RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2022.53.1.79-84

Molecular detection and control of non-regenerative anaemia associated with *Babesia gibsoni* and *Anaplasma platys* co-infection in a dog

Arun George[1*], Usha Narayana Pillai[2], S. Ajithkumar[3], T. V. Aravindakshan[4], K. K. Jayavardhanan[5], N. Madhavan Unny[6] and Bindu Lakshmanan[7]
Department of Clinical Medicine, Ethics and Jurisprudence College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651,
Kerala Veterinary and Animal Sciences University, Kerala, India.

Citation: Arun, G. Usha, N.P., Ajithkumar, S., Aravindakshan, T.V., Jayavardhanan, K.K., Madhavan, U. N. and Bindu, L. 2022. Molecular detection and control of non-regenerative anaemia associated with *Babesia gibsoni* and *Anaplasma platys* co-infection in a dog. *J. Vet. Anim. Sci.* **53**(1): 79-84.
DOI: https://doi.org/10.51966/jvas.2022.53.1.79-84

Received: 14.06.2021 *Accepted: 15.08.2021* *Published: 31.03.2022*

Abstract

Anaemia and thrombocytopenia are the common clinical features of blood parasite infection in dogs. Blood parasites previously identified in dogs in Kerala were Babesia gibsoni, Babesia canis vogeli, Ehrlichia canis and Trypanosoma evansi. A two-year old female Labrador Retriever was brought to the University Veterinary Hospital, Mannuthy, Thrissur, with a complaint of anorexia for the past two weeks. A thorough clinical examination did not give any evidence of involvement of other body systems, except for a splenomegaly which was confirmed by radiography. The dog was negative for microfilaria and intestinal parasites. Haematological findings revealed moderate anaemia and thrombocytopenia. Anaemia was classified as non-regenerative based on reticulocyte count. Blood smear was positive for basophilic intra-erythrocytic inclusions suggestive of small babesia and basophilic inclusions in platelets suggestive of morula of Anaplasma spp. Species-specific PCR on whole blood genomic DNA-showed specific amplification for B. gibsoni and A. platys organisms and negative for B. canis vogeli, E. canis and T. evansi. The present study reports a case of non-regenerative anaemia due to B. gibsoni and A. platys co-infection in a dog and its successful therapeutic management. This is the second report of A. platys infection in dogs in Kerala, India.

Keywords: *Anaplasma platys, Babesia gibsoni, non-regenerative anaemia, basophilic platelet inclusions*

1. *Assistant Professor and Ph.D. Scholar*
2. *Professor and Head*
3. *Professor and Head, Department of Clinical Medicine, Ethics & Jurisprudence, CVAS, Pookode, and Director of Clinics, KVASU*
4. *Director, Centre for Advanced Studies in Animal Genetics and Breeding, CVAS, Mannuthy*
5. *Professor and Head, Department of Veterinary Biochemistry*
6. *Associate Professor*
7. *Associate Professor and Head, Department of Veterinary Parasitology*

**Corresponding author: arun@kvasu.ac.in*

Natural tick-borne blood parasite infections are often reported in dogs all over the world. The common blood parasites identified in dogs in the state of Kerala in India were *B. gibsoni*, *B. canis vogeli*, *E. canis* and rarely *T. evansi* (Vishnurahav, 2014, Augustine *et al*. 2017, Jain *et al*., 2018, and Vismaya *et al*., 2020). Kavitha *et al*. (2020) first reported *A. platys* infection in dogs in Kerala by molecular methods though there were earlier reports in other states of India (Abd Rani *et al*., 2011, Manoj *et al*., 2020) as well as other countries (Arraga-Alvarado *et al*., 2003).

Natural blood parasite infections occur either as mono-infection or co-infection. In a pan-India study in dogs, Abd Rani *et al*. (2011) reported co-infections with two or more blood parasites such as *B. canis vogeli*, *E. canis*, *Hepatozoon canis*, *A. platys* and *Mycoplasma haemocanis*. Jain *et al.* (2018) reported co-infections with *B. gibsoni* and *B. canis vogeli*, *B. gibsoni* and *E. canis*, and *B. canis vogeli* and *E. canis* in dogs in Kerala.

Anaemia associated with blood parasites were often regarded as regenerative anaemia. However, chronic infections produce non-regenerative anaemia due to suppression of bone marrow (Couto, 2014).

The present study reports a case of non-regenerative anaemia due to concurrent *B. gibsoni* and *A. platys* infection in a dog, its molecular confirmation and successful therapeutic management.

