesia-positive dogs.

Teodorowski et al (2021) found no significant difference in GSH values in Babesia-positive dogs.

Otsuka et al (2001) found increases in the value of GSH and Crnogaj et al (2017) found a decrease in the value of GSH in Babesia-positive dogs in comparison to healthy control.

**Table 4.17 Mean ± SE values of CAT (U/gm Hb) of healthy & Babesia positive dogs in different groups at various time interval.**

| Days | Control Group | Group-1 | Group-2 | Group-3 |
|---|---|---|---|---|
| 0 Day | $72.81 \pm 0.35^{ax}$ | $126.43 \pm 3.92^{bx}$ | $156.41 \pm 1.85^{cx}$ | $171.88 \pm 2.63^{dx}$ |
| $7^{th}$ Day | $71.86 \pm 0.24^{ax}$ | $100.32 \pm 0.50^{by}$ | $113.72 \pm 0.29^{cy}$ | $116.72 \pm 0.57^{dy}$ |
| $14^{th}$ Day | $62.12 \pm 0.29^{ay}$ | $71.96 \pm 0.25^{bz}$ | $75.07 \pm 0.25^{cz}$ | $75.49 \pm 0.26^{dz}$ |

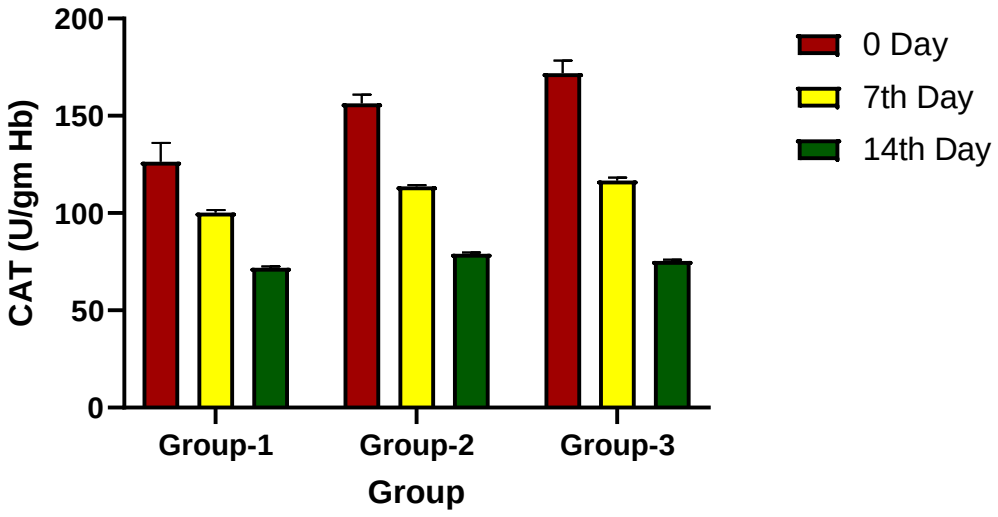


**Fig.4.17.1 Bar diagram of CAT level in different treatment groups of Babesia positive dog.**

## Results: -

In the present study, the CAT level was measured in pre-treatment and post-treatment in the healthy and different treatment groups.

Variation in CAT value in different groups and days of treatment on 0,7,14 days were found respectively.

The value of CAT in group-1 on different time interval on 0,7,14 days were found 126.43 ± 3.92, 100.32 ± 0.50 and 71.96 ± 0.25 respectively.

The value of CAT in group-2 on different time interval on 0, 7, 14 days were found 156.41 ± 1.85, 113.72 ± 0.29 and 75.07± 0.25 respectively.

The value of CAT in group-3 on different time interval on 0, 7, 14 days were found 171.88 ± 2.63, 116.72 ± 0.57, 75.49 ± 0.26 and respectively.

Mean ± SE values of CAT in all treatment groups were significantly higher than the healthythe control group on day zero; day 7th and day 14th of treatment.

