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Occurrence, clinical and haemato-biochemical profiling of *Anaplasma platys* infection in naturally infected dogs#

Gowri Venugopal[1], P.V. Tresamol[1*], K. Vinodkumar[1], V.H Shyma[1], P.M Priya[2] and Y. Ajith[3]

[1]Department of Veterinary Epidemiology and Preventive Medicine, [2] Department of Veterinary Microbiology, [3] Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, Pookode, Kerala.

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Abstract

Anaplasma platys, the causative agent of infectious cyclic thrombocytopaenia in dogs, is an obligate intracellular bacterium transmitted primarily by Rhipicephalus sanguineus ticks. The organism invades circulating platelets, forming intracytoplasmic morulae and inducing a cyclic pattern of thrombocytopaenia. The present study was conducted to assess the occurrence and to evaluate the clinical presentations and haemato-biochemical alterations in dogs naturally infected with A. platys. Twelve dogs presented to the University Veterinary Hospital, Kokkalai and Teaching Veterinary Clinical Complex, Mannuthy, positive for A. platys infection were selected for this study. The more frequent clinical signs observed in the affected dogs included anorexia, lethargy, pyrexia and lymphadenopathy. Epistaxis, icterus and seizures were also observed infrequently. Tick infestation was reported in all the affected dogs. Among the positive cases, more numbers were males and of the age three years or more. Majority of dogs were housed outdoors in cages or kennels and more number of cases was observed in summer months. The major haematological abnormalities observed in infected dogs included normocytic normochromic anaemia and marked thrombocytopaenia. Among the biochemical changes, significant hyperglobulinemia was noticed when compared to the control group. The study indicated the presence of A.platys infection among the dogs in this region. It also highlighted the usefulness of clinical and haemato-biochemical profiling in the diagnosis and clinical management of A. platys infections, enabling timely intervention and improved prognosis in the affected dogs.

Keywords: Canine, *Anaplasma platys*, thrombocytopaenia, anaemia

Infectious cyclic thrombocytopenia is a canine tick-borne disease caused by *Anaplasma platys*, an obligate intracellular bacterium that targets circulating platelets and forms distinct morulae within them (Harvey *et al.,* 1978). The infection is primarily transmitted by *Rhipicephalus sanguineus* and the molecular detection of *A. platys* DNA in semi-engorged ticks has suggested its potential vector role (Inokuma *et al*., 2000). The disease typically follows a cyclic pattern of thrombocytopaenia, initially due to direct destruction of platelets by the pathogen and later influenced by immune-mediated mechanisms (Dyachenko *et al*., 2012).

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**Corresponding author: tresamol@kvasu.ac.in , Ph. 9947659891*

The clinical course of *A. platys* infection in dogs may range from subclinical to overt bleeding manifestations such as epistaxis, petechiae and ecchymosis (Gaunt *et al*., 2010). Common haematological abnormalities included thrombocytopaenia, mild to moderate non-regenerative normocytic normochromic anaemia, hyperglobulinaemia and hypoalbuminaemia (Sainz *et al*., 2015). Limited research has been conducted on the occurrence, haematological and biochemical profile and risk factor analysis of *A. platys* in dogs from Kerala. Therefore, the present investigation was designed to assess the occurrence of *A. platys* among dogs in and around Thrissur district of Kerala and to evaluate the haematological and biochemical profiles, as well as various risk factors associated with *A. platys* in naturally infected dogs.

Materials and methods

The current study was conducted on dogs brought to the University Veterinary Hospital (UVH), Kokkalai and the Teaching Veterinary Clinical Complex (TVCC), Mannuthy. Dogs with a history of tick infestation and symptoms suggestive of haemoparasitic infections such as fever, inappetance, lymphadenopathy, epistaxis, pale mucous membranes, cutaneous petechiae or ecchymoses were considered. A total of 120 dogs were screened for the presence of haemoprotozoan infections, including *A. platys*, *Ehrlichia canis*, *Hepatozoon canis, Babesia vogeli* and *Babesia gibsoni*. Out of these, *A. platys* infection was confirmed in seven and 12 dogs by blood smear and PCR examinations, respectively and were subsequently included in the present study.

The clinical parameters included detailed signalment and host parameters such as breed, age, sex, housing and diet. Vital parameters, including rectal temperature, pulse rate and respiration rate were recorded. Superficial lymph nodes were palpated and the findings were documented. Additionally, a control group comprising ten apparently healthy dogs was included to establish the normal haematological and biochemical reference values.

