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Therapeutic management and Haemato-biochemical changes of *Babesiosis* in dogs

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Abstract
This study was done to investigate the therapeutic efficacy of imidocarb dipropionate against babesiosis in dogs. A total of eighteen dogs diagnosed positive for babesiosis were taken for study which were presented at Department of Veterinary Medicine, Ranchi Veterinary College, Kanke, Ranchi, Jharkhand, India. They were diagnosed on history of ticks' infestation, characteristic clinical features and hematological examinations. These 18 dogs were divided randomly into 3 groups having 6 dogs in each. Group 1 was treated with Imidocarb @ 6 mg/kg; S/C, whereas Group 2 with Oxytetracycline & Doxycycline @ 10mg/kg; I/V & 6.6 mg/kg; PO, respectively and Group 3 with Diminazine @ 0.1 ml/2kg; I/M. Group 1 showed 100% recovery on 3rd day, while Group 2 & 3 showed 66.6%, recovery on 10th day and 7th day, respectively on blood smear examination for babesiosis. Since, the use of Imidocarb dipropionate has a number of advantages over that of Doxycycline, Oxytetracycline and Diminazine, comparative hematological tests were carried out to more definitively determine the efficacy of Imidocarb dipropionate in canine babesia. All the hemato-biochemical values came to normalcy at the treatment of 10th day in gr 1 whereas in group 2 & 3 normal parameters were observed on 14th day.

Keywords: Babesia, dog, Imidocarb, haematobiochemical

Introduction
Canine babesiosis is a protozoal, tickborne hemolytic disease with worldwide dissemination and global importance. Babesiosis is considered an emerging disease, as numerous cases are being reported in new areas [8]. More than hundered Babesia species have been distinguished, however mostly *Babesia canis* and *Babesia gibsoni* have been proven to infect dogs [16]. Canine babesiosis is a significantly important and probably dangerous lethal disease in India, caused by *B. canis* or *B. gibsoni.* Clinical manifestations due to babesiosis may vary with the different species, strains of babesia involved and their specific virulence and additionally with component that confirm the host's response to infection comparable to age, status of individual immune system and also the presence of concurrent infections of different diseases [12] [13]. Hemolytic anemia with destruction of red blood cell and an intrinsic inflammatory response may lead to organ dysfunction, account formost of the clinical signs determined babesia in canine [6]. Normocytic normochromic anaemia was prevalent in canine babesiosis [25]. In certain animals which is infected with some infections of Babesia, there will be an immune mediated part to the anemia and/or an extreme inflammatory reaction associated. High morbidity and mortality are associated with this complicated form of canine babesiosis [14]. Stained microscopic blood smear examination is conventionally used for the diagnosis of canine babesiosis which was relied mainly in most of the resource constrained settings within developing countries [1]. Babesia are in particular tick transmitted disease; sporozoites precisely infect RBCs resulting into the development of merozoites that is termed as piroplasms, that can be visible by light microscope [3]. Simple or conventional PCR protocols are thought-about to be perfect techniques for diagnosis of canine babesiosis at laboratory but nested PCR alternatively of simple PCR used to be relied upon as a screening assay and also for epidemiological examination as statistically significant false-negative were observed among the latter results [17]. Many drugs and its combinations have been accounted for to be viable against canine babesiosis [7]. It is therefore important to treat babesial infections with the most effective anti-protozoal drugs to recognize the treatment's limitations. So, this work had been designed to evaluate the efficacy of imidocarb for the treatment of babesiosis in dogs.

