



ISSN (E): 2277-7695

ISSN (P): 2349-8242

NAAS Rating: 5.23

TPI 2023; 12(4): 05-07

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Received: 08-01-2023

Accepted: 11-02-2023

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## Diagnosis of *Anaplasma platys* infection in a dog from Palakkad district, Kerala by direct microscopy and real time PCR: A case report

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### Abstract

*Anaplasma platys* is a tick-borne obligate intracellular rickettsial organism which has tropism for platelets, resulting in infectious canine cyclic thrombocytopenia. This case is the first microscopic and molecular confirmed case of *Anaplasma platys* infection in a dog from Palakkad District, Kerala State. In the present case, Giemsa-stained peripheral blood smear revealed giant platelets containing pleomorphic basophilic inclusion bodies. Molecular confirmation of pathogen was done by using Taqman based quantitative real-time PCR (qPCR) for *Anaplasma platys* targeting *gltA* gene. Anaemia and thrombocytopenia are the important hematological alterations noticed. Treatment was initiated with Doxycycline along with supportive therapy and the animal had an uneventful recovery.

**Keywords:** *Anaplasma platys*, canine, real-time PCR, doxycycline

### 1. Introduction

Infectious canine cyclic thrombocytopenia (ICCT) is a disease condition caused by an obligate intracellular bacterium *Anaplasma platys*, which has tropism for platelets and form pleomorphic basophilic inclusions (morulae) within platelets [1]. *A. platys* is more prevalent in tropic and subtropical climate and the brown dog tick, *Rhipicephalus sanguineus* is the probable vector responsible for transmission of this organism, as in many studies [2, 3]. Incubation period of the disease is 1 to 2 weeks and the main clinical manifestations are anorexia, lethargy, fever, weight loss, pale mucous membrane, lymphadenomegaly, mucopurulent nasal discharge, cutaneous petechiae, ecchymoses and bleeding disorders [4]. Cyclic episodes of thrombocytopenia are the characteristic of this infection, due to which the detection of inclusion bodies inside platelets by blood smear examination is not always possible in most of the times and is usually an accidental finding. Molecular technique is most specific and sensitive in diagnosis of *A. platys* compared to conventional blood smear examination [5]. In this case, Taqman based quantitative real-time PCR (qPCR) method targeting the citrate synthase gene as a specific target for *A. platys* detection was adopted. Real Time PCR was proven to be the more specific and sensitive for detecting *A. platys* when compared to nested PCR [6, 7]. Occurrence of this pathogen in dogs of Kerala has been previously reported [7, 8]. This case is the microscopic and molecular confirmation of *A. platys* infection in a dog from Palakkad district of Kerala.

### 2. Materials and Methods

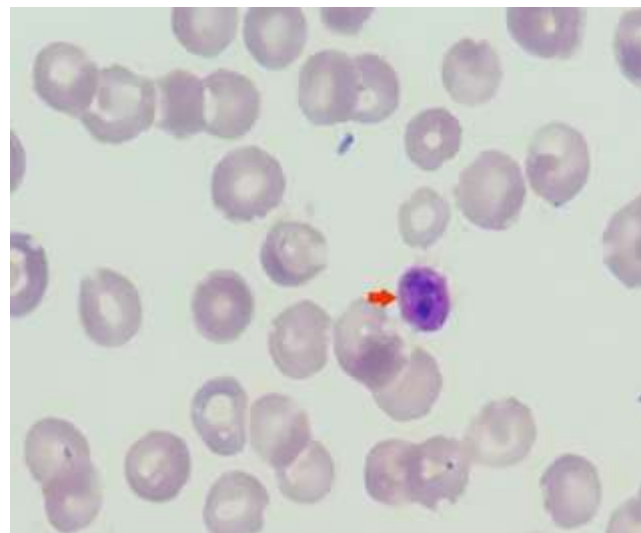
A two-year-old female pug was presented at District Veterinary Centre, Palakkad, Kerala with the history of inappetence, lethargy and respiratory distress. Clinical examination revealed pyrexia (104°F), tachycardia, nasal discharge, pale mucous membrane, petechiae, generalised lymphadenopathy and limb oedema. Peripheral thin blood smear was prepared from ear tip and stained with Giemsa stain. Around 2ml of blood was collected from cephalic vein in EDTA coated vial for hematological analysis. Peripheral blood was also collected from ear tip in EDTA vial and sent to Clinical Laboratory, Animal Disease Control Project, Department of Animal Husbandry, Thrissur, Kerala for PCR analysis. Peripheral blood smear examination and hematology was repeated on 14<sup>th</sup> day of post treatment.

