



***Anaplasma platys* in dog from Forest Zone of Minas Gerais State – Case Report**

*Anaplasma platys* em cão na Zona da Mata Mineira – Relato de Caso

Relato

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**Abstract:** The present study describes the clinical case of a mixed breed dog from Juiz