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Therapeutic Studies and Post Therapeutic Follow-up Evaluation of Dogs Affected by Cerebral Babesiosis

Gollapalli Nagarjuna[1], Bhavanam Sudhakara Reddy[1], Gungi Saritha[1] and Sirigireddy Sivajothi[2]

[1]Dept. of Veterinary Medicine, [2]Dept. of Veterinary Parasitology, College of Veterinary Science, Proddatur, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh (516 360), India

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Corresponding bhavanamvet@gmail.com

0000-0003-1002-3778

ABSTRACT

The present study was carried out from October, 2023 to September, 2024 at Department of Veterinary Medicine, College of Veterinary Science, Proddatur. The research study was carried out to investigate cerebral babesiosis in dogs to record the therapeutic response and post therapeutic assessment. The study was carried out on the dogs presented to the different veterinary polyclinics in YSR and Krishna districts of Andhra Pradesh. Confirmation of the babesiosis was done by microscopic examination of Giemsa-stained blood smears and further by polymerase chain reaction assay as detection of 670 bp amplicons specific to the *Babesia gibsoni* in blood. Based on the presence of the neurological signs, demonstration of *Babesia* organisms in red blood cells of cerebrospinal fluid, 8.08% (11/136) of dogs with babesiosis diagnosis had the cerebral babesiosis. Neurological symptoms observed in dogs with cerebral form included ataxia, stiffness of hind limbs, paddling, opisthotonus, twitching of hind limbs, seizures, head pressing, stupor, decerebrate rigidity, decerebellate rigidity, circling, nystagmus, blindness and paraplegia. Dogs were administered with deep intramuscular injection of diminazene aceturate, in two doses at 48-hour intervals at a dose of 3.5 mg kg^{-1} body weight, tablet doxycycline @ 5 mg kg^{-1} body weight every 12 hours PO, tablet enrofloxacin @ 10 mg kg^{-1} body weight every 24 hours PO, tablet metronidazole @ 15 mg kg^{-1} body weight BID, PO along with supportive and symptomatic therapy. Two of the eleven dogs in the study recovered after receiving appropriate standard therapy, according to a post-therapeutic assessment of dogs with cerebral babesiosis.