### 3. Results and Discussion

Giemsa-stained peripheral blood smear on microscopic examination revealed giant platelets containing pleomorphic basophilic inclusion bodies which were suspected of being *A. platys*

inclusions (Fig.1). Microscopic detection of such inclusion bodies in platelets is possible in early stage of infection only and is non-specific later as there are chances for getting non-parasitic inclusions in platelets [9]. So, blood collected from ear tip in EDTA vial was sent to Clinical Laboratory, Animal Disease Control Project, Department of Animal Husbandry, Thrissur, Kerala for PCR analysis. Molecular confirmation of pathogen was done by using Taqman based quantitative real-time PCR (qPCR). Template prepared from peripheral EDTA blood in duplicate using DNA preparation kit and qPCR was done for *A. platys* targeting *gltA* gene. Duplicate templates tested with Ct value 20.93 and 20.31 were considered as positive. Anaemia and thrombocytopenia are the major hematological alterations noticed in this case (Table. 1). Such alterations in dog infected with *A. platys* have also been documented earlier [10, 11, 12]. Thrombocytopenia in *A. platys* is cyclic in nature and the initial thrombocytopenia is due to the destruction of platelets by the multiplying pathogen which may cause an immune response as the infection progresses [13]. After an incubation period of 1 to 2 weeks, the platelet count reduces drastically within a few days and recovery occur at 7 to 14 days interval and the severity of thrombocytopenia gradually decrease in subsequent cycles [14]. Total leukocyte count and differential leukocyte count were within normal reference range [12]. Based on these findings, this case was confirmed as infectious canine cyclic thrombocytopenia and treatment was initiated with Doxycycline @ 10 mg/kg body weight IV for 5 days, followed by oral administration of Doxycycline at the same

dose rate for next 9 days. Advised oral platelet boosters (THROMBOFIT 200ml- Sihil pharma) 5ml BID orally for a month. The hematological parameters showed improvement and the peripheral blood smear was found negative on 14<sup>th</sup> day of post treatment. Successful treatment of dog infected with *A. platys* with Doxycycline has been reported earlier [10, 11] and the animal recovered uneventfully.



**Fig 1:** Giant platelets with pleomorphic basophilic inclusion bodies of *Anaplasma platys* (red arrow) 100X

**Table 1:** Hematological parameters of affected dog before and after therapy

Parameters	First day of presentation of animal	Day 14 (After treatment)	Reference range	Key findings on first day of presentation of animal
Hb (g/dl)	9.3	12.8	12-18	Anaemia
PCV (%)	30.1	37.8	37-55	
Total RBC Count (million/ $\mu$ l)	3.8	5.7	5.5-8.5	
Total WBC count (thousand/ $\mu$ l)	12	12.2	6-17	Normal leukocyte count with mild granulocytosis and moderate lymphopenia
Neutrophils (%)	83.5	82	58-85	
Lymphocytes (%)	10.8	12	8-21	
Monocytes (%)	5.7	6	2-10	Thrombocytopenia
Platelets (lakhs/ $\mu$ l)	0.44	2.18	2-6	

#### 4. Conclusion

Cyclic nature of thrombocytopenia in *A. platys* decreases the chance of identification of this organism in peripheral blood smear and adoption of molecular diagnostic technique only provide an accurate result. In this case animal showed complete recovery after treatment with Doxycycline.