Materials and methods

A two-year-old, female Labrador Retriever weighing 32 kilograms was brought to the University Veterinary Hospital, Mannuthy, Kerala, India, with the complaint of decreased food intake, yet normal water consumption, for the last two weeks. Administration of a dewormer two-weeks back did not bring any relief. Anti-rabies (Inj. Raksharab, Indian Immunologicals, Limited) and multi-component (Inj. Nobivac DHPPi, Intervet) vaccinations were regular. Defecation and urination were reported to be normal. Temperature, mucous membrane and capillary refill time were within normal limits. A detailed examination of the patient and a faecal microscopical evaluation was carried out. Wet blood film and blood smear were prepared by puncture of ear margin using a sterile lancet. Blood from cephalic vein was collected in a K-EDTA tube for haematology, reticulocyte count, and molecular diagnosis and in a clot-activator tube for serum biochemical analysis. PCR was carried out on whole blood genomic DNA using *B. gibsoni*-specific primer sets, BAGI-F and BAGI-R targeting 18S rRNA as per the protocol of Jain *et al*. (2018). *A. platys* specific PCR was done using primers targeting a partial segment of 16S rRNA as per Abd Rani *et al*. (2011). The occurrence of other blood parasites was also probed using species-specific PCR protocols targeting 18S rRNA gene for *B. canis vogeli* (Duarte *et al*., 2008), 16S rRNA gene for *E. canis* (Gal *et al*., 2008) and a repetitive nuclear sequence of *T. evansi* (Wuyts *et al*., 1994). The primer sequences are provided in Table 1.

Table 1. Primer sequences of *B. gibsoni*, *A. platys*, *B. canis vogeli*, *E. canis* and *T. evansi*

Species	Primer	Sequences	Expected product size
B. gibsoni	Forward	BAGI F-5'-TTGGCGGCGTTTATTAGTTC-3'	468 bp
	Reverse	BAGI R-5'-AAAGGGGAAAACCCCAAAAG-3'	
A. platys	Forward	PLATYS: 5'-GATTTTTGTCGTAGCTTGCTATG-3'	238 bp
	Reverse	EHR16SR 5'TAGCACTCATCGTTTACAGC-3'	
B. canis vogeli	Forward	BAB1 F-5'- GTG AAC CTT ATC ACT TAA AGG- 3'	584 bp
	Reverse	BAB4 R-5' CAACTCCTCCACGCAATCG- 3'	
E. canis	Forward	ECA F-5'-AACACATGCAAGTCGAACGGA-3'	390 bp
	Reverse	HE3R-5'-TATAGGTACCGTCATTATCTTCCCTAT-3'	
T. evansi	Forward	TRYP-F 5'- TGCAGACGACCTGACGCTACT-3'	229 bp
	Reverse	TRYP-R 5'- CTCCTAGAAGCTTCGGTGTCCT-3'	

Results and discussion

On physical examination all the body systems were normal except for a suspected splenomegaly which was confirmed by radiography.

Wet blood film and faecal sample were negative for parasites or parasitic stages. Leucogram was normal but haemogram revealed moderate anaemia (Hb: 8.5 g/dL, reference interval (RI): 12-18; PCV: 24.5 %; RI: 37-55; RBC: 4.10 x 10^6 cells/µL, RI: 5.5 – 8.5 x 10^6; MCV: 59.8 fL, RI: 60 -77) and thrombocytopenia (83 x 10^3/µL, RI: 200 – 500 x 10^3). Stained peripheral blood smear revealed large platelets (Fig. 1). The absolute reticulocyte count calculated manually as per Briggs and Bain (2017) was significantly low (46,617 reticulocytes/µl, normal > 60,000/µL). A reticulocyte count of less than 60,000/µL in an anaemic dog is regarded as non-regenerative anaemia (Thrall, 2012). Serum biochemical parameters such as ALT, total protein, albumin, BUN, and creatinine were within normal range.

Peripheral blood smear examination revealed the presence of organisms and/ or inclusions within erythrocytes as well as thrombocytes. The morphology of intra-erythrocytic basophilic inclusion was suggestive of small *Babesia* spp. (Fig. 2), whereas the basophilic inclusion within the platelets (Fig. 3) was suggestive of morula of *Anaplasma* spp. PCR on whole-blood genomic DNA confirmed the presence of *B. gibsoni* by yielding a 468 bp amplicon (Fig. 4) and *A. platys* by amplifying a 238 bp (Fig. 5) sequence.

The occurrence of other blood parasites such as *B. canis vogeli*, *E. canis* and *T. evansi* were ruled by species-specific PCR.