KEYWORDS: Dogs, *Babesia*, nervous signs, tremors, ataxia, treatment, prognosis

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1. INTRODUCTION

Dogs with central nervous systems are impacted by canine cerebral babesiosis, a rare but serious form of *Babesia* infection that usually results in neurological symptoms like seizures, ataxia, and altered mental status. *Babesia canis* or *Babesia gibsoni* are more likely to cause it, and when it occurs in the brain, it is frequently linked to a serious systemic infection (Liu et al., 2022). A dog's urogenital hemorrhage caused by *Babesia gibsoni*-induced immune thrombocytopenia was treated with romiplostim and prednisolone, according to Khan et al. (2025). Infection can result in mild, moderate, or severe illness, depending on the parasite species and the host's immune system. The most severe form of the disease in domestic dogs is caused by *B. rossi* (Zygner et al., 2023). According to Schafer et al. (2023), the tick Dermacentor reticulatus, a possible vector for canine babesiosis, is most active in the spring and fall in Germany, so the risk of canine infection with B. canis needs to be particularly taken into account during these times. In Germany, dog importation and travel are thought to be significant contributors to canine babesiosis. However, a sizable portion of German dogs also contract autochthonous *Babesia* spp. infections. Babesiosis is an emerging infectious disease, according to Bajer et al. (2022), and some of the causative species are spreading as a result of their tick vector hosts' expanding range. We provide an overview of the incidence and prevalence of babesiosis in 20 European countries in this review, taking into account both recent research and historical data. According to Miranda et al. (2022), the acute course typically consists of pyrexia, lethargy, inappetence, gastrointestinal symptoms, and infrequently, hematuria. Additionally, it is associated with common hematological abnormalities like anemia and thrombocytopenia, as well as, to a lesser extent, biochemical abnormalities like elevated liver enzymes, hypoalbuminemia, and hyperbilirubinemia. According to this study, *B. gibsoni* is an endemic hemoparasite that can cause a range of clinical symptoms in dogs. Very few case reports have reported cerebral involvement in babesiosis, despite it not being the most common manifestation (Schoeman et al., 2009). Only a small number of clinical traits and laboratory results pertaining to naturally infected *B. gibsoni* have been published, despite the fact that the parasite is increasingly being acknowledged worldwide as the cause of anemic canine tick-borne disease. Numerous clinical characteristics, including fever, anorexia, depression, pale mucous membranes, reddish to dark urine, splenomegaly, icterus, and a lack of symptoms, were observed in the majority of infected dogs. Infections can present as asymptomatic or as complex symptomatic cases, and their presentations can vary from hyperacute and acute to chronic. The disease's hyperacute form is uncommon and primarily affects young puppies. Although they are uncommon, shock and significant tissue damage have been documented in fighting dogs with hyperacute onsets. Pallor, fever, anorexia, weakness, and splenomegaly are among the non-specific clinical symptoms that are frequently seen in the acute form of the illness. The most frequent symptoms in infected dogs are thrombocytopenia and hemolytic anemia. Accordingly, complications have been documented for bicavitary effusion, disseminated intravascular coagulation (DIC), glomerulonephritis, renal failure, pulmonary edema, and acute respiratory distress syndrome. Cerebral babesiosis is suspected when dogs exhibit the neurological signs, along with diagnosis of *Babesia* infection, concomitant infections should be ruled out and in case of death of dogs, presence of protozoa in the brain have been confirmed by histopathological or molecular tests (Adaszek et al., 2012). There is no systemic research has been done on dogs with cerebral babesiosis and its therapeutic response till now in the India. Thus, the research study was carried out to investigate cerebral babesiosis in dogs to record the therapeutic response and post therapeutic assessment.

2. MATERIALS AND METHODS

The study was conducted during October, 2023 and September, 2024 at Department of Veterinary Medicine, College of Veterinary Science, Proddatur between. To provide normal reference data for comparing the parameters under study, ten healthy dogs which were chosen at random from those brought in for regular examinations and vaccinations served as the control group. Dogs with symptoms like fever, anemia, lymphadenopathy, tick infestation, and nervous signs were incorporated. All the dogs included in the present study had the regular annual vaccinations against canine distemper, canine adeno virus, canine parvo virus, canine parainfluenza, canine leptospiral serovars and rabies. The study excluded dogs with a history of trauma, uraemic encephalopathy, congestive heart failure, idiopathic epilepsy, canine distemper, rabies, or episodic seizures. Babesiosis was confirmed by microscopic examination of stained blood smears (Reddy et al., 2014; Sivajothi et al., 2023). Standard morphological features were used to confirm the presence of *Babesia* (Sivajothi et al., 2014; Sivajothi and Reddy, 2017). In order to extract genomic DNA, blood samples from 11 dogs displaying babesiosis-related nervous symptoms were drawn from the cephalic vein and placed in EDTA-coated blood collection vials (Almería et al., 2001; Kopparthi et al., 2021). Dogs in the current study received a variety of medications according to predetermined dosages and schedules. Berenil, a deep intramuscular (IM) injection that contained diminazene aceturate (70 mg ml^{-1}), was administered in two doses at

