



Studies on Haemato- Biochemical Changes and Evaluation of Combination Therapy Against *B. gibsoni*

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Abstract

Babesiosis is one of the main causes of anemia in dogs and lot of research has been proposed for its pathogenesis. Present study was envisaged to study the hemato-biochemical changes with different therapeutic interventions against Babesia gibsoni in canines. During the study period, 2236 dogs were presented to TVCC, IVRI, of which 48 dogs were suspected for hemoprotozoan diseases and finally 15 dogs were found positive for B. gibsoni by microscopic examination. Blood samples were taken to study hematological and serum changes. Positive dogs were randomly divided into 3 groups (n=5) for different therapeutic interventions. Dogs of Gr. I received Berenil (5mg/Kg IM) and was repeated after 7 days. Dogs of Gr. II were treated with combination of Doxycycline @ 10mg/Kg PO q12h and Clindamycin @ 25mg/Kg q12h for 21 days. Dogs of Gr. III received combination of Doxycycline @ 10mg/Kg q12h, Clindamycin @25mg/Kg q12h, Metronidazole @ 7mg/Kg q12h for 21 days. All the treated dogs received need based supportive therapy in addition to the specific therapy.

Keywords: Babesia, Canine, Clindamycin, Doxycycline, Metronidazole, Therapy

Introduction

Canine babesiosis is a tick-borne hemoprotozoan disease typically caused by *Babesia canis*, a large piroplasm or *Babesia gibsoni*, a small piroplasm. Canine babesiosis was known to cause hemolytic changes which ranged from hypochromic to normochromic anemia and this change is associated with reduction in erythrogram parameters and alterations in serum biochemical values (Welzl *et al.*, 2000). Severity of babesiosis ranges from subclinical infection to widespread organ failure and death. Most dogs with babesiosis develop hemolytic anemia and/or thrombocytopenia, together with varying degrees of anorexia, fever, splenomegaly, and icterus (Boozer and Macintire, 2005). Acute infection is characterized by remittent fever, lethargy, thrombocytopenia, and anemia whereas chronic form may be completely asymptomatic or may be characterized by intermittent fever, lethargy and weight loss (Abdullahi *et al.*, 1990). Clinical signs in severe form may be due to excessive systemic inflammatory response syndrome (SIRS) which results in multiple organ dysfunction syndromes (MODS) (Welzl *et al.*, 2000).

The major complications of canine babesiosis are multiple organ dysfunction syndromes (MODS) involving hepatic and kidney failure, disseminated intravascular coagulation, pancreatitis, oxidative damage on erythrocyte, immune mediated hemolytic anemia and pulmonary edema (Jacobson *et al.*, 1994; Strombeck and Gribble, 1978). The microscopic blood smear examination considered as the cheapest method for diagnosis of hemoprotozoan disease during acute stage of disease and this ability decrease sharply in other situations like carrier animals (Bhatia *et al.*, 2000; Ettinger and Feldman, 2000). The present study has emphasized on the clinical and laboratory alterations in *Babesia gibsoni* infected dogs receiving different therapeutic modalities.

Materials and Methods

Screening of Dogs for *Babesia gibsoni*

Dogs presented to TVCC, IVRI were randomly screened for hemoprotozoan disease based on characteristic clinical signs. Dogs found positive for *B. gibsoni* by Giemsa's staining based on the morphology (small form, mostly single) were randomly selected. Blood, serum samples were collected for laboratory investigation of organ profile. Considerate number *B. gibsoni* positive dogs with definite breed, sex and age group was taken in account for the present study.

Microscopic Examination of Blood Smears

Peripheral blood smear from ear vein stained with Giemsa's stain and examined under oil immersion (100x).

Changes in Complete Blood Count (CBC)

Around 1 ml of blood was collected from the *B. gibsoni* infected dogs. Complete blood count (CBC) was performed by using EDTA coated blood in auto-analyzer (Junior Vet Abacus). The parameters studied were Hb, RBC, WBC, PCV and platelet count.

Changes in Serum Biochemistry

Around 3 ml of blood was collected in plain tube and serum was separated after centrifugation at 3000 rpm for 10 minutes. The parameters studied were ALT, ALP, TP, Albumin, BUN and creatinine.

Evaluation of Combination Therapy against *B. gibsoni*

Dogs of Gr. I received Diminazene aceturate (5mg/Kg IM) which was repeated after 7 days. Dogs of Gr. II were treated with combination of Doxycycline @ 10mg/Kg q 12h and Clindamycin @ 25 mg/Kg q12h for 21 days. Dogs of Gr. III received combination of Doxycycline @10mg/Kg q 12h and Clindamycin @ 25 mg/Kg q 12h and Metronidazole @ 7 mg/Kg q 12h for 21 days. All the treated dogs received need based supportive therapy in addition to the disease specific therapy. In addition, other interfering conditions, infections etc. has been ruled out during the entire study by blood and serum biochemistry. Dogs were monitored for relapse of clinical condition during treatment period.