The case was diagnosed as non-regenerative anaemia due to *B. gibsoni* and *A. platys* co-infection based on clinical features, haematology, low reticulocyte count and molecular tests. This is the second report on *A. platys* infection in dogs in Kerala.

The dog responded to parenteral administration of clindamycin @ 11 mg/kg IV, OD, for three days with an increase in food intake. The thrombocyte count increased from 83 x 10^3/ µl to 150 x 10^3/ µl though other haematological parameters remain unchanged. Supportive therapy included fluids, electrolytes, and B-complex vitamins. Further, treatment was continued *per os* with Tab. Clindamycin @ 11 mg/kg PO, OD, Tab. Doxycycline @ 10 mg/ kg PO OD, and Tab. Pantoprazole @ 0.5 mg/kg PO, OD for fourteen days.

In this case of *B. gibsoni* and *A. platys* co-infection clinical manifestations were less apparent. The only notable finding was a gradual decrease in food intake over a period of two-weeks and was otherwise active. Clinical signs of *B. gibsoni* infection vary greatly between individuals, but the common findings were lethargy, inappetence, pyrexia, pale mucous membrane, and splenomegaly (Solano-Gallego *et al*., 2016). Signs of ill-health in *A. platys* infection were pyrexia in mild cases and bleeding episodes of petechiae, ecchymoses, epistaxis or haematochezia in severe cases. Some of the infected dogs remained asymptomatic (Harrus *et al*., 2012).

The major haematological alterations observed were anaemia, thrombocytopaenia, a low reticulocyte count and the presence of abnormally large platelets of the size of red cells on stained blood smear. Anaemia and thrombocytopenia were similar to the observations of Harrus *et al*. (2012). The presence of large thrombocytes in blood smear, as reported by Beaufils *et al*. (2002) in *A. platys* infection, was a notable thrombocyte morphological variation. Anaemia due to blood parasitic infection was generally regarded as regenerative (Couto, 2014). Anaemia with bone marrow regeneration in *B. gibsoni* infection has been reported by Solango-Gallego (2016). Shah *et al*. (2011) observed two per cent reticulocytosis in natural *B. gibsoni* infection in dogs. Reports on regenerative status of *A. platys* infection based on reticulocyte count could not be found on a review of related literature. The non-regenerative anaemia observed in this study can be attributed to anaemia of chronic inflammation (Couto, 2014) due to blood parasites.

The *B. gibsoni* and *A. platys* co-infected dog responded to clindamycin, and

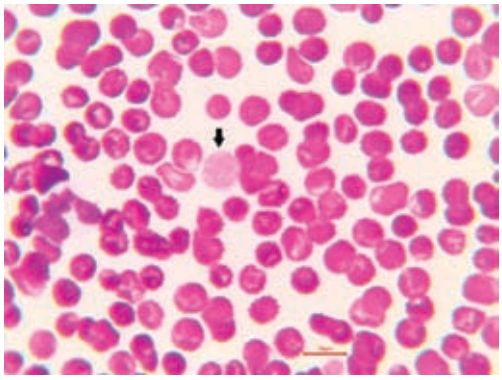

Fig. 1. A platelet (black arrow) larger than the size of an average erythrocyte in peripheral blood smear (Diff Quik stain; 100 X objective).

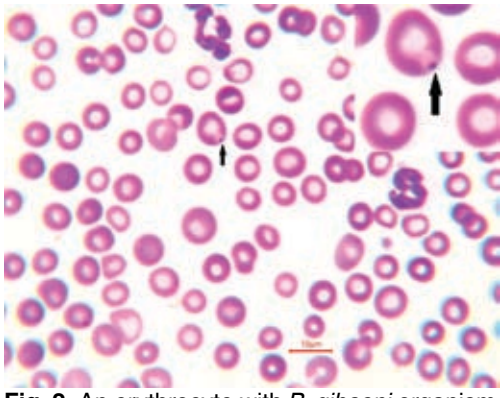

Fig. 2. An erythrocyte with *B. gibsoni* organism (black arrow) in peripheral blood smear (Diff Quik stain; 100 X objective).

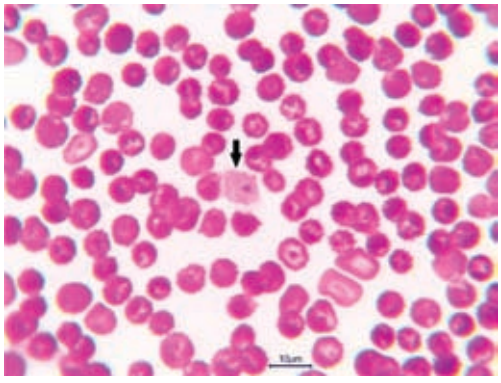

Fig. 3. Platelet (black arrow) with basophilic *Anaplasma platys* morula in peripheral blood smear (Diff Quik stain; 100 X objective).

