

Evaluation of the therapeutic efficacy of Yacasyn[®] tablet as a platelet supplement in thrombocytopenic dogs

A. Sudha Naga Chittitalli¹, N. Devadevi^{1*}, K. Rajkumar², S. Vivek², P. Vijayalakshmi¹ and A. Abiramy Alias Prabavathy¹

¹Department of Veterinary Medicine, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry-605 009, India; ²Department of Veterinary Clinical Complex, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry- 605 009, India

Abstract

Dogs of different breeds which were presented to the Veterinary Clinical Complex, RIVER, Puducherry, with the clinical signs of anorexia, fever, lethargy, congested or pale mucous membrane lymphadenomegaly, bleeding disorders and tick infestation were suggestive of tick-borne diseases which were included under the study. Diagnosis of the tick-borne disease was done by haemato-biochemical examination and PCR. Haemato-biochemistry revealed significant reduction of the Hb, PCV, TLC and platelet count. Molecular diagnosis revealed *B. gibsoni* and *H. canis* in the tick-infected dogs, which were treated with standard treatment protocol with the supportive of Tab. Yacasyn as a platelet enhancer. After four weeks of treatment, haemato-biochemistry revealed significant increase in the Hb, PCV, RBC and platelet count without alteration in liver function test and renal function test.

Keywords: *B. gibsoni*, Dog, *H. canis*, Thrombocytopenia, Yacasyn

Highlights

- Tick-borne diseases manifest as fever, congested or pale mucous membranes, lymphadenomegaly, and bleeding disorders.
- The blood parasites encountered in the present study were *B. gibsoni* and *H. canis* by PCR.
- The standard treatment protocol gave good recovery with supportive therapy.
- Tab. Yacasyn significantly increases the thrombocyte count within four weeks.
- It's a safe drug for anemic and thrombocytopenic dogs.

INTRODUCTION

Tick-transmitted diseases are an emerging problem in dogs. Ticks and fleas are significant carriers of various viral, bacterial, and protozoan infections that affect both humans and animals due to their blood-feeding behavior (Day, 2016). Ticks are attached to their host and are successfully transmitted to the infectious agents. The transmission of microorganisms to various geographic environments via travelling of dogs with their infections. Ticks may transmit the pathogens via trans-stadial and/or trans-ovarian routes. Female ticks are reproducing rapidly, which facilitates the spread of infectious pathogens in reservoir populations that interact with pets and their owners. Babesiosis, ehrlichiosis, and hepatozoonosis are the three infectious diseases that are most commonly transmitted by ticks and cause severe clinical illnesses in dogs. Jena *et al.* (2021) reported that *Rhipicephalus sanguineus* (brown dog tick) is a widespread tick in the world, including India. Bhadesiya *et al.* (2016) and Anish *et al.* (2020) reported

that *Haemophysalis* spp., *Dermacentor* spp., and *Otobius magnini* are prevalent ticks in India. Bhowmik *et al.* (2024) reported that brown dog tick is 62.21% prevalent in Assam which was confirmed by 16S rRNA gene amplification and sequence analysis. Ticks have been commonly associated with protozoal infections like *Babesia canis*, *Ehrlichia canis*, and *Hepatozoon canis* (Dantas-Torres and Otranto, 2015; Taylor *et al.*, 2016; Dantas-Torres *et al.*, 2018). Several infections, including *Babesia canis* and *Haemobartonella canis* have been identified as challenges to the health and production of the animals (Shitta *et al.*, 2018). The present study objective is to study the efficacy of Tab. Yacasyn[®] (Mutt Nature Private Ltd., Tamil Nadu) in the treatment of thrombocytopenic dogs affected with tick-borne diseases.