Results and Discussion

Epidemiology of *Babesia gibsoni*

Out of 2236 dogs presented during the present study period, 48 dogs were suspected for hemoprotozoan diseases with clinical signs like anemia, fever, tick exposure, lymph node enlargement. Among these suspected dogs, 15 (31.25%) dogs were found positive for *Babesia gibsoni* infection.

Changes in Complete Blood Count (CBC)

Non significantly increased level of hemoglobin (Hb) and packed cellular volume (PCV) was observed in all the groups at 7th day of treatment as compared to day 0. No significant difference was noticed in the total erythrocyte count (TEC) on day 0 and day 7 in all the groups. With respect to total leukocyte count (TLC), non-significant increased level of TLC was observed at 7th day as compared to day 0 in all the groups. To conclude, blood changes were not significantly conclusive before and after treatment in all three treatment groups. CBC details have been shown in Table 1.

Table 1: Haematological changes in dogs with *Babesia gibsoni* infection (Mean± SE)

Group	Day	Hb (gm/dl)	PCV (%)	TEC ($\times 10^6/\mu\text{l}$)	TLC ($\times 10^3/\mu\text{l}$)
1	0	9.52(±1.49) (AB)	28.44(±4.51) (AB)	3.7(±0.88) (AB)	4.94(±1.47)
	7	11.56(±0.99) (A)	33.82(±3.01) (A)	4.32(±0.57) (A)	6.96(±2.09)
2	0	10.66(±0.93) (AB)	31.92(±3.32) (A)	4.63(±0.31) (A)	6.62(±2.06)
	7	12.56 (±0.64) (A)	36.14(±1.65) (A)	5.17 (±0.31) (A)	11.89(±2.29)
3	0	4.66(±0.64) (C)	14.9 (±2.02) (B)	2.09(±0.29) (B)	5.78(±1.36)
	7	6.88(±0.84) (BC)	24.34(±3.98) (AB)	4.08(±0.11) (AB)	7.85(±0.67)

Data was analyzed by using two-way ANOVA using SPS trail version 20.0. (Value bears different capital letters are significantly different in row wise)

Changes in Serum Biochemistry

No significant change was observed for ALT in Gr. I and Gr. III as compared to Gr II. No significant change was noticed for total protein (TP) in Gr. I, II and III after 7 days of treatment. Similar trend was observed with albumin (ALB) and creatinine level after 7 days of treatment. Non-significant change was noticed for BUN in Gr. I and Gr. II as compared to Gr. III on day 7th. In nutshell, serum biochemistry changes were not significantly conclusive before and after treatment in all three treatment groups. Serum biochemistry details have been shown in Table 2.

Table 2. Serum biochemical values in dogs with *Babesia gibsoni* infection (Mean± SE)

Group	Day	ALT (IU/L)	ALP	Total protein	Albumin	BUN	Creatinine
			(IU/L)	(g/dl)	(g/dl)	(mg/dl)	(mg/dl)
1	0	68.2(±13.98) (A)	166.6(42.45) (A)	5.72(±0.49) (A)	2.63(±0.29)	17.28(±1.62) (B)	1.02(±0.12)
	7	61.2 (±8.96) (A)	188.6(±37.76) (A)	6.26(±0.27) (A)	2.36(±0.17)	27.8(±4.72) (AB)	0.84(±0.13)
2	0	32.6(±7.83) (A)	114.6(±14.92) (A)	5.9(±0.23) (A)	2.82(±0.28)	34.4(±9.63) (AB)	1.66(±0.37)
	7	46.8(±7.43) (A)	123.6(±14.27) (A)	5.91(±0.42) (A)	2.43(±0.26)	49.6(±7.09) (AB)	1.5(±0.40)
3	0	63.8(±12.35) (A)	328.6(±160.11) (A)	6.31(±0.52) (A)	2.76 (±0.41)	50.52(±13.15) (A)	1.64 (±0.29)
	7	56.2(±1.66) (A)	214.8(±94.73) (A)	6.48(±0.37) (A)	2.58(±0.27)	21.6(±10.33) (AB)	1.02 (±0.19)

Data was analyzed by using two-way ANOVA using SPS trail version 20.0. (Value bears different capital letters are significantly different in row wise).