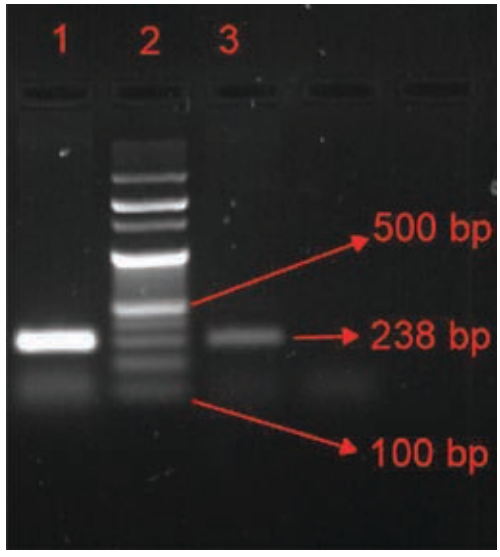

Fig. 5. PCR gel image of *Anaplasma platys* in 1.2 % agarose; Lane 1: Sample, Lane 2: Ladder (Himedia MBT049, 100bp), Lane 3: Positive Control.

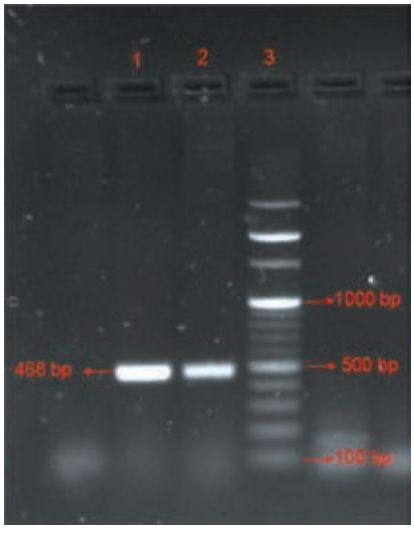

Fig. 4. PCR gel image of *Babesia gibsoni* in 1.2 % agarose; Lane 1: Sample, Lane 2: Positive Control, Lane 3: Ladder (Himedia MBT049, 100bp).

doxycycline along with supportive therapy. The different chemotherapeutic agents used to control *B. gibsoni* infection included imidocarb, clindamycin, doxycycline, and atovaquone, as a monotherapy or as combination therapy. *A. platys* was often treated with oxytetracycline, doxycycline and enrofloxacin (Harrus *et al*., 2012).

A follow up of the case after a period of fourteen days confirmed a complete clinical recovery. A further tele-review one-year-and-a-half later, found the animal to be healthy and active without any history of relapse. Meanwhile,

during this period, the dog conceived once, whelped, and had healthy litters.

Conclusion

A case of non-regenerative anaemia due to *B. gibsoni* and *A. platys* co-infection, confirmed by PCR, was reported in a dog in Thrissur district of Kerala in India. Chronic inappetence was the only notable clinical sign whereas other important findings were splenomegaly, moderate anaemia, thrombocytopenia, and a low reticulocyte count. Intraerythrocytic and intraplatelet basophilic inclusions seen on stained peripheral blood smear were confirmed as *B. gibsoni* and *A. platys* organisms by PCR test. The dog was negative for microfilaria on wet film examination and for *B. canis vogeli*, *E. canis* and *T. evansi* by PCR. Therapeutic management with clindamycin, doxycycline and supportive drugs cured the infection. Relapse of infection was not reported on a review after one-and-a-half years.

Acknowledgement

The authors gratefully acknowledge the support of Kerala Veterinary and Animal Sciences University, Kerala, India for providing the research infrastructure.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

References

Abd Rani, P.A.M., Irwin, P.J., Coleman, G.T., Gatne, M. and Traub, R.J. 2011. A survey of canine tick-borne diseases in India. *Parasit. Vectors*. **4**: 141-148.

Arraga-Alvarado, C., Palmar, M., Parra, O. and Salas, P. 2003. *Ehrlichia platys* (*Anaplasma platys*) in dogs from Maracaibo, Venezuela: an ultrastructural study of experimental and natural infections. *Vet. Pathol.* **40**: 149-156.

Augustine, S., Sabu, L. and Lakshmanan, B. 2017. Molecular identification of *Babesia* spp. in naturally infected dogs of Kerala, South India. *J. Parasit. Dis.* **41**: 459-462.