48 h intervals at a dose of 3.5 mg kg^{-1} body weight (Thakur et al., 2024). For 21 days, Tab. Doxypet, which contained 100 mg, 200 mg, and 300 mg of doxycycline and lactic acid bacillus $tablet^{-1}$, was taken orally at a dose of 5 mg kg^{-1} body weight every 12 h (Gonde et al., 2016). Likewise, Tab. Enrohat-DT 200, which contained 50 mg, 150 mg, and 200 mg of enrofloxacin $tablet^{-1}$, was administered orally for 21 days at a dose of 10 mg kg^{-1} body weight every 24 h (Birkenheuer et al., 2004). The active ingredient in Tab. Flagyl 200, metronidazole (200 mg $tablet^{-1}$), was taken orally every 12 h for 21 days at a dose of 15 mg kg^{-1} body weight (Nandini et al., 2016). Once daily until the fever went away, 0.2 mg kg^{-1} body weight of Inj. Melonex, which contained meloxicam (5 mg ml^{-1}), was injected into the muscle. Inj. Iron Dextran was administered intramuscularly once weekly for four doses at a dose of 10 mg kg^{-1} body weight. It contained ferric hydroxide and dextran (100 mg elemental iron ml^{-1}). Susp. Hatplat, which contains extracts of Carica papaya, goat milk powder, almond, wheatgrass, kiwi, and pomegranate, is another supportive therapy that is administered. Its dosage was based on body weight and was 5 ml for animals weighing up to 10 kg, 10 ml for animals weighing 10–20 kg, and 20 ml for animals weighing more than 20 kg. With folic acid, ferric ammonium chloride (12.2 mg 5 ml^{-1}), and vitamins B1, B2, B6, and B12, Susp. Ferrimin was also given according to body weight: 5 ml for animals weighing up to 10 kg, 10 ml for animals weighing 10–20 kg, and 20 ml for animals weighing more than 20 kg. For four days, Tab. Pantop 40, which contained 40 mg of pantoprazole $tablet^{-1}$, was taken orally at a dose of 1 mg kg^{-1} body weight every 24 h. Animals weighing less than 5 kg received 5 ml of Syrup Silytek, which contained silymarin buclizine hydrochloride (35 mg (5 ml)$^{-1}$). Animals weighing more than 10 kg received 15 ml, while those weighing more than 20 kg received 20 ml twice a day (Koster et al., 2015; Malinovska, 2024). On the 0^{th}, 3^{rd}, 7^{th}, 14^{th}, and 28^{th} days following treatment, the resolution of clinical symptoms and the improvement in haematological and biochemical parameters were used to gauge the clinical recovery of dogs with cerebral babesiosis. Post-treatment evaluations were conducted on the 0^{th}, 7^{th}, 14^{th} and 28^{th} days, with haematological and serum biochemical parameters obtained the means to compare the response to the therapy. Using SPSS version 20.00, the results were statistically analysed using the procedures.

3. RESULTS AND DISCUSSION

Among the 620 dogs, 136 samples tested positive for *Babesia* spp. through microscopic examination of Giemsa-stained blood smears. The organisms were identified based on their morphology, appearing as pleomorphic, oval, or signet ring-shaped forms confirming the presence of *Babesia gibsoni*. The eleven blood samples from dogs diagnosed with babesiosis and exhibiting neurological signs were further analysed using polymerase chain reaction (PCR) for confirmatory diagnosis. DNA extracted from the blood of dogs with cerebral babesiosis produced a 670 bp amplicon specific to the *Babesia gibsoni*. This assay, targeting a portion of the 18S rRNA gene, was optimized to amplify a *Babesia gibsoni*-specific 670 bp amplicon.

In this study, 11 dogs with cerebral babesiosis were treated with standard therapeutic medications, and post-treatment evaluations were conducted on the 0^{th}, 3^{rd}, 7^{th}, 14^{th}, and 28^{th} day of post treatment. Neurological examinations were performed on all 11 dogs at the time of presentation; 9 dogs on the third day of examination; 8 dogs on seventh day of examination, 4 dogs on fourteen day of examination 2 dogs on twenty-eight day of examination. During the study period two dogs, three dogs, seven dogs, nine dogs were died on the third day, seventh day, fourteenth day and twenty-eight day of post therapeutic assessment period. Results of the findings depicted in Table 1. Tremors may results from the direct neurological involvement caused by *Babesia* infection or as a results of severe systemic illness

Table 1: Post therapeutic assessment of dogs with cerebral babesiosis by clinical examination