In treatment group-1, group-2, and group-3 the Mean ± SE values of CAT significantly decreases in all the three groups from day 0 to day 7th and from day 7th to day 14th.

Erythrocytic Antioxidant enzymes act as a scavenger of free radicals during the oxidative process of Babesia infection. Catalase is the major enzyme present in RBC to counteract toxic effects of ROS such as hydrogen peroxide; Moral et al. (1997). CAT is responsible for the breakdown of $H_2O_2$, an important ROS produced during metabolism; hence CAT value increases in Babesia positive dogs.

The increased activity of CAT may also be due to the higher number of reticulocytes in the blood of infected animals produced by the bone marrow in response to the destruction of mature erythrocytes by protozoa.

Chudhuri et al (2007); Teodorowski et al (2021); Otsuka et al (2001) increase Catalase value and Crnogaj et al (2017) found a decreased value of catalase in Babesia positive dogs in comparison to healthy control.

## Table 4.18 Organ damage

| Serial No | No of Babesia Positive dogs showing MODS= 6 or 33.3% | SGPT > 80 | Creatinine > 0.9 |
|---|---|---|---|
| 1 | | 136.25 | 2.85 |
| 2 | | 138.38 | 2.81 |
| 3 | | 139.01 | 2.75 |
| 4 | | 135.94 | 2.64 |
| 5 | | 137.89 | 2.98 |
| 6 | | 136.68 | 2.92 |

In the present study, the MODS were assessed based on the involvement of hematological alteration (as per Table No-4.1,4.2 &4.3); LFT (as per Table No- 4.4); KFT (as per Table No-4.7) in all the treated groups of dogs and it was observed that in six babesia affected dogs; multiorgan dysfunction was evident.

Lipid peroxidation of erythrocytes also decrease RBC membrane pliability; resulting in the slowed passage and further damage to the erythrocyte as it traverses capillary beds (Taboada and Lobetti,2006).

The capillary sludging of erythrocytes in combination with soluble parasite proteases activate the kallikrein system leading to the production of fibrinogen-like protein. This promotes vascular stasis; which leads to ischemia, thrombosis, and end-organ damage. The CNS; Kidney; and muscle appears to be the organs most affected by the resultant tissue hypoxia (Uilenberg et al.,1989; Jacobson and Lobetti,1996; Lobetti et al.,1996).