MATERIALS AND METHODS

A total of 18 dogs which were presented to the Small Animal Medicine Unit, Veterinary Clinical

*Corresponding Author, E-mail: devadevivmd@river.edu.in

Complex, RIVER, Puducherry, divided into the control group (n=6) which were apparently healthy dogs and were present for general checkup and vaccination. Dogs with thrombocytopenia (less than $150 \times 10^3/\mu\text{L}$) due to tick-borne diseases were taken under the study group (n=12). All the animals were subjected to complete clinical examination. Five millilitres of whole blood was collected for haematology, serum biochemical analysis and PCR. Haematology includes Haemoglobin (Hb), Red Blood Cell (RBC) count, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC) and Thrombocyte count, were estimated using haematology autoanalyzer. Serum samples were subjected to estimation of liver function test (total bilirubin, total protein, albumin, globulin, A:G, ALP, AST, ALT) and renal function test (BUN and creatinine) using a semi-autoanalyzer. For PCR, DNA extraction was done using the QIAmp Blood and Tissue Extraction Kit from Qiagen, Hilden, Germany. 200 μL of whole blood was utilized to extract the DNA as per the manufacturer's guidelines. The extracted DNA was stored at -20°C until further use. Details about the primer, target gene, size of *Babesia gibsoni* and *Hepatozoan canis* as per reference of Peddineni *et al.* (2023) and Inokuma *et al.* (2002), respectively which was given in Table 1. The total volume of the PCR reaction mixture was 25 μL , which consisted of 12.5 μL of 2 x PCR Master mix, 1 μL of forward and 1

μL of reverse primer, 4 μL of DNA template and 6.5 μL of ultrapure water. The PCR conditions for the amplification of *Babesia gibsoni* and *Hepatozoan canis* are given in Table 2. The amplified products were analyzed in 1.0% agarose gel electrophoresis using 5 μL of PCR product at 100 volts for 30 minutes along with 100 bp ladder. Subsequently, the DNA bands were visualized using a UV transilluminator and documented using the Bio-Rad gel documentation system. The anticipated size products were 662 bp for *B. gibsoni* and 666 bp for *H. canis*. Dogs which were positive for *B. gibsoni* were treated with triple-drug therapy, which includes doxycycline (10 mg/kg BW PO, SID), metronidazole (20 mg/kg BW PO BID) and clindamycin (11 mg/kg BW PO BID) for 2 weeks. *H. canis* infection, dogs were treated with doxycycline (10 mg/kg BW PO, SID) for 2 weeks. Tab. Yacasyn® is a potent platelet enhancer which is manufactured by Mutt Nature Private Ltd., Tamil Nadu. All 12 dogs were treated with the platelet supplement Tab. Yacasyn® @ 1tab/30 kg BW PO SID for 4 weeks. Blood samples were collected after four weeks of treatment and subjected to haemato-biochemical estimation. The data obtained were subjected to statistical analysis using paired t-test (Peddineni *et al.*, 2023).

RESULT

Dogs with tick-borne diseases exhibited the clinical signs of dullness, anorexia, general weakness, fever, lymphadenopathy, pale to blanched mucus membrane, bleeding disorders, and increased heart and respiration rate with severe tick infestation. Haemato-biochemistry of the control group and study

Table 1. Primer sequence and target gene of the blood parasites (*B. gibsoni* and *H. canis*)

Blood parasites	Primers	Target gene	Size	Reference
<i>Babesia gibsoni</i>	Gib599 F: 5'-CTCGGCTACTTGCCTTGTC-3'	18S rRNA	662bp	Peddineni <i>et al.</i> (2023)
	Gib1270 F: 5'-CCGAAACTGAAATAACGGC-3'			
<i>Hepatozoan canis</i>	Hep F: 5'-ATACATGAGCAAAATCTCAA-3'	18S rRNA	666bp	Inokuma <i>et al.</i> (2002)
	Hep R: 5'-CTTATTATTCCATGCTGCAG-3'			

Table 2. PCR conditions for amplification of *B. gibsoni* and *H. canis*

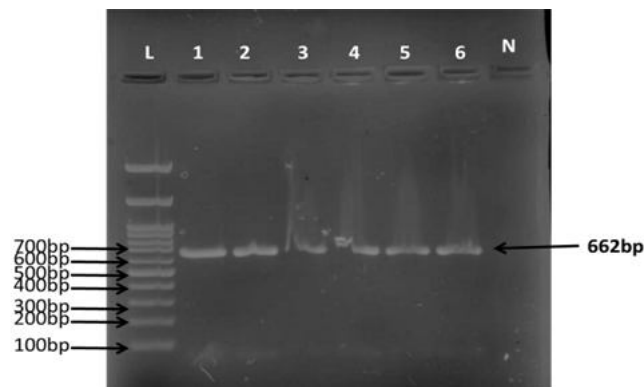
Sl. No	Blood parasites	Initial denaturation	Denaturation	Annealing	Extension	Final extension
1	<i>B. gibsoni</i>	95°C 5 min	95°C 30 sec	60°C 30 sec	72°C 105 sec	72°C 5 min
2	<i>H. canis</i>	95°C 5 min	95°C 30 sec	60°C 30 sec	72°C 90 sec	72°C 5 min

group (before and after treatment) were given in Table 3. PCR amplification of the DNA samples of dogs suspected of tick-borne disease revealed positive for *Babesia gibsoni* and *Hepatozoan canis*, as shown in Fig. 1 and 2.