#### 5. Acknowledgment

The authors are grateful to the Chief Veterinary Officer, District Veterinary Centre, Palakkad, Kerala and District Laboratory Officer, Animal Disease Control Project, Department of Animal Husbandry, Thrissur, Kerala for providing necessary facility for conducting this work.

#### 6. References

- Harvey JW, Simpson CF, Gaskin JM. Cyclic thrombocytopenia induced by a Rickettsia-like agent in dogs. *J infect Dis.* 1978;137(2):182-188.
- Inokuma H, Raoult D, Brouqui P. Detection of Ehrlichia platys DNA in brown dog ticks (*Rhipicephalus sanguineus*) in Okinawa Island, Japan. *J Clin Microbiol.* 2000;38(11):4219-4221.
- Cohn LA. Ehrlichiosis and related infections. *Vet Clin North Am Small Anim Pract.* 2003;33(4):863-884.
- Sainz A, Roura X, Miro G, Estrada-Pena A, Kohn B, Harrus S, *et al.* Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. *Parasites and Vectors.* 2015;75(8):1-20.
- Bhoopathy D, Latha BR, Palavesam A. Molecular detection of *Anaplasma platys* infection in dogs in Chennai, Tamil Nadu, India- A pioneer report. *J Entomol Zool.* 2017;5(3):1608-1610.
- Da Silva CB, Pires MS, Vilela JAR, Peckle M, Da Costa RL, Vitari GLV, *et al.* A new quantitative PCR method for the detection of *Anaplasma platys* in dogs based on the citrate synthase gene. *J Vet Diagn Invest.* 2016;28(5):529-535.
- Karunakaran S, Gopinathan GT, Sasidharan H. Molecular evidence for the presence of *Borrelia burgdorferi* and *Anaplasma platys* among domesticated animal population- Study based on real time PCR methodology - in Thrissur District-Kerala, India. *Newest Updates in*

- Agriculture and Veterinary Science. 2023;3(Chapter 4):42-50.
8. Kavitha S, Panicker VP, Vismaya KK, Narayanan A, Ajith KS, Lakshmanan B. Molecular identification and phylogenetic analysis of *Anaplasma platys* from naturally infected dogs of South India. J Entomol Zool. 2020;8(2):15-19.
  9. Ferreira RF, Cerqueira AMF, Pereira AM, Guimaraes CM, Garcia de Sa A, Aberu FS, *et al.* *Anaplasma platys* diagnosis in dogs: comparison between morphological and molecular tests. Intern J Appl Res Vet Med. 2007;5(3):113-119.
  10. Maurya PS, Sahu S, Jithin MV, Chandra G, Devi A. Conventional and nucleic acid-based confirmation of *Anaplasma platys* infection in a dog from Meerut, Western Uttar Pradesh: A case report. Haryana Vet. 2021;60(1):140-141.
  11. Arun A, Reena KK, Rafiqui SI, Jithin MV, Sharma DK, Mahendran K, *et al.* Molecular and parasitological evidence of *Anaplasma platys* infection in a dog: A case report. Ann Clin Cytol Pathol. 2017;3(3):1059.
  12. Dyachenko V, Pantchev N, Balzer HJ, Meyersen A, Straubinger RK. First case of *Anaplasma platys* infection in a dog from Croatia. Parasites and Vectors. 2012;49(5):1-7.
  13. French TW, Harvey JW. Serologic diagnosis of infectious cyclic thrombocytopenia in dogs using an indirect fluorescent antibody test. Am J Vet Res. 1983;44(12):2407-2411.
  14. Eddlestone SM, Gaunt SD, Neer TM, Boudreaux CM, Gill A, Haschke E, *et al.* PCR detection of *Anaplasma platys* in blood and tissue of dogs during acute phase of experimental infection. Exp Parasitol 2007;115(2):205-210.