## USG FINDINGS

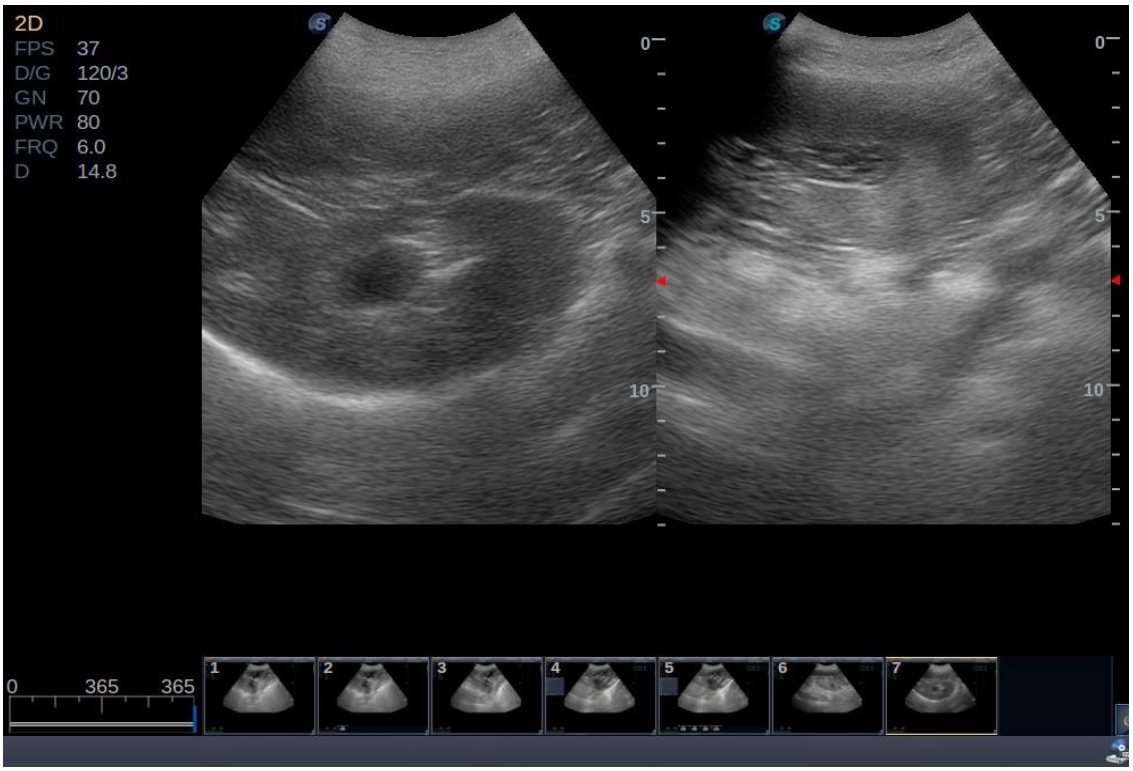


**Fig: -3.7 Ultrasonographic images of Kidney of dog suffereing from canine babesiosis (Iso-echoic to spleen, cortex diameter increases and loss of corticomedullary junction).**