Sl. No.	Neurological findings	Percentage of dogs with cerebral babesiosis				
		Post therapeutic day of observation				
		0^{th} day (11)	3^{rd} day (9)	7^{th} day (8)	14^{th} day (4)	28^{th} day (2)
1.	Ataxia	72.72% (8/11)	77.77% (7/9)	62.50% (5/8)	50.00% (2/4)	50% (1/2)
2.	Stiffness of hind limbs	63.63% (7/11)	77.77% (7/9)	62.50% (5/8)	25.00% (1/4)	0% (0/2)
3.	Paddling	63.63% (7/11)	77.77% (7/9)	75.00% (6/8)	25.00% (1/4)	0% (0/2)
4.	Opisthotonus	54.54% (6/11)	55.55% (5/9)	62.50% (5/8)	25.00% (1/4)	0% (0/2)
5.	Twitching of hind limbs	54.54% (6/11)	66.66% (6/9)	62.50% (5/8)	25.00% (1/4)	0% (0/2)
6.	Seizures	54.54% (6/11)	55.55% (5/9)	37.50% (3/8)	50.00% (2/4)	50% (1/2)
7.	Head pressing	36.36% (4/11)	22.22% (2/9)	25.00% (2/8)	25.00% (1/4)	0% (0/2)

Table 1: Continue...

Sl. No.	Neurological findings	Percentage of dogs with cerebral babesiosis				
		Post therapeutic day of observation				
		0th day (11)	3rd day (9)	7th day (8)	14th day (4)	28th day (2)
8.	Stupor	36.36% (4/11)	33.33% (3/9)	25.00% (2/8)	0.00% (0/4)	0% (0/2)
9.	Decerebrate rigidity	36.36% (4/11)	33.33% (3/9)	37.50% (3/8)	50.00% (2/4)	0% (0/2)
10.	Decerebellate rigidity	36.36% (4/11)	44.44% (4/9)	25.00% (2/8)	25.00% (1/4)	0% (0/2)
11.	Circling	27.27% (3/11)	22.22% (2/9)	25.00% (2/8)	50.00% (2/4)	0% (0/2)
12.	Nystagmus	18.18% (2/11)	22.22% (2/9)	12.50% (1/8)	25.00% (1/4)	0% (0/2)
13.	Blindness	18.18% (2/11)	22.22% (2/9)	25.00% (2/8)	25.00% (1/4)	0% (0/2)
14.	Paraplegia	18.18% (2/11)	22.22% (2/9)	12.50% (1/8)	0% (0/4)	0% (0/2)

affecting nervous system, while paresis may arise from nerve damage due to *Babesia* induced inflammation or severe anemia leading to reduced oxygen supply to nervous tissue. These neurological manifestations collectively indicate the cerebral form of babesiosis.

Hematological exams were performed on all 11 dogs at the time of presentation; 9 dogs on the third day of examination; 8 dogs on seventh day of examination, 4 dogs on fourteenth day of examination 2 dogs on twenty-eight day of examination was carried out. A significant decrease (p<0.05) was observed in the mean values of total leukocyte count, percentage of eosinophils, percentage of monocytes, absolute neutrophil count, absolute lymphocyte count, absolute eosinophil count, and absolute monocyte count when comparing dogs with cerebral babesiosis to apparently healthy dogs. On the other hand, a significant increase (p<0.05) was found in the mean values of hemoglobin, total erythrocyte count, packed cell volume, percentage of lymphocytes, and platelet count. No significant variation (p<0.05) was noted in the mean values of mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and percentage of neutrophils. Details of the study findings presented in Table 2. Reported haematological parameters associated in the study, might

Table 2: Post therapeutic assessment of dogs with cerebral babesiosis by hematological examination