Two millilitres of blood were aseptically collected from the medial cephalic or saphenous vein of each dog and transferred into EDTA-coated tubes (Hi Media). Haematological evaluation was carried out using a five-part automated analyser (ORPHEE Mythic 5Vet PRO), which measured haemoglobin (g/dL), volume of packed red cells (VPRC) (%), total erythrocyte count (10^6/µL), total leukocyte counts (10^3/ µL), differential leucocyte count (%), mean corpuscular volume (MCV) (fL), mean corpuscular haemoglobin (MCH) (pg) and platelet count (10^5/µL). All procedures followed the standard protocol described by Feldman *et al.* (2000).

Two millilitres of blood were aseptically drawn from the medial cephalic or saphenous vein of each animal and transferred into clot-activator vials (HiMedia, Mumbai). The samples were allowed to clot and serum was subsequently separated and stored in sterile microcentrifuge tubes at –20°C until analysis. Biochemical parameters including serum creatinine (mg/dL), alkaline phosphatase (ALP) (IU/L), alanine aminotransferase (ALT) (IU/L), total protein, albumin (g/dL) and total and direct bilirubin (mg/dL) were measured using commercially available kits (Alpha Technologies). All assays were carried out using a semi-automated biochemical analyser (RMS Analytica 705i / 3000 Evolution Semi-automated clinical chemistry analyzer).

The haematological and biochemical parameters of *A. platys*-positive dogs (n=12) were statistically compared with those of a healthy control group (n=10) using an independent t-test performed in IBM-SPSS software (version 24).

Results and discussion

Out of the 120 dogs screened, seven were positive on blood smear examination for *A. platys* morulae in the platelets (Fig. 1) and 12 were positive on species specific PCR for the same (Fig. 2).

The clinical profile of dogs diagnosed with *A. platys* is given in Fig. 3. The most frequently seen clinical signs were lymphadenopathy (83.33%), anorexia (75%), pyrexia (66.66%) and lethargy (58.33%). Splenomegaly was frequently noted in 11 dogs (91.66%) in the present study. Epistaxis was observed in two dogs (16.66 %). Icterus and seizure were observed in a single case each (8.33%). It was observed that ticks were present on all dogs infected with *A. platys*.

Bouzzoura *et al*. (2016) also reported anorexia and splenomegaly as predominant signs in 32 dogs

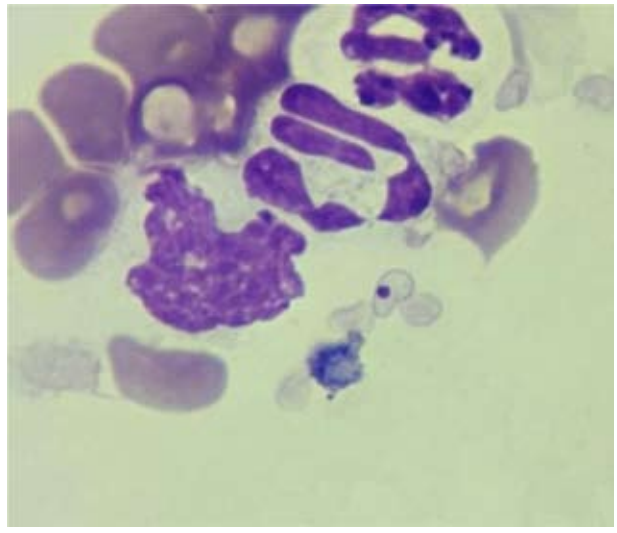

Fig. 1. Distinct blue-coloured basophilic morulae of ***A. platys*** observed within the platelets of infected dogs under oil immersion objective(100x) of microscope using field stain