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Material and Method

This work was conducted in dogs brought to Department of Veterinary Medicine, Ranchi Veterinary College., Kanke, Ranchi, Jharkhand, India which were examined clinically irrespective of their age, breed and sex. Peripheral blood smears were made from suspected dogs and examined for the presence of babesiosis from dogs having the history of tick's infestation, characteristic clinical features, lethargy, weakness and discolouration of the urine (haemoglobinuria). Blood was collected from them aseptically. Peripheral blood smears were made for diagnosis of babesiosis and was confirmed by cytological examination by standard procedure. Eighteen dogs positive for babesiosis, on the basis of presence of *Babesia* in blood smears was selected for the study. Approximately, 6 ml of blood samples was collected from positive dogs in a sterile anticoagulant vial containing ethylenediaminetetraacetic acid (EDTA) and another 6ml of blood was also collected for serum on day 0, 3, 7, 10, 14 and 21th through cephalic or recurrent tarsal vein puncture. For haematological parameters CBC were estimated following standard procedure including TEC (total erythrocyte count, millions/u1), Hb (haemoglobin, g/ dl), PCV (packed cell volume, %), TLC (total leukocyte count), DLC (differential leukocyte count, %) and platelets (thousands/u1). Biochemical parameters including alanine transaminase (ALT, U/L), aspartate transaminase (AST, U/L), alkaline phosphatase (ALP, U/L), bilirubin (mg/dl), total protein (g/dl) and albumin (g/dl), blood urea nitrogen (mg/dl), glucose (mg/dl) and creatinine (mg/dl), were determined using Blood Chemistry Semi Auto Analyzer using standard kits. Treatment schedule was as follows (Table 1):

Table 1: Treatment Schedule

Group	No. of animals	Drug used	Dose	Days of treatment
1	6	Imidocarb	6 mg/kg; S/C	once
2	6	Oxytetracycline Followed by Doxycycline	10mg/kg ; I/V 6.6 mg/kg; PO	7days 14 days
3	6	Diminazine	0.1 ml/2kg; I/M	once

Result and Discussion

Clinical findings noted in this study included fever, anorexia, dullness, hemoglobinuria, pale mucous membrane, haemoglobinuria, which were in agreement with Roopali *et al.,* (2018) [19], however after treatment, the dogs in group 1 started showing clinical recovery and became completely normal within 7 days. Presence of organism as evident in blood smear, came negative by 3rd day of treatment in gr 1 and 7th day in gr 3 and 10th days in gr 3.

The finding revels that the RBC, Hb, PCV and platelets values were below the reference values and increase level of WBC value (Table 2) in all groups on day 0. These parameters came to normal in 10 days of treatment in gr 1 and 14 days in gr 2 & 3 which are in accordance with the finding of Al Izzi *et al.*, (2013) [2]. Anemia and thrombocytopenia were the most common hematological alterations observed in the present study which concurred with the report of Roopali *et al.,* (2018) [19], Bhrahma *et al.,* (2019) [9]. Intravascular and extravascular haemolysis may occur due the destruction of circulating RBC by auto antibodies which are directed against infected and non-infected erythrocytes [9]. According to Taboada and Lobetti, (2006) [23], direct parasitic damage contributes to anaemia. Low in erythrocytes count, packed cell volume percentage and reduced haemoglobin concentration define anemia. Antibody mediated cytotoxic destruction of erythrocytes may initiate severe microcytic-hypochromic anemia and/or may also be initiated by auto-antibody directed against components of the membranes of infected and uninfected erythrocytes which has also been reported by Aysul *et al.,* 2013 [4]. Anemia due to babesiosis may occur from an increased osmotic fragility of red blood cells, increased activity of erythrophagocyte of macrophages immune-mediated cleavage and thrombocytopenia due to immune-mediated platelets destruction [24]. Lower neutrophils and monocytes values and increased lymphocyte and eosinophil values were observed on day 0 may be due to alteration in hemostasis as well as parasite involvement, which elucidate immune responses and hence overproduction of lymphocytes and monocytes [5]. These values came to normal on 10 days of treatment in gr 1 and 14 days in gr 2 & 3 (Table 2).

