
NON-REGENERATIVE ANAEMIA AND THROMBOCYTOPENIA IN A CASE OF *TRYPANOSOMA EVANSI* - *BABESIA GIBSONI* CO- INFECTION IN A CLIENT-OWNED DOG

Arun George*, Usha N. P., Ajith Kumar S., Aravindakshan T.V., Jayavardhanan
K.K., Madhavan Unny N. and Bindu Lakshmanan

*Department of Clinical Medicine, Ethics and Jurisprudence,
College of Veterinary and Animal Sciences (KVASU), Thrissur, Kerala – 680651*

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

ABSTRACT

Anaemia and thrombocytopenia are common in blood parasite infection in dogs. The diagnostic investigation of non-regenerative anaemia (NRA) in canine practice is more challenging than regenerative anaemia. A 4-year-old, intact non-descript male dog weighing 20 kg was presented to the clinics at Thrissur, Kerala with the complaint of continuous weight loss and inappetence for the past few weeks. Major findings were splenomegaly, non-regenerative anaemia and thrombocytopenia. The dog was positive for *Trypanosoma evansi* and *Babesia gibsoni* by species-specific PCR. The dog responded to treatment with diminazene aceturate, quinapyramine chloride/ sulphate and doxycycline. This article reports non-regenerative anaemia and thrombocytopenia in a *T. evansi* –

B. gibsoni natural mixed infection in a dog and its successful management. This article reinforces the importance of PCR-based tests in the diagnostic investigation of non-regenerative anaemia and thrombocytopenia.

Keywords: *Trypanosoma evansi*, *Babesia gibsoni*, non-regenerative anaemia, dog

INTRODUCTION

Diagnostic investigation of non-regenerative anaemia (NRA) in canine practice is more challenging than regenerative anaemia. The establishment of NRA on the basis of mean corpuscular volume or based on the presence of polychromatophilic erythrocytes in a stained peripheral blood smear have given inconsistent results. To obtain a true reflection of erythroid regeneration in the bone marrow, an assessment of absolute

reticulocyte count in the peripheral blood by automated haematology analyser or manual counting, or bone marrow evaluation is required. Of these techniques, absolute reticulocyte count provides results consistent with a bone marrow evaluation and is less cumbersome (Thrall, 2012).

Factors for the development of NRA in dogs are many. Chronic inflammatory process is an important factor whereas other factors include iron deficiency anaemia and anaemia of chronic kidney disease (Couto, 2014). Blood parasites are notorious for bringing about chronic inflammatory reactions owing to their long incubation period, prolonged presence in the body, and an evolutionary preference to infect blood cells or inhabit bone marrow. Though there is a greater inclination to consider infections associated with blood parasites as regenerative, this is not always true.

Thrombocytopenia resulted from either decreased production or increased consumption of platelets or a combination of both. Thrombocytopenia associated with anaemia has been noticed in chronic blood parasitic infections (Russell, 2010)

The common blood parasites reported from the state of Kerala in India were *Babesia gibsoni* (Vishnurahav, 2014), *Babesia canis* (Augustine, 2017) and *Ehrlichia canis* (Jain et al., 2018), and

the less common haemoparasites were *Trypanosoma evansi* (Vismaya et al., 2020), *Anaplasma platys* (Kavitha et al., 2020), and *Hepatozoon canis* (Lakshmanan et al., 2018).

This article reports a case of non-regenerative anaemia and thrombocytopenia due to *T. evansi* and *B. gibsoni* co-infection in a non-descript client-owned dog and its therapeutic management.

CASE HISTORY AND OBSERVATIONS

A four-year-old, intact non-descript male dog weighing 20 kg was presented to the clinics at Thrissur, Kerala with the complaint of continuous weight loss and inappetence for the past few weeks. The dog was neither vaccinated nor dewormed. The dog was dull and lethargic. The vital parameters were sub-normal body temperature (100.5 °F), normal pulse rate (78 bpm), a panting respiration and a pale-moist conjunctival mucous membrane. Splenomegaly was noticed on abdominal palpation and confirmed by radiography. No abnormality could be detected on examination of other body systems.

Wet film, blood smear and buffy coat smear examination were positive for the presence of trypanosoma organisms. Haematology revealed severe anaemia (HCT: 9.4%) and thrombocytopenia ($33 \times 10^3/\mu\text{l}$). The absolute reticulocyte count

(Briggs and Bain, 2017) was 37,125/ μ l (Reference interval, RI: >60,000/ μ l). The trypanosoma organisms in the blood were confirmed as *Trypanosoma evansi* by species-specific PCR (Table 1) on whole blood genomic DNA.