All the dogs recovered from the tick-borne disease without any completion and well tolerated the Tab. Yacasyn (Mutt Nature Private Ltd., Tamil Nadu). There was a significant decrease in Hb, PCV, RBC, and platelet count in the study group. After four weeks of treatment, the haemato-biochemistry results of pre-treatment and post-treatment were compared, which revealed significant ($p<0.01$) increased Hb, PCV, RBC and platelets count and significant ($p<0.05$) increased TLC, MCHC and albumin, and significant decreased ($p<0.05$) MCV, MCH and total protein. Tab. Yacasyn® (Mutt Nature Private Ltd., Tamil Nadu) treatment showed no change in the values of the liver function test and renal function test.

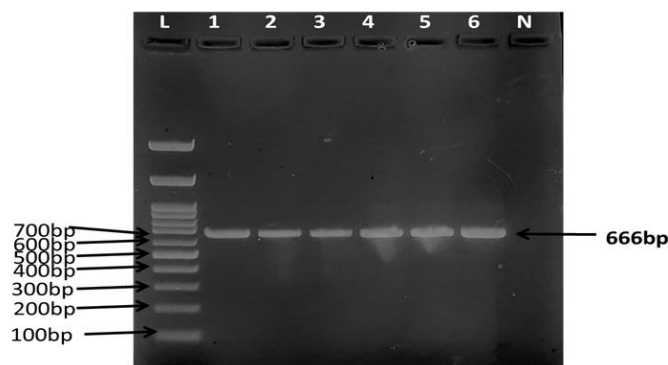
DISCUSSION

Acute thrombocytopenia can result due to decrease in the median life of platelets, immune-mediated destruction, or an increase in platelet consumption by the inflamed vascular endothelium (Pasqualetti *et al.*, 2021). In dogs, *B. gibsoni* infections are clinically severe or mild, and they are frequently unintentionally found during regular screenings for unrelated illnesses. Thrombo-cytopenia and hemolytic anaemia are the most frequent findings and hallmarks of tick-borne



[Lane L: 100bp ladder, Lane 1-6: Unknown samples, Lane N: Negative control]

Fig. 1. Gel picture of PCR amplified products of *B. gibsoni* with size of 662bp



[Lane L: 100bp ladder, Lane 1-6: Unknown samples, Lane N: Negative control]

Fig. 2. Gel picture of PCR amplified products of *H. canis* with size of 666bp

Table 3. Comparison of haemato-biochemical parameters of the control group and study group (before and after treatment) in dogs with tick-borne diseases

Parameters	Control group (n=6) (Mean $\pm$ SE)	Study group (n=12)		't' test
		Before treatment (Mean $\pm$ SE)	After treatment (Mean $\pm$ SE)	
TLC ($\times 10^3/\mu\text{L}$)	10.27 $\pm$ 0.44	8.38 $\pm$ 0.84	10.25 $\pm$ 0.63	0.016*
Neutrophils (%)	61.33 $\pm$ 1.64	63.40 $\pm$ 3.50	67.50 $\pm$ 1.50	0.149 ^{NS}
Lymphocytes (%)	34.66 $\pm$ 3.68	30.41 $\pm$ 3.40	26.70 $\pm$ 1.60	0.114 ^{NS}
Monocytes (%)	0.66 $\pm$ 0.21	3.41 $\pm$ 0.47	2.66 $\pm$ 0.41	0.056 ^{NS}
Eosinophils (%)	1.50 $\pm$ 0.33	2.75 $\pm$ 1.30	3.08 $\pm$ 0.60	0.782 ^{NS}
RBC ($\times 10^6/\mu\text{L}$)	7.50 $\pm$ 0.52	3.70 $\pm$ 0.30	5.80 $\pm$ 0.29	0.0001**
Haemoglobin (g/dL)	14.52 $\pm$ 0.84	8.10 $\pm$ 0.70	11.50 $\pm$ 0.30	0.0001**
PCV (%)	44.95 $\pm$ 3.17	25.10 $\pm$ 1.90	34.90 $\pm$ 1.08	0.0001**
MCV (fl)	60.30 $\pm$ 0.37	67.50 $\pm$ 2.70	61.30 $\pm$ 2.70	0.034*
MCH (pg)	19.43 $\pm$ 0.31	22 $\pm$ 0.70	20.10 $\pm$ 0.70	0.027*
MCHC (%)	32.33 $\pm$ 0.54	32.60 $\pm$ 0.36	33.10 $\pm$ 0.61	0.505 ^{NS}
Platelets ($\times 10^3/\mu\text{L}$)	218.33 $\pm$ 8.33	101.50 $\pm$ 13.60	238.30 $\pm$ 28.1	0.0001**