Table 2: Haematological parameter in groups of babasia positive dogs

Parameters	Group	0th Day	7th Day	10th Day	14st Day	21th Day
Hb	1	8.1±0.63	10.9±0.43	12.9±0.04	14.5±0.15	14.6±0.18
	2	8.7±0.49	9.1±0.12	9.9±0.25	12.1±0.21	13.1±0.14
	3	9.2±0.91	9.4±0.16	10.4±0.3	13.2±0.17	13.6±0.16
PCV	1	23.2±0.24	32.7±0.41	39.8±0.12	41.6±0.47	48.4±0.81
	2	22.1±0.47	26.8±0.37	32.7±0.77	36.5±0.6	39.7±0.32
	3	24.8±0.18	28.3±0.35	36.7±0.53	39.1±0.3	44.2±1.11
Neutrophil	1	31.8±1.21	47.32±2.12	57.36±2.17	62.81±2.19	62.97±1.76
	2	34.8±1.15	41.19±2.71	46.01±1.62	63.39±2.03	63.11±1.45
	3	33.8±1.51	44.61±2.23	51.21±2.21	61.61±2.14	62.62±1.11
Eosinophil	1	3.19±0.78	1.31±0.22	1.26±0.92	1.59±0.43	1.12±0.17
	2	4.28±0.81	3.19±0.67	2.31±0.28	1.65±0.27	1.61±0.32
	3	4.17±0.42	2.56±0.38	1.73±0.51	1.51±0.41	1.83±0.29
Lymphocyte	1	63.1±1.78	47.63±2.1	35.66±2.9	34.21±1.54	37.84±0.47
	2	58.2±2.54	52.28±1.7	47.81±1.71	41.18±1.19	38.81±0.71
	3	59.9±1.91	49.56±3.27	42.14±1.83	39.28±1.41	38.15±0.27
Monocyte	1	2.16±0.13	3.86±0.23	5.39±0.31	3.8±0.16	3.76±0.25
	2	2.31±0.31	2.71±0.39	4.03±0.43	3.62±0.25	3.17±0.36
	3	2.14±0.23	3.11±0.42	4.92±0.36	3.11±0.36	3.59±0.36
Platelet count($\times 10^5/ml$)	1	1.9±0.23	2.9±0.21	3.7±0.27	4.4±0.18	6.8±0.3
	2	2.2±0.27	3.7±0.16	5.4±0.17	6.9±0.26	8.3±0.13
	3	2.1±0.2	4.9±0.14	5.8±0.2	7.6±0.19	8.3±0.07

Platelets dysfunction have occurred, due to early removal of platelets at an accelerated rate by antiplatelet antibodies which were formed as a result of interaction of B cells antibody receptor with foreign antigen, which is also described by Smitha *et. al.*, (2002) [22] and Shekhar *et al.,* (2011) [21].

There was increase in levels of ALT, AST, ALP and bilirubin in the infected dogs on day 0 (Table 3). These changes may be due to the haemolysis and cellular damage to the hepatic cells. These findings were in accordance with Roopali *et al.,* (2018) [19] and Al Izzi *et al.*, (2013) [2]. Increase in level of ALP was may be due to damage or abnormal function of biliary system [10]. Escape of AST and ALT enzymes from the damaged hepatic parenchymal cells with necrosis or altered membrane permeability may cause increased activities of these enzymes indicating hepatic dysfunction [11]. Increased alanine aminotransferase alkaline phosphatase and bilirubin are indicative of hepatic hypoxia whereas elevation of creatinine are indicative of degenerative changes in kidneys [9]. All these parameters came to normalcy at the 10th day of treatment in gr 1 whereas in group 2 & 3 normal parameters were observed on 14th day (Table – 3).