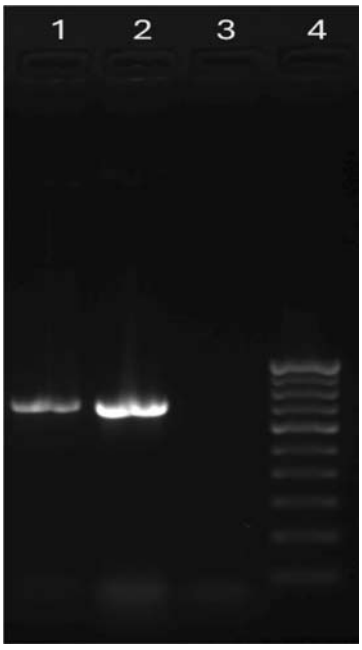

Fig. 2. Species specific PCR for *A. platys*- Lane 1: Sample, Lane 2: Positive control, Lane 3: Negative control, Lane 4: Ladder

infected with *A. platys* in the Mediterranean basin. Lethargy observed in *A. platys* infected dogs aligns with the weakness and apathy as described by Dantas-Torres and Otranto (2016). The epistaxis observed in two dogs corroborate the haemorrhagic manifestations as reported by Selim *et al*. (2021) in *A. platys* positive cases in a study conducted in Egypt. Marked lymphadenopathy was reported as a consistent finding by Rar *et al*. (2021) in previously reported *A. platys* cases. Neurological disorders in *A. platys* infections were noted by Ahmed *et al*. (2021).

The distribution of *A. platys* positive cases with respect to risk factors such as age, breed, sex, housing, seasonality etc. is given in Table 1. The infected animals were predominantly adults and geriatric dogs over 3 years of age, with ages ranging up to 15 years. This aligns with the findings of Selim *et al*. (2021), who noted increased susceptibility in older dogs, likely due to greater cumulative tick exposure. Most of the affected dogs were non-descript or crossbred, a pattern supported by Pesapane *et al*. (2019), who associated lower infection rates in purebreds with their indoor housing and reduced ectoparasite exposure. Majority of the dogs affected were males, consistent with findings by De La Fuente *et al*. (2006) and Jerez-Sulvaran *et al*. (2024), who reported higher infection rates in males, possibly due to greater outdoor exposure, particularly among hunting dogs. The majority of cases were recorded during summer months, corresponding with the seasonal tick activity peak reported by Chao *et al*. (2024) in brown dog ticks. Notably, most infected dogs in the current study were kept in kennels or cages rather than indoors, echoing the observations of De La Fuente *et al*. (2006), who attributed higher infection prevalence in pound or outdoor dogs due to increased tick contact.

The key haematological changes observed in this study were anaemia and thrombocytopaenia, as evidenced by significantly reduced values of red blood cell