Cont. Table 3.

Table 3., Cont. ...

Parameters	Control group (n=6) (Mean ± SE)	Study group (n=12)		't' test
		Before treatment (Mean ± SE)	After treatment (Mean ± SE)	
BUN (mg/dL)	27.70±4.19	15.40±4.50	14.60±2.80	0.665 ^{NS}
Creatinine (mg/dL)	0.83±0.10	1.12±0.30	0.94±0.16	0.346 ^{NS}
Total Bilirubin (mg/dL)	0.17±0.01	0.61±0.78	0.48±0.42	0.036*
SGOT (IU/L)	36.15±4.99	57.50±14.70	45.70±6.70	0.211 ^{NS}
SGPT (IU/L)	39.83±3.55	52±16.70	50.50±11.30	0.905 ^{NS}
ALP (IU/L)	61.58±5.40	179.70±51.20	130.80±24.60	0.276 ^{NS}
Total protein (g/dL)	6.94±0.58	6.64±0.43	6.69±0.39	0.762 ^{NS}
Albumin (g/dL)	3.27±0.07	2.33±0.14	2.67±0.12	0.014*
Globulin (g/dL)	3.66±0.57	4.32±0.46	4.01±0.34	0.084 ^{NS}
A:G ratio	0.53 ±0.18	0.652±0.11	0.74±0.10	0.062 ^{NS}

Note: ** Significant $p < 0.01$, * Significant $p < 0.05$, ^{NS} Non-significant $p > 0.05$

disease, which is in accordance with the present study. Almendros and Burchell (2021), Sudhakara *et al.* (2016) and Vidhya *et al.* (2024) reported that dogs exhibit pallor mucus membrane, weakness, fever, anorexia, lymphadenomegaly, splenomegaly, and icterus, which were recorded in the present study. Epistaxis and gingival haemorrhage are infrequently recorded, while petechiae and ecchymoses are uncommon. *Babesia gibsoni* infection occurs as per-acute, acute, or chronic form of disease. In the present study, PCR diagnosis of *B. gibsoni* and *H. canis* was carried out as per the recommendation of Peddineni *et al.* (2023) and Inokuma *et al.* (2002), respectively. Peddineni *et al.* (2023) reported that there was a significant reduction in the Hb, PCV, TEC and platelet count in *B. gibsoni*, which was in accordance with the present study. All the dogs responded well to the therapy without any complications. Tab. Yacasyn® (Mutt Nature Private Ltd., Tamil Nadu) is a platelet supplement which is used to increase the platelet count within 4-6 weeks. All dogs showed a rapid increase in the platelet count within 4 weeks and without any alteration in the liver function test and renal function test values, which indicates it's an ideal drug for platelet enhancement, which is non-toxic and very well

tolerated by the dogs.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: AS: Carried out the data collection, analysis and drafted the manuscript; ND: Supervised, analyzed the work and revised the manuscript; KR, SV, PV, AA: Read, corrected and approved the final manuscript.

Data availability statement: All the data supporting the research findings have been presented in this paper. The corresponding author is willing to provide the raw data upon reasonable request.

ACKNOWLEDGEMENTS

The authors thank the Dean and Department of Veterinary Medicine, Veterinary Clinical Complex, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry for providing the necessary facilities. We are grateful to Mutt Nature Private Ltd, Triton Healthcare Pvt. Ltd., No. 50, 1st Floor, GST Road, Kallapranpuram, Padalam Via, Madurantagam Talk, Chengalpattu Dist.- 603 308, Tamil Nadu for their financial support.