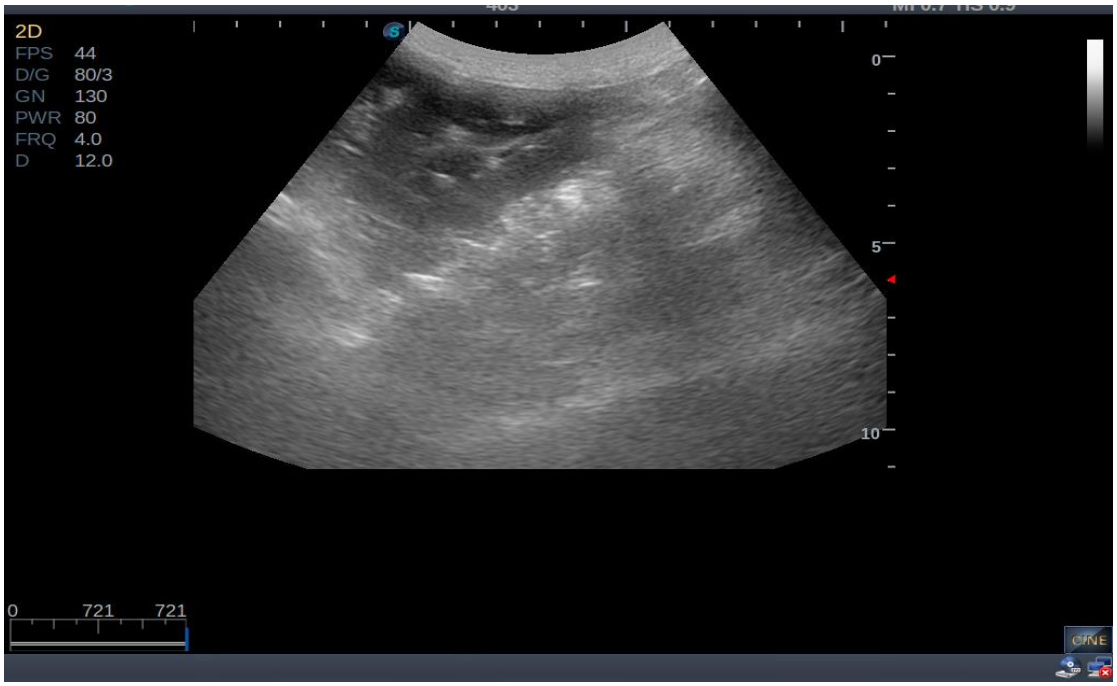


**Fig: -3.8 USG showing hyper echoic liver lobe in dog suffering from canine babesiosis**

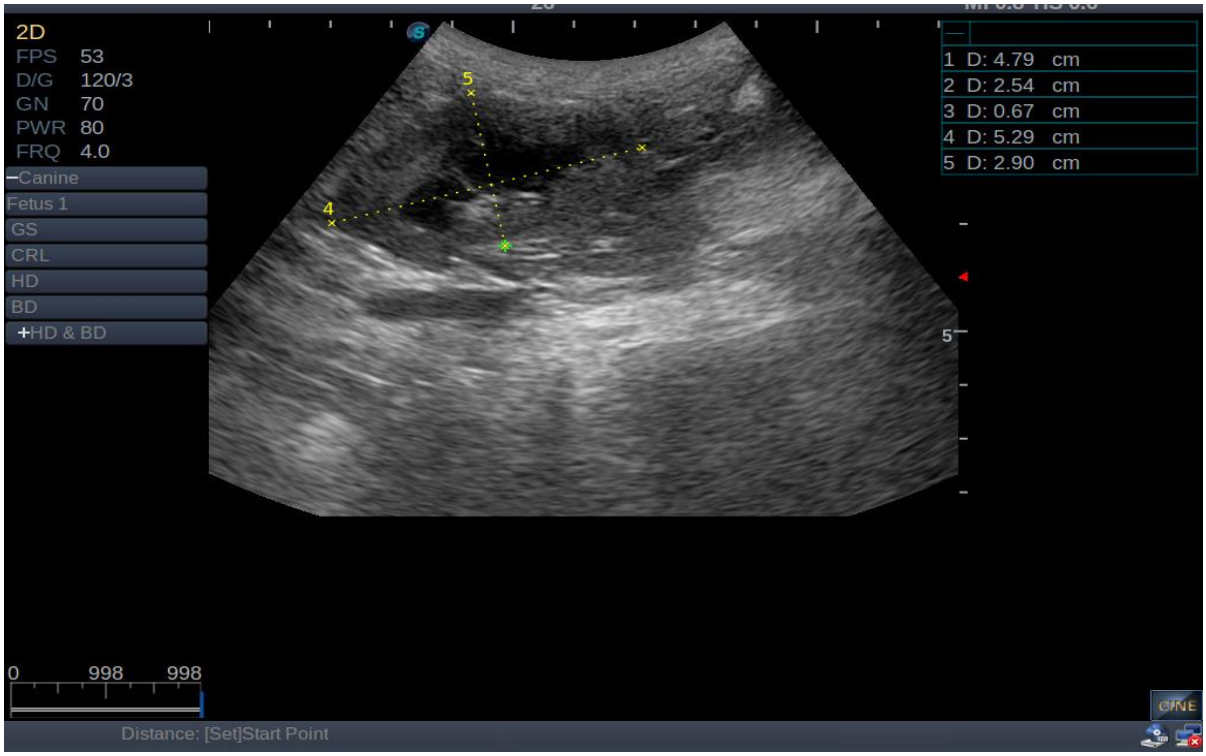


**Fig: -3.9 USG of Kidney showing hydronephrosis, loss of corticomedullary junction and hyper echoic cortex.**

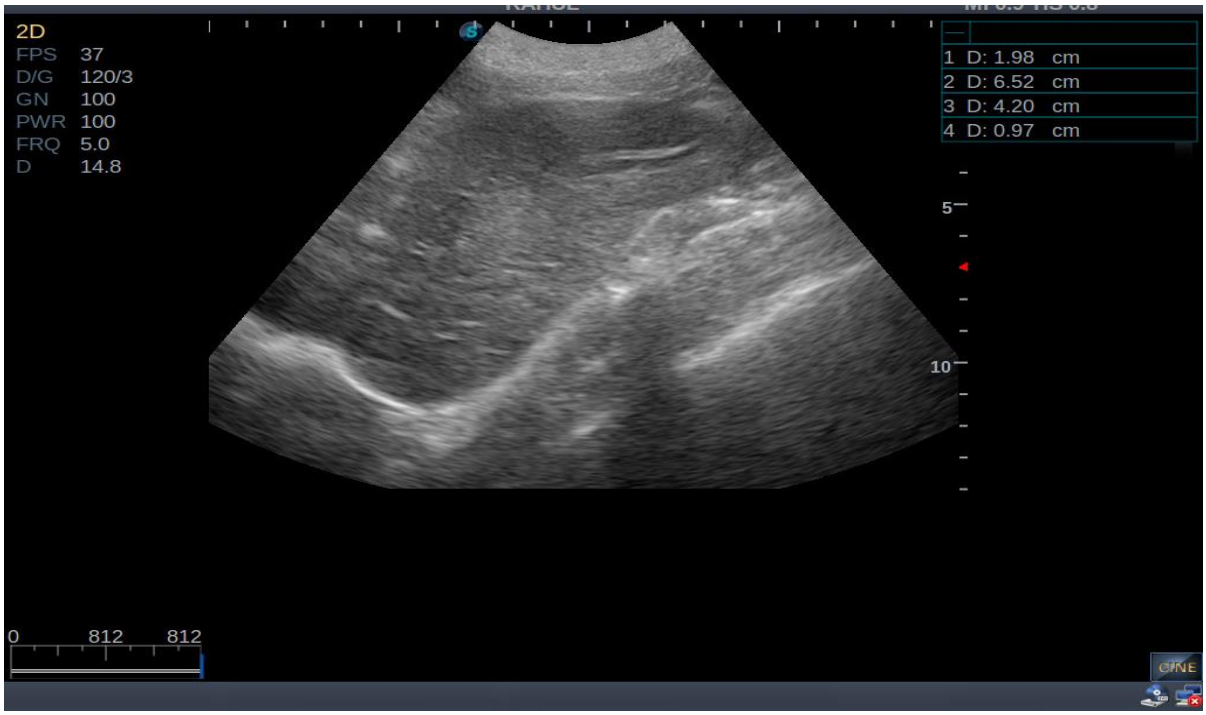


**Fig: -3.10 USG showing starring of liver with roundish boundary suggestive of hepatomegaly in dog suffering from canine Babesiosis.**

## Table 4.19 SIRS POSITIVE

| Serial No | No of Dogs Showing SIRS Positive = 5 | WBC >16000 | HR>120 | RT>103.46 |
|---|---|---|---|---|
| 1 | | 16220 | 129 | 105.5 |
| 2 | | 16750 | 131 | 105.9 |
| 3 | | 16370 | 125 | 104.7 |
| 4 | | 16890 | 134 | 104.5 |
| 5 | | 16750 | 132 | 104.7 |

For the assessment of SIRS; 3 parameters were considered for the study; among them WBC; Heart rate and Rectal temperature were parameters. In this study, we found that if the values of WBC in clinically affected dogs were > 16000(×103/µl); Heart Rate > 120 bpm and Rectal temperature> 103.46˚F were affected with SIRS.

Out of 18 dogs that were found to be positive for *Babesia gibsoni*; 5 dogs were positive for SIRS, as per criteria for considering SIRS positive were WBC> 16000(×103/µl); Heart Rate > 120 bpm and Rectal temperature> 103.46˚F. SIRS is frequently present in canine babesiosis. Since the SIRS criteria concept has been widely used in prognosis in emergency and intensive care fields Becoz of its simplicity and usefulness.

The same criteria were used by Vesna *et al*., (2010). The cut-off values for SIRS criteria are a major issue in veterinary medicine. Since normal values for rectal temperature, heart rate, and respiratory rate vary in dogs due to significant variation in their size (HOUSTON and RADOSTITS,2000) and my finding is in agreement with the above authors.