Sl. No.	Parameters	Apparently healthy dogs (10)	Percentage of dogs with cerebral babesiosis			
			Post therapeutic day of observation			
			0th day (11)	7th day (8)	14th day (4)	28th day (2)
1.	Haemoglobin (g/dl)	13.03±0.42	10.21±0.18	11.02±0.80	11.91±0.04	12.22±0.02
2.	Total erythrocyte count (×10^6/µl)	6.51±0.19	5.09±0.08	5.51±0.66	5.69±0.62	6.10±0.03
3.	Packed cell volume (%)	39.29±2.14	31.23±2.10	33.01±2.65	34.03±1.12	36.41±1.34
4.	Mean corpuscular volume (fl)	60.35±4.26	61.35±3.69	59.90±4.22	59.80±4.32	59.68±3.28
5.	Mean corpuscular hemoglobin (pg)	20.01±0.92	20.16±0.82	20.00±2.32	20.93±1.72	20.03±2.01
6.	Mean corpuscular hemoglobin concentration (g/dl)	33.16±2.54	32.69±2.14	33.38±0.32	34.99±0.32	33.56±0.32
7.	Total leucocyte (×10^3/µl)	9.33±0.81	14.32±1.22	13.79±1.39	11.08±0.78	9.27±0.08
8.	Neutrophils (%)	73.67±1.20	71.18±0.54	76.11±2.81	74.31±1.80	73.91±0.71
9.	Lymphocytes (%)	20.43±0.85	17.02±0.75	15.09±0.66	19.97±0.52	21.04±0.09
10.	Eosinophils (%)	3.81±0.23	6.72±0.65	5.08±0.31	2.87±0.09	3.01±0.01
11.	Monocytes (%)	2.09±0.27	5.08±0.01	3.72±0.01	2.89±0.01	2.04±0.01
12.	Absolute neutrophil count (×10^3/µl)	6.89±1.23	10.19±1.09	10.49±1.13	8.24±0.89	6.85±0.70
13.	Absolute lymphocytes count (×10^3/µl)	1.90±0.09	2.44±0.09	2.09±0.08	2.21±0.10	1.95±0.04
14.	Absolute eosinophils count (×10^3/µl)	0.35±0.07	0.96±0.03	0.70±0.02	0.32±0.03	0.28±0.01
15.	Absolute monocytes count (×10^3/µl)	0.19±0.01	0.73±0.01	0.51±0.01	0.31±0.01	0.19±0.01
16.	Platelet count (×10^3/µl)	2.71±0.07	1.94±0.24	2.01±0.31	2.11±0.31	2.28±0.02

be due to hosts immune response against *Babesia* infection, systemic inflammation, bone marrow suppression, intra-erythrocytic parasitic replication which leads to haemolysis.

In this study, 11 dogs with cerebral babesiosis were selected from a total of 136 dogs for post-therapeutic assessment on the 0th, 7th, 14th, and 28th days of presentation. Serum biochemical tests were performed on all 11 dogs at the time of presentation; 9 dogs on the third day of examination; 8 dogs on seventh day of examination, 4 dogs on 14th day of examination 2 dogs on 28th day of examination was carried out. During the study period two dogs, three dogs, seven dogs, nine dogs on the third day, seventh day, fourteenth day and twenty-eight day of post therapeutic assessment period. The serum biochemical changes in these dogs on the 0th, 7th, 14th, and 28th day of presentation are presented in Table 3. A significant decrease (p<0.05) in the mean values of alanine aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin, blood urea nitrogen, creatinine, and magnesium were observed when comparing dogs with cerebral babesiosis to apparently healthy dogs. On the other hand, there was a significant increase (p<0.05) in the mean values of total protein, albumin, A/G ratio, and glucose in dogs with cerebral babesiosis compared to healthy dogs. However, no significant difference (p<0.05) was found in the mean values of globulin and indirect bilirubin between dogs with cerebral babesiosis and healthy dogs. Elevated creatinine levels results from haemoglobinuria, hypotension or immune-mediated kidney damage associated with the systemic effects of the disease. Elevated liver enzymes due to hepatocellular injury caused by the *Babesia* infection and it is depends on the severity and stages of infection. Elevated bilirubin levels attributed to haemolysis, impaired hepatic clearance during the course of infection.

Table 3: Post therapeutic assessment of dogs with cerebral babesiosis by biochemical examination