Beaufils, J.P., Inokuma, H., Martin-Granel, J., Jumelle, Ph., Barbault-Jumelle, M. and Brouqui, P. 2002. *Anaplasma platys* (*Ehrlichia platys*) infection in a dog in France: description of the case, and characterization of the agent. *Revue Méd. Vét.* **153**: 85-90.

Briggs, C., and Bain, B.J. 2017. Basic haematological techniques. In: Bain, B.J., Bates, I., Laffan, M.A. and Lewis, S.M. (eds.), *Dacie and Lewis Practical Haematology*, (12th Ed.) Elsevier Limited, pp.18-49.

Couto, C.G. 2014. Anaemia. In: Nelson, R.W. and Couto, C.G. (eds.), *Small Animal Internal Medicine*, (5th Ed.). Mosby Elsevier, Missourie, pp.1201-1224.

Duarte, S.C., Linhares, G.F.C., Romanowsky, T.N., da Silveira Neto, O.J. and Borges, L.M.F. 2008. Assessment of primers designed for the subspecies-specific discrimination among *Babesia canis*, *Babesia canis vogeli* and *Babesia canis rossi* by PCR assay. *Vet. Parasitol.* **152**: 16-20.

Gal, A., Loeb, E., Yisaschar-Mekuzas, Y. and Baneth, G. 2008. Detection of *Ehrlichia canis* by PCR in different tissues obtained during necropsy from dogs surveyed for naturally occurring canine monocytic ehrlichiosis. *The Vet. J*. 175: 212-217.

Harrus, H., Waner, T. and Neer, T.M., Cocayne, C.G., Cohn, L.A, Diniz, P.P., Breitschwerdt, E.B., Lappin, M.R. and Harvey, J.W. 2012. *Ehrlichia* and *Anaplasma* infections. In: Greene, C.E. (eds.), *Infectious diseases of the dog and cat*, (4th Ed.). Elsevier-Saunders, Missourie. pp.227-259.

Jain, J., Bindhu, L., Hitaishi, V.N. Jose, E.P., Syamala, K. and Aravindakshan, T.V. 2018. Detection of *Babesia canis vogeli*, *Babesia gibsoni* and *Ehrlichia canis* by

multiplex PCR in naturally infected dogs in South India. *Vet. Arch.*, **88**: 215-224.

Kavitha, S., Panicker, V.P., Vismaya, K.K., Athira Narayanan, A.K.S. and Lakshmanan, B. 2020. Molecular identification and phylogenetic analysis of *Anaplasma platys* from naturally infected dogs of South India. *J. Entomol. Zool. Stud.* **8**: 15-19.

Manoj, R.R.S., Iatta, R., Latrofa, M.S., Capozzi, L., Raman, M., Colella, V. and Otranto, D. 2020. Canine vector-borne pathogens from dogs and ticks from Tamil Nadu, India. *Acta Trop.* **203**: 1-11.

Shah, S.A., Sood, N.K. and Tumati, S.R. 2011. Haemato-biochemical changes in natural cases of canine babesiosis. *Asian J. Anim. Sci*. 5: 385-392.

Solano-Gallego, L., Sainz, Á., Roura, X., Estrada-Peña, A. and Miró, G. 2016. A review of canine babesiosis: the European perspective. *Parasit. Vectors.* **9**: 336-354.

Thrall, M.A. 2012. Classification of and diagnostic approach to anaemia. In: Thrall, M.A., Weiser, G., Allison, R.W. and Campbell, T.W. (eds.), *Veterinary Hematology and Clinical Chemistry*, (2nd Ed.), Wiley-Blackwell publishers, pp: 75-80.

Vishnurahav, R. B. 2014. Clinico-therapeutic Studies on *Babesia gibsoni* Infection in Dogs. *M.V.Sc. thesis*, Kerala Veterinary and Animal Sciences University, Pookode, 93p.

Vismaya, K.K., Varuna, P.P., Kumar, S.A., Narayanan, A., Kavitha, S. and Lakshmanan, B. 2020. Clinico-haematological evaluation and molecular identification of *Babesia gibsoni*, *Babesia canis vogeli*, *Ehrlichia canis* and *Trypanosoma evansi* in dogs. *J. Vet. Parasitol.* **34**: 17-26.

Wuyts, N., Chokesajjawatee, N. and Panyim, S. 1994. A simplified and highly sensitive detection of *Trypanosoma evansi* by DNA amplification. *Southeast Asian J. Trop. Med. Public Health*, **25**: 266-266. ■