## Table 4.20 Recovery percentage with treatment protocol Number

| Group | Treatment Protocol | Recovery Percentage |
|---|---|---|
| Group-1 | Imidocarb | 50 |
| Group-2 | Berenil + Clindamycin | 66.6 |
| Group-3 | Azithral + Clindamycin | 83 |

Based on various physiological; hematological and biochemical parameters all the three treatment groups were compared for the efficacy of drugs which is more successful for the treatment of *B. gibsoni* were found to be 50% and 66.6 % in group-1 and group-2 respectively. In group-3, the recovery percent is maximum i.e 83 %.In conclusion, it can be said that Azithral and Clindamycin is the best module of treatment against B.gibsoni infected dogs.

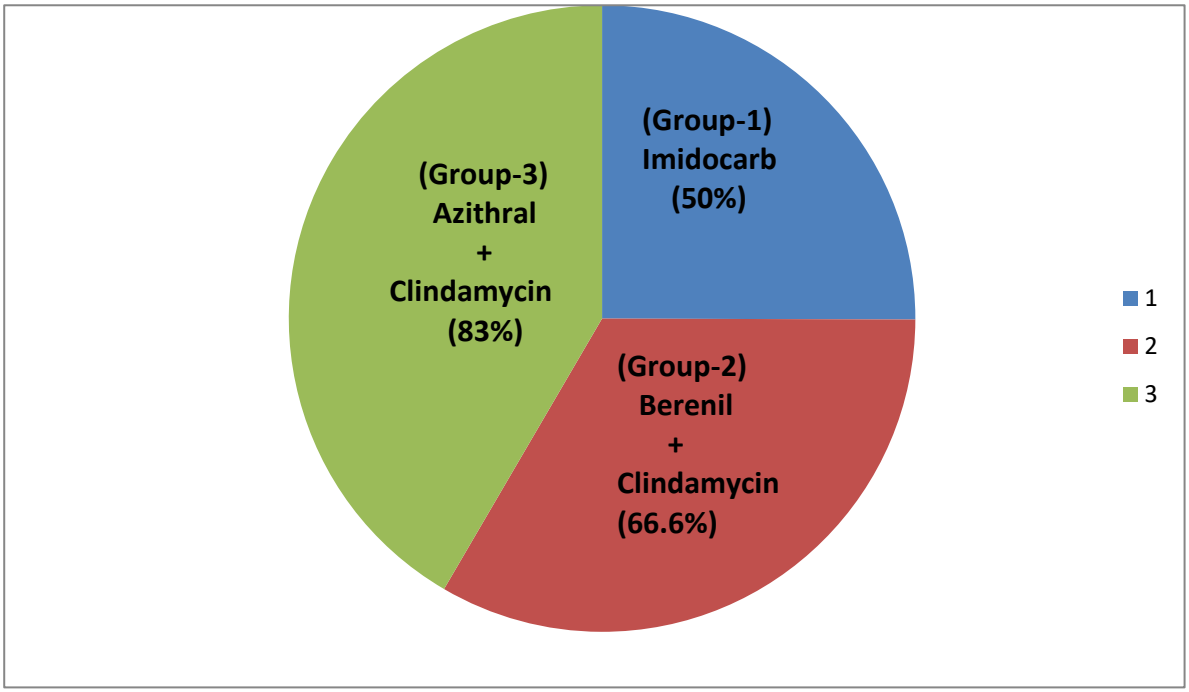


**Fig: - 3.11 Pie diagram showing recovery percent in different treatment group of dogs suffering from *Babesia gibsoni* infection.**

# 5 SUMMARY AND CONCLUSION

Multiple Organ dysfunctions Syndrome is a condition in which two or more organ systems are simultaneously affected cause severe illness in the patient. Many atypical signs are associated with multiple organ dysfunction are recorded in dogs. Unresponsive diarrhoea, nasal bleeding, vomition, muscular weakness, and posterior paresis are important clinical signs observed in cases suspected to be suffering from multi-organ dysfunction. The large number of dogs presented to the teaching veterinary hospital is suffering from MODS. It has been observed that the percentage of canine babesiosis is high in the vicinity of Patna. Babesiosis is a life-threatening disease of dogs caused by Haemoprotozoan parasites of genus babesia. It leads to anemia and high mortality in complicated cases. Few cases of acute renal failure, hepatopathy, and myopathy were recorded in clinical cases of a dog suffering from canine babesiosis. Therefore, keeping these facts in view this research work was planned to evaluate the effect of babesiosis on single and multiple organ damage and its therapeutic management.

Dogs irrespective of age, sex, and breed suffering from fever, anemia, and jaundice were screened for babesia infection. Screening of the babesia-positive infection in dogs was done by blood smear examination. Animals found positive for babesia positive in blood smear examination were subjected for clinical examination and haemato- biochemical examination. A study was done to ascertain multiple organ dysfunction. Affected dogs with dysfunction of more than one organ were selected for further study. Twenty-four selected dogs were divided into four groups consisting of six dogs in the group. Dogs naturally infected with babesiosis were grouped irrespective of species in the control group and three treatment groups (i.e., Group -1, 2, 3). Eighteen positive cases of canine babesiosis were selected for therapeutic management with different drugs as well as supportive therapy.

The mean values of rectal temperature; heart rate and respiration rate in dogs with canine babesiosis increased significantly ($p<0.05$) than healthy dogs. The mean values of haematological parameters; HB, TEC decreased significantly ($p<0.05$) but TLC levels increased significantly ($p<0.05$) in dogs with canine babesiosis than healthy control.

Among various biochemical parameters; the levels of ALP, AST, ALT, BUN, CRE increased significantly ($p<0.05$) but TP and ALB values decreased significantly ($P<0.05$) in dogs with babesiosis than healthy control dogs.

Among oxidative stress parameters the values of LPO, CAT, SOD increased significantly ($p<0.05$) but GSH values were found to be decreased significantly ($P< 0.05$) in comparison to healthy control.

In USG finding of Kidney of some dogs suffering from canine babesiosis is found to be iso-echoic to spleen, cortex diameter increases and loss of corticomedullary junction; hydronephrosis, hyperechoic cortex.

In USG finding of Liver of some dogs suffering from canine babesiosis is hyper-echoic than spleen; roundish boundary suggestive of hepatomegaly.

Out of 18 dogs that were found to be positive for *Babesia gibsoni*, five dogs were SIRS (Systemic inflammatory response syndrome) positive. The criteria for considering SIRS positive were WBC > 16000(x103/µl), HR>120 bpm and RT > 103.4 ˚F.

In the present study MODS (Multiorgan dysfunction syndrome) were assessed based on the involvement of hematological alteration in all the treated groups of dogs and it was observed that in six (33.3%) babesia affected dogs MODS was evident.

Based on various physiological; hematological and biochemical parameters all the three treatment groups were compared for the efficacy of drugs treatment were 50 % and 66.6 % in group-1 and group-2 respectively. While in group-3; the recovery percent is maximum i.e 83 %. In conclusion, it can be said that Azithral and clindamycin along with supportive therapy is the best module of treatment against *B. gibsoni* infection in dogs.

## Conclusion: -

- **Present study reveals that there is a involvement of liver; kidney in the infected dog which further complicates the clinical situations.**
- **There was significant decrease in different parameters of haemogram and significant increase in inflammatory liver and kidney markers.**
- **It has been observed that there is a significant increase in LPO; SOD, Catalase and decrease in GSH in Babesia infected dogs.**
- **On the basis of comparative study of three different drugs combination for the treatment of *Babesia gibsoni*; group-3 was found to be most affective followed by group-2 and least was group-1.**
- **Most suitable package of Practice for MODS affected dog suffering from *Babesia gibsoni* was found to be combination therapy consisting of Azithromycin and Clindamycin along with supportive drugs.**

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