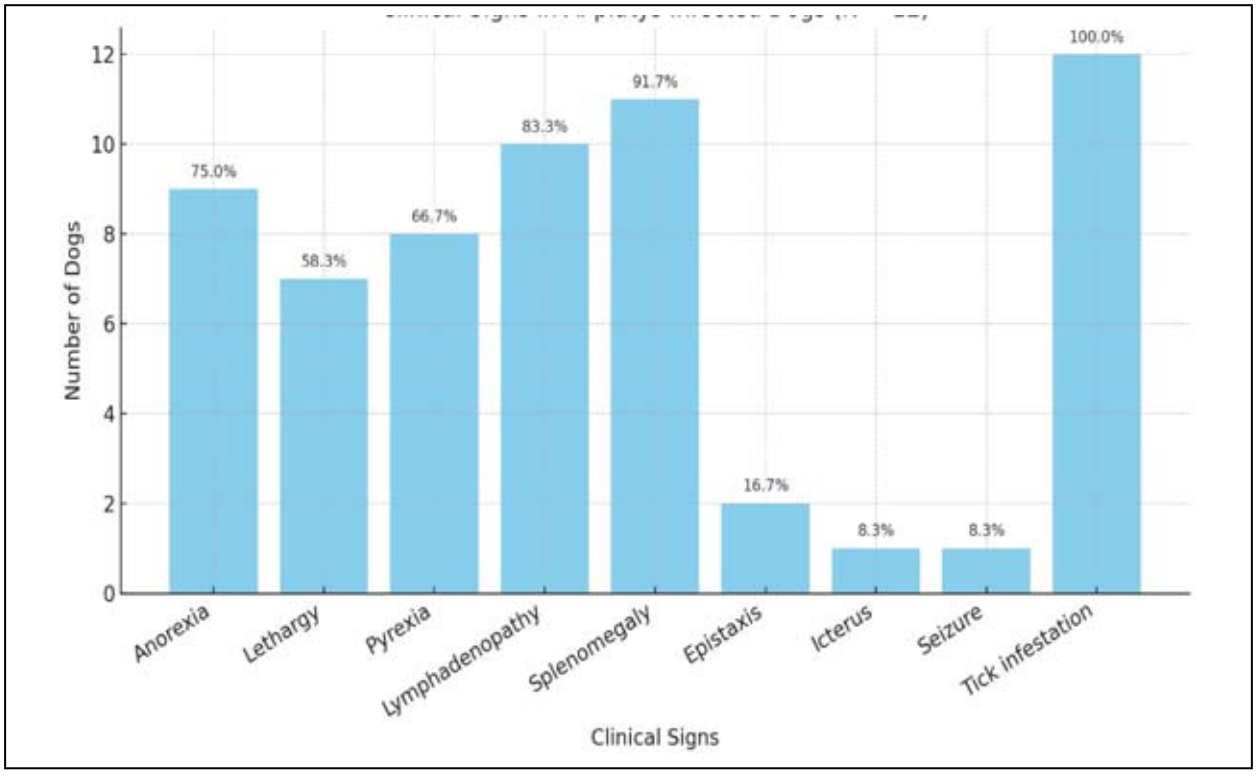

Fig. 3. Clinical signs depicted by animals infected with *A. platys* (n=12)

Table 1. Distribution of risk factors in *A. platys* infected dogs (n=12)

Factor	Levels	Number of animals	Percentage (%) of animals
Breed	Non-descript	4	33.33
	Labrador Retriever	2	16.66
	Golden Retriever	2	16.66
	Others	4	8.33 (each)
Sex	Male	8	66.66
	Female	4	33.33
Age	<1 year	1	8.33
	1-2 years	2	16.66
	3-5 years	4	33.33
	5-10 years	2	16.66
	10-15 years	3	25
Season	Summer	8	66.66
	Winter	2	16.66
	Monsoon	2	16.66
Housing	Indoor	5	41.66
	Caged/Kennelled	7	58.33

Table 2. Haematological parameters of dogs infected with *A. platys*

Haematological parameters	Mean values ± SE		t value	p value
	Infected group (n = 12)	Control group (n = 10)		
Total White blood cell count (WBC) (10^3/µL)	10.656 ± 1.32	10.416 ± 1.06	0.137^{ns}	0.892
Lymphocytes (%)	18.261 ± 3.65	16.914 ± 1.83	0.069^{ns}	0.760
Monocytes (%)	6.716 ± 1.22	5.210 ± 0.705	2.725^{ns}	0.326
Neutrophils (%)	71.47 ± 4.28	71.79 ± 1.91	1.043^{ns}	0.946
Eosinophils (%)	3.21 ± 0.729	5.85 ± 0.598	0.310*	0.013
Basophils (%)	0.29 ± 0.0747	0.192 ± 0.055	1.008^{ns}	0.309
Red Blood Cell (RBC) count (10^6/µL)	4.92 ± 0.387	6.38 ± 0.231	3.080*	0.006
Haemoglobin (Hb) (g/dL)	11.97 ± 0.387	14.57 ± 0.231	2.088*	0.050
Volume of Packed Red Cells (VPRC) (%)	33.94 ± 2.69	43.15 ± 1.76	2.732*	0.013
Mean Corpuscular Volume (MCV) (fL)	69.58 ± 0.931	67.85 ± 1.336	1.091^{ns}	0.288
Mean Corpuscular Haemoglobin (MCH) (pg)	24.24 ± 0.360	23.63 ± 1.051	0.591^{ns}	0.561
Platelet (PLT) Count(10^3/µL)	134.84 ± 49.308	279.60 ± 27.261	2.428*	0.025

*Significant at p<0.05, ^{ns} non significant

Table 3. Biochemical parameters of dogs infected with *A. platys*

Biochemical parameters	Mean values ± SE		t value	p-value
	Infected group n = 12	Control group n = 10		
Creatinine (mg/dL)	1.21 ± 0.088	1.08 ± 0.0939	1.032^{ns}	0.314
Alanine aminotransferase (ALT) (IU/L)	35.65 ± 5.003	27.34 ± 5.691	1.099^{ns}	0.285
Alkaline phosphatase (ALP) (IU/L)	53.56 ± 9.954	31.87 ± 7.011	1.713^{ns}	0.102
Total protein (g/dL)	7.66 ± 0.491	6.34 ± 0.619	1.693^{ns}	0.106
Albumin (g/dL)	3.83 ± 0.292	3.47 ± 0.355	0.779^{ns}	0.445
Globulin (g/dL)	3.82 ± 0.256	2.86 ± 0.297	2.451^{*}	0.024
Total Bilirubin	0.35 ± 0.065	0.27 ± 0.033	0.948^{ns}	0.355
Direct bilirubin	0.13 ± 0.0247	0.14 ± 0.023	0.204^{ns}	0.841