REFERENCES

- Almendros A and Burchell R, 2021. Multiple complications in a dog infected with *Babesia gibsoni*. Vet Rec Case Rep, 9(3): e126, doi: 10.1002/vrc2.126
- Anish RK, Venu R, Rayulu VC, Jacob SS, Srilatha CH *et al.*, 2020. Prevalence and diversity of ixodid tick fauna in domestic animals of Andhra Pradesh state, India. J Entomol Zool Stud, 8: 2346-2351, doi: 10.22271/j.ento.2020.v8.i5af.7825
- Bhadesiya CM, Raval SK, Hasnani JJ and Neha R, 2016. Epidemiological aspects of tick infestation by *Otobius magnini* and *Dermacentor variabilis* in dogs of Anand, Gujarat. Environ Ecol, 34(2A): 636-639
- Bhowmik P, Islam S, De A, Tamuly S and Bouragohain L, 2024. Prevalence and molecular characterization of ticks infesting dogs in Agartala, Tripura. Indian J Anim Sci, 94(8): 669-673, doi: 10.56093/ijans.v94i8.151086
- Dantas-Torres F and Otranto D, 2015. Further thoughts on the taxonomy and vector role of *Rhipicephalus*

- sanguineus* group ticks. Vet Parasitol, 208: 9-13, doi: 10.1016/j.vetpar.2014.12.014
- Dantas-Torres F, Latrofa MS, Ramos RA, Lia RP, Capelli G *et al.*, 2018. Biological compatibility between two temperate lineages of brown dog ticks, *Rhipicephalus sanguineus* (sensu lato). Parasit Vectors, 11: 398-408, doi: 10.1186/s13071-018-2941-2
- Day MJ, 2016. Arthropod-Borne Infectious Diseases of the Dog and Cat. 2nd edn., CRC Press, Bristol UK, pp 1-14, doi: 10.1201/b19686
- Inokuma H, Okuda M, Ohno K, Shimoda K and Onishi T, 2002. Analysis of the 18S rRNA gene sequence of a Hepatozoon detected in two Japanese dogs. Vet Parasitol, 106(3): 265-271, doi: 10.1016/s0304-4017(02)00065-1
- Jena A, Baidya S, Pandit S, Jas R, Mandal SC *et al.*, 2021. Incidence of canine tick vectors and molecular detection of haemoparasites in vectors and hosts. Indian J Anim Res, 55(10): 1215-1223, doi: 10.18805/IJAR.B-4229
- Pasqualetti VB, Gandolfi AM, dos Santos Pessini PG and da Silva EB, 2021. Clinical and hematological changes in dogs with canine monocytic Ehrlichiosis attended in a private clinic in São Bernardo do Campo, São Paulo. J Vet Anim Res, 4: 101
- Peddineni SKS, Vijayalakshmi P, Abiramy Prabavathy A, Devadevi N, Rajkumar K *et al.*, 2023. Clinical, haemato-biochemical evaluation and comparison of PCR with conventional blood smear examination for the detection of *Babesia gibsoni* infection in dogs. Ind J Vet Sci Biotech, 19(5): 33-36, doi: 10.48165/ijvsbt.19.5.06
- Shitta KB, James-Rugu NN and Badaki JA, 2018. Prevalence of ticks on dogs in Jos, Plateau State, Nigeria. Bayero J Pure Appl Sci, 11(1): 451-454, doi: 10.4314/bajopas.v11i1.71S
- Sudhakara Reddy B, Sivajothi S, Varaprasad Reddy LS and Solmon Raju KG, 2016. Clinical and laboratory findings of Babesia infection in dogs. J Parasit Dis, 40: 268-272, doi: 10.1007/s12639-014-0491-x
- Taylor MA, Coop RL and Wall RL, 2016. Veterinary Parasitology, 4th edn., Black Well Oxford, United Kingdom, pp 340
- Vidhya MS, Chandrasekar M, Arunkumar S, Subapriya S and Sathish G, 2024. Clinico pathological evaluation of *Babesia gibsoni* associated renal failure in dogs. Indian J Vet Sci Biotechnol, 20(3): 77-81, doi: 10.48165/ijvsbt.20.3.15