Table 3: Haematological parameter in groups of Babasia positive dogs

Parameters	Group	0th Day	7th Day	10th Day	14st Day	21th Day
ALT, U/L	1	113.76±8.44	62.54±5.56	61.12±3.22	59.02±4.28	58.54±5.63
	2	129.82±9.73	81.02±4.99	77.87±3.37	70.13±5.66	69.02±4.24
	3	141.41±10.21	73.24±4.11	66.69±3.09	61.23±4.28	61.24±4.99
AST, U/L	1	83.76±3. 27	63.22±3.17	60.63±4.02	57.33±2.19	58.12±3.22
	2	78.84±3.11	72.71±3.64	68.33±4.37	61.46±2.46	60.87±3.37
	3	89.23±3.96	72.56±3.71	62.86±3.93	58.23±2.33	58.69±3.09
ALP, U/L	1	280.25±13.89	98.25±17.36	93.76±08.27	91.71±03.63	93.76±04.27
	2	256.59±19.48	171.33±13.81	178.84±11.11	111.29±11.68	98.84±3.11
	3	274.61±14.57	123.27±16.72	149.23±12.99	99.57±08.37	94.23±3.96
Bilirubin (mg/dl),	1	1.22±0.19	0.62±0.08	0.61±0.01	0.59±0.07	0.61±0.02
	2	1.19±0.23	0.99±0.03	0.82±0.02	0.74±0.08	0.69±0.06
	3	0.96±0.12	0.76±0.02	0.66±0.02	0.61±0.04	0.62±0.02
Total protein (g/dl)	1	5.73 ± 0.43	5.93 ± 0.27	6.21 ± 0.41	6.67 ± 0.15	6.78 ± 0.51
	2	5.27±0.52	5.79±0.49	5.96±0.67	6.01±0.57	6.16±0.45
	3	5.93±0.22	5.99±0.31	5.93±0.44	6.27±00.22	6.32±0.66
Albumin (g/dl),	1	1.53 ± 0.19	2.61 ± 0.28	3.31 ± 0.37	3.87 ± 0.06	3.93 ± 0.29
	2	1.22±0.09	1.91±0.11	2.42±0.23	2.99±0.18	3.22±0.13
	3	1.71±0.07	2.18±0.17	2.97±0.05	3.77±00.19	3.79±0.42
blood Glucose (mg/dl)	1	58.91±5.63	78.91±4.91	88.91±4.77	88.43±3.72	88.91±3.54
	2	66.88±4.97	71.34±4.03	74.88±4.09	79.02±3.58	86.08±3.36
	3	63.24±4.01	73.24±3.62	83.24±4.56	86.18±3.27	83.41±3.92
Creatinine (mg/dl)	1	2.13±0.30	1.19±0.17	1.16±0.18	1.13±0.16	1.09±0.21
	2	2.02±0.24	1.72±0.12	1.69±0.11	1.26±0.13	1.26±0.12
	3	1.98±0.19	1.56±0.24	1.38±0.14	1.24±0.14	1.18±0.11

Decrease in the blood glucose level was observed in this experiment on day 0 in all groups. The lower levels may be due to starvation and hepatic dysfunction. Same findings were also reported by Kumar & Kumar, 2018 [15], Bhrahma *et al.*, (2019) [9] and Roopali *et al.,* (2018) [19]. The level of serum creatinine was higher in the infected dogs on day 0. Increase in creatinine in canine babesiosis might have resulted due to acute renal failure [20]. Reddy *et al.* (2014) [18] reported significant increase in creatinine in babesiosis positive dogs. There was a decrease in serum total protein and albumin levels in affected dogs due to liver damage in affected animals on day 0. These findings were in accordance with Reddy *et al.* (2014) [18]. All these parameters came to normalcy at the 10th day of treatment in gr 1 whereas in group 2 & 3 normal parameters were observed on 14th day. After the end of this study all haemato-biochemical parameters ultimately came to normal with cessation of all observed clinical signs resulting into complete recovery of dogs.

Group 1 showed 100% recovery on 3rd day, while Group 2 & 3 showed 66.6%, recovery on 10th day and 7th day, respectively. On 28th day all the animal recovered in all groups on the basis of blood smear examination.

Table 3: Post treatment recovery

	Group	0th Day	3rd Day	7th Day	10th Day	14th Day
Treatment	1	0	6	6	6	6
	2	0	4	5	6	6
	3	0	4	6	6	6

Conclusion
Group 1 showed 100% recovery on 3rd day, while Group 2 & 3 showed 66.6%, recovery on 10th day and 7th day, respectively on the basis of blood smear examination. All the dogs become normal on 10 days in Group 1. The dogs returned to normal appetite and improved blood count by 10 days in gr 1 and 14 days in gr 2 & 3. Though therapy with oxytetracycline & doxycycline and Diminazine had shown to be effective in the treatment of canine babesiosis as a result of elimination of parasites, treatment with imidocarb dipropionate was found best by decreasing the severity in this experiment.

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