Pathogenic tick born hemoprotozoan canine babesiosis caused by *Babesia gibsoni*, is widely distributed throughout the world (Ettinger and Feldman, 2000). In India, *B. gibsoni* is more commonly recorded than *B. canis* (Bhatia *et al.*, 2000). Pleomorphic small form of babesia in canines caused by *B. gibsoni* infection may occur in acute or chronic form. The arthropod borne disease caused by *Babesia* spp. has different susceptibility to anti-protozoal drugs either alone or in combination (Vial and Gorenflot, 2006; Irwin, 2009; Solano-Gallego and Baneth, 2011; Beugnet and Moreau, 2015). Large form of babesiosis such as *Babesia canis*, *Babesia rossi*, and *Babesia vogeli* is quite sensitive to diminazine aceturate and imidocarb but small form as well most pathogenic form *Babesia gibsoni* is resistant to these drugs (Jefferies *et al.*, 2007; Lin *et al.*, 2012; Kirk *et al.*, 2017). Line of treatment for canine babesiosis varies with the severity of infection and accompanying complications. Researchers have explored various therapeutic regimens using combination of antibiotic and anti-protozoal drug against *Babesia gibsoni*. Combination of Clindamycin, Diminazine aceturate and Imidocarb dipropionate was also effective against *B. gibsoni* (Lin *et al.*, 2012; Islam *et al.*, 2017). Newer successful treatment modalities are the choice of present era. Ultimate success of any treatment protocol lies with the positive prognostic response correlating the instituted therapy. Hence, it is much essential to evaluate the regimens of novel drug combinations for its synergistic effects if any against *Babesia gibsoni* infection with special emphasis on prognostic biomarkers as a success indicator of any effective therapy. Study concludes that no single drug has been reported effective against of *B. gibsoni* in dog. Triple (combined) therapy with Doxycycline @ 10 mg/Kg along with Clindamycin 25mg/Kg and Metronidazole @ 5-7 mg/Kg PO in combination with need based supportive therapy had better prognosis cum survivability of animal.

Conclusion

Gr. III dogs had better recovery followed by Gr. II and I. because there was no persistence of parasitemia and relapse of clinical condition in Gr. III as contrary to the dogs of Gr. I and Gr. II.

Conflict of Interests

There is no conflict of interest.

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References

1. Abdullahi, S.U., Mohammed. A.A., Trimnell, A.R., Sannusi. A. and Alafiatayo. R. (1990). Clinical and haematological findings in 70 naturally occurring cases of canine babesiosis. *J. Small Anim. Pract.* 31, 145-147.
2. Beugnet, F., Moreau, Y. (2015). Babesiosis. *Rev. Sci. Tech.* 34, 627–639
3. Bhatia, B. B., Pathak, K. M. L. and Juyal, P.D. (2000). Text book of Veterinary Parasitology, Kalyani Publisher, 289-311.
4. Bick, R.L. Arun, B. and Frenkel, E.P. (1999). Disseminated intravascular coagulopathy: clinical and pathophysiological mechanism and manifestations. *Hemostasis*, 29, 111- 134.
5. Boozer, L. and Macintire, D. (2005). *Babesia gibsoni*: An Emerging Pathogen in Dogs. Compendium, Article 2. Auburn University. 33-42.
6. Ettinger. S.J. and Feldman, E.C. (2000). Text book of veterinary internal medicine: Disease of dog and cat. Philadelphia: W.B. Saunders Co 7th Ed., Elseiver, 916-917.
7. Irwin, P.J. (2009). Canine babesiosis: from molecular taxonomy to control. *Parasit.* 2, S4
8. Islam, S., Kalita, D and Dutta, T.C. (2017). Comparative efficacy of combination therapeutics for management of canine babesiosis. *Intas Polivet*, 19.
9. Jacobson, L.S and Clark, I.A. 1994. Review/Oorsig. The pathophysiology of canine babesiosis: new approaches to an old puzzle. *J. South African Vet. Assoc.* 65, 134-145.
10. Jefferies, R., Ryan, U.M., Jardine, J., Robertson, I.D., Irwin, P.J. (2007b). *Babesia gibsoni*: detection during experimental infections and after combined atovaquone and azithromycin therapy. *Exp. Parasitol.* 117, 115–

123.

11. Kirk, S.K., Levy, J.K., Crawford, P.C. (2017). Efficacy of azithromycin and compounded atovaquone for treatment of in dogs. *J. Vet. Intern. Med.* <http://dx.doi.org/10.1111/jvim.14777>
12. Lin, E.C., Chueh, L.L., Lin, C.N., Hsieh, L.E., Su, B.L. (2012). The therapeutic efficacy of two antibabesial strategies against *Babesia gibsoni*. *Vet. Parasitol*, 186, 159–164.
13. Murase, T, Ueda, T., Yamato, O., Tajima, T., Maede, Y. (1996). Oxidative damage and enhanced erythrophagocytosis in canine erythrocytes infected with *Babesia gibsoni*. *Journal of Veterinary Medical Science*, 58, 259-261.
14. Solano-Gallego, L., Baneth, G. (2011). Babesiosis in dogs and cats—expanding parasitological and clinical spectra. *Vet. Parasitol*, 181, 48–60.
15. Strombeck, D.R. and Gribble, D. (1978). Chronic active hepatitis in the dog. *J. Am. Vet. Med. Assoc.* 173, 380-386.
16. Van mourik, J.A. Romani de wit, T. (2001). Von willebrand factor propeptide in vascular disorders. *Thromb haemostat*, 86, 164-71.
17. Vial, H.J. and Gorenflot, A. (2006). Chemotherapy against babesiosis. *Vet. Parasitol*, 138, 147–160.
18. Welzl C, Leisewitz A.L, Jacobson, L.S., Vaughan-Scott, T., Myburgh, E. (2000). Systemic inflammatory response syndrome and multiple-organ damage/ dysfunction in complicated canine babesiosis. *J S Afr Vet Assoc*, 72, 158–162.