An initial diagnosis of *T. evansi* infection was made and treated with a combination of Inj. Berenil (diminazene aceturate) @ 3.5 mg/kg deep IM on day-1 and Inj. Triquin 2.5 g (quinapyramine sulphate, 1.5 g and quinapyramine chloride, 1.0) @ 5 mg/kg SC on day-3. Supportive therapy and haematinics were supplemented. On a review two weeks later, the dog was found alert and active with moderate food intake and a marginal improvement in haematocrit (HCT: 14.1

%) and thrombocyte count ($127 \times 10^3/\mu$ l). A repeat wet film, blood smear and buffy coat smear examination was negative for blood parasites and hence other blood parasites prevalent in this area were looked for by PCR on whole-blood genomic DNA obtained earlier. The *T. evansi* positive dog was found co-infected with *Babesia gibsoni* by species-specific PCR and negative for *Babesia canis vogeli* and *Ehrlichia canis* by PCR (Table 1). Advised doxycycline @ 10 mg/kg for two weeks along with haematinics and nutritional supplements. The dog was not presented for re-examination two-weeks later, but on a telephonic review, the dog was reported to be clinically alert and active with normal appetite and better performance.

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

Organism	Primer	Sequences	Expected product size
<i>B. gibsoni</i> (Jain et al., 2018)	Forward	BAGI F-5'-TTG GCG GCG TTT ATT AGT TC-3'	468 bp
	Reverse	BAGI R-5'-AAA GGG GAA AAC CCC AAA AG-3'	
<i>B. canis vogeli</i> (Duarte et al., 2008)	Forward	BAB1 F-5' -GTG AAC CTT ATC ACT TAA AGG- 3'	584 bp
	Reverse	BAB4 R-5' -CAA CTC CTC CAC GCA ATC G- 3'	
<i>E. canis</i> (Gal et al., 2008)	Forward	ECA F-5'-AAC ACA TGC AAG TCG AAC GGA-3'	390 bp
	Reverse	HE3 R-5'-TAT AGG TAC CGT CAT TAT CTT CCC TAT-3'	
<i>T. evansi</i> (Wuyts et al., 1994)	Forward	TRYP-F 5' – TGC AGA CGA CCT GAC GCT ACT - 3'	229 bp
	Reverse	TRYP-R 5' – CTC CTA GAA GCT TCG GTG TCC T - 3'	

TREATMENT AND DISCUSSION

The case was confirmed as *T. evansi* and *B. gibsoni* co-infection. The primary focus of therapeutic management of blood parasites is its elimination from the host, and if elimination cannot be achieved, attention is given to reduce parasitaemia so that the host can maintain a near normal healthy life. Drugs commonly used for the management of *T. evansi* infection in dogs were diminazene aceturate @ 3.5 mg/kg IM, single dose and quinapyramine chloride/ sulphate @ 4 mg/kg SC, single dose (Singh *et al.*, 1993). A second dose was required in some instances. In the present case both diminazene aceturate and quinapyramine chloride/ sulphate were administered. Response to therapy was assessed two weeks later, based on a negative peripheral blood smear, and an increase in haematocrit percentage and thrombocyte count.

B. gibsoni caused moderate to severe anaemia and thrombocytopenia even with low parasitaemia and hence a negative peripheral blood smear and a positive species-specific PCR was not surprising. The routinely used choice of drugs for *B. gibsoni* included imidocarb, doxycycline, clindamycin, metronidazole or a combination of these (Birkenheuer,

2012). In this study the dog responded to doxycycline @ 10 mg/kg body weight for 10 days by a normal food intake and an improvement in general activity.

Persistent anaemia and thrombocytopenia are common in blood parasite infection in dogs (Russell, 2010). However, anaemia is generally regarded as regenerative in blood parasite infections (Couto, 2014). The non-regenerative anaemia recorded in this study can be attributed to suppression of bone marrow by *T. evansi* as well as *B. gibsoni*.

CONCLUSION

Non-regenerative anaemia and thrombocytopenia was documented in a case of *Trypanosoma evansi* and *Babesia gibsoni* mixed-infection in a non-descript, client-owned dog. A partial response to therapy directed against *T. evansi*, and the persistence of anaemia and thrombocytopenia raised suspicion of mixed blood parasite infection. Co-infection with *Babesia gibsoni* was confirmed by species-specific PCR.

ACKNOWLEDGEMENT

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

REFERENCES

- 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.
- Birkenheuer, A.J. 2012. Babesiosis. In: Greene, C.E. (eds.), *Infectious Diseases of the Dog and Cat*, (4th Ed.). Elsevier-Saunders, Missouri. pp.771-784.
- 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, Missouri, 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.
- Jain, J., Bindhu, L., Hitaishi, V.N. Jose, E.P., Syamala, K. and Aravindakshan, T. 2018. Detection of *Babesia canis vogeli*, *Babesia gibsoni* and *Ehrlichia canis* by multiplex PCR in naturally infected dogs in South India. *Vet. Arh.*, **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.
- Lakshmanan, B., Jose, K.J., George, A., Usha, N.P. and Devada, K., 2018. Molecular detection of Hepatozoon canis in dogs from Kerala. *J. Parasit. Dis.* **42**: 287-290.
- Russell, K.E. 2010. Platelet kinetics and laboratory evaluation of thrombocytoenia. In: Weiss, D.J. and Wardrop, K.J. (eds.). *Schalm's Veterinary Haematology*. (6th Ed.).

- Blackwell Publishing Ltd, USA, pp.576–585.
- Singh, B., Kalra, I.S., Gupta, M.P. and Nauriyal, D.C. 1993. Trypanosoma evansi infection in dogs: seasonal prevalence and chemotherapy. *Vet. Parasitol.* 50: 137-141.
- 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.