*Significant at p<0.05, ^{ns} non-significant

count, haemoglobin concentration, packed cell volume and platelet count in the infected group compared to the healthy controls (Table 2). Similar haematological abnormalities were observed by Arun *et al.* (2022) in a dog co-infected with *Babesia gibsoni* and *A. platys*. Among the biochemical parameters, hyperglobulinemia emerged as the most significant alteration (Table 3).

Dogs infected with *A. platys* exhibited significant reductions in red blood cell (RBC) count, haemoglobin concentration (Hb) and volume of packed red cells (VPRC) when compared to healthy controls, indicating the presence of anaemia. Since mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values remained within normal limits, the anaemia was classified as normocytic and normochromic. Such a pattern typically suggests a non-regenerative anaemia. The underlying mechanisms proposed in earlier studies include immune-mediated erythrophagocytosis, suppression of erythropoiesis by pro-inflammatory cytokines and functional iron deficiency caused by iron sequestration within macrophages despite adequate iron stores in the bone marrow (Lasta *et al*., 2013; Sainz *et al*., 2015; Bouzourra *et al*., 2016)

Thrombocytopaenia was one of the most striking haematological abnormalities detected in *A. platys*-positive animals, reflecting the pathogen's unique tropism for platelets. The organism replicates within circulating thrombocytes, forming intracytoplasmic morulae and causing their direct destruction. Additionally, as the infection progresses, immune-mediated mechanisms may be triggered, leading to enhanced clearance of platelets from circulation (Rar *et al*., 2021). This dual mechanism of platelet loss, initially due to direct cytopathic effects and subsequently *via* immune responses, explains the marked thrombocytopaenia observed in affected animals. The infection typically follows a cyclic pattern, with alternating periods of thrombocytopaenia corresponding to waves of parasitaemia, as documented by Harvey *et al*. (1978) and Dyachenko *et al.* (2012). The severity of platelet reduction may also predispose infected dogs to clinical bleeding manifestations such as epistaxis, which was observed in few dogs included in this study.

A significant reduction in eosinophil percentage was also recorded in the infected group, indicative of eosinopenia. This is most likely associated with a stress-induced elevation in endogenous cortisol levels, which suppresses eosinophil production in the bone marrow and promote their peripheral destruction. Van Zyl *et al*. (2023), in their study on *Babesia rossi* infection in dogs, reported a similar pattern of eosinopenia linked to heightened cortisol responses under systemic stress. Although the current study focussed on *Anaplasma platys*, a different tick-borne pathogen, the stress-mediated mechanism is likely comparable, reflecting the body's systemic response to acute or chronic infection.

Hyperglobulinemia was the most notable serum biochemical alteration observed in *A. platys*-infected dogs when compared to healthy controls. The elevated globulin levels are likely a result of sustained antigenic stimulation and immune system activation in response to the infection, reflecting a chronic inflammatory state (Sainz *et al*., 2015; Bouzzoura *et al.,* 2016).

The findings of the present study highlighted the importance of clinical, haematological and biochemical evaluations in diagnosing and managing *A. platys* infection and association of various risk factors, emphasising the need for increased awareness and control measures for this neglected tick-borne disease in canines in this region.

Conclusion

Dogs infected with *Anaplasma platys* predominantly exhibited clinical signs such as pyrexia, lethargy, inappetence, lymphadenopathy, splenomegaly and haemorrhagic manifestations including epistaxis. Haematological evaluation revealed normocytic normochromic anaemia, significant thrombocytopaenia and eosinopaenia, while serum biochemical analysis indicated hyperglobulinemia as a consistent abnormality in the infected group compared to healthy controls. These findings underscored the diagnostic value of haemato-biochemical profiling in identifying and managing *A. platys* infections in canine patients.

Conflict of interest

There are no conflicts of interest reported by the authors.

Acknowledgement

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