Sl. No.	Parameters	Apparently healthy dogs (11)	Percentage of dogs with cerebral babesiosis			
			Post therapeutic day of observation			
			0th day (11)	7th day (8)	14th day (4)	28th day (2)
1.	Total protein (g/dl)	6.81±0.22	5.08±0.91	5.48±0.21	6.01±0.31	6.23±0.09
2.	Albumin (g/dl)	3.25±0.04	1.84±0.11	2.12±0.09	2.43±0.21	2.79±0.08
3.	Globulin (g/dl)	3.56±0.08	3.24±0.41	3.36±0.38	3.58±0.42	3.44±0.17
4.	A/G ratio	0.91±0.20	0.58±0.22	0.63±0.07	0.68±0.03	0.81±0.08
5.	ALT (IU/l)	18.71±1.56	44.86±11.3	53.00±8.2	43.12±7.10	38.08±6.6
6.	Alkaline Phosphatase (IU/l)	87.05±6.31	281.9±7.08	139.5±34.83	127.6±22.08	96.8±8.08
7.	Total bilirubin (mg/dl)	0.84±0.05	1.27±0.09	0.96±0.12	1.02±0.03	0.89±0.01
8.	Direct bilirubin (mg/dl)	0.44±0.02	0.76±0.04	0.49±0.03	0.61±0.12	0.47±0.23
9.	Indirect bilirubin (mg/dl)	0.40±0.03	0.51±0.05	0.47±0.08	0.41±0.07	0.42±0.08
10.	Blood urea nitrogen (mg/dl)	16.56±0.95	28.81±0.63	25.67±2.55	24.77±2.02	23.08±0.08
11.	Creatinine (mg/dl)	0.91±0.07	1.47±0.37	1.37±0.14	1.08±0.22	1.06±0.02
12.	Glucose (mg/dl)	102.27±0.56	91.08±4.09	96.27±1.56	95.08±2.08	99.03±0.41
13.	Magnesium (mg/dl)	1.94±0.20	2.72±0.44	2.39±0.03	2.08±0.41	2.33±0.52

In the present study, combination of medications was administered to treat the cerebral babesiosis in dogs. Administered medications were diminazene aceturate at 3.5 mg kg^{-1} body weight through deep intramuscular injection in two doses, oral doxycycline and lactic acid bacillus at 5 mg kg^{-1} body weight every 24 h for 21 days, enrofloxacin at 10 mg kg^{-1} body weight every 24 h for 21 days, metronidazole at 15 mg kg^{-1} body weight orally every 24 h for 21 days along with supportive and symptomatic medications.

Azithromycin every 24 h combined with atovaquone every 8 h for 10 days was the current recommended treatment for *Babesia gibsoni* (Birkenheuer et al., 2004; Solano-Gallego et al., 2016; Baneth, 2018). Diminazene diaceturate, along with other supportive medications such as probiotic and prebiotic capsules, iron supplements, 20% mannitol, doxycycline, prednisolone, furosemide, and kidney and liver cleanser. For 21 days, a single dose of diminazene aceturate at 3.5 mg kg^{-1} body weight deep I/M was administered along with an oral combination of doxycycline, enrofloxacin, and metronidazole at dose rates of 5, 3, and 10 mg kg^{-1} body weight (Lin and Huang, 2010). Clindamycin-metronidazole-doxycycline has been shown in some studies to be effective; however, this regimen was time-consuming and frequently necessitates supportive therapy (Suzuki et al., 2007). The combination of imidocarb, diminazene, and clindamycin was another alternate treatment plan (Lin et al., 2012). Antibiotics (doxycycline) could reduce

the disease's clinical symptoms, but they could not totally eradicate piroplasms (Lin and Huang, 2010). Although the prognosis for dogs with cerebral babesiosis appeared to be grave (Maele et al., 2008) reported full neuro-logical recovery of a dog with cerebral babesiosis after treatment with imidocarb dipropionate (Maele et al., 2008).

On the first day of presentation, all 11 dogs underwent clinical evaluations, which included neurological, haematological, and biochemical tests. Nine dogs underwent post-therapy evaluations on the third day, eight dogs on the seventh day, four dogs on the fourteenth day, and two dogs on the twenty-eighth day of post treatment. These results showed that dogs with cerebral babesiosis did not respond well to treatment. The post-treatment period documented the notable changes in hemoglobin, leukocyte count, eosinophils, total protein, albumin, liver enzymes, and magnesium levels, among other haemato-biochemical changes.

4. CONCLUSION

Dogs with neurological symptoms associated with *Babesia* infection might not respond well to treatment, and early detection of the illness might increase the chances of the dogs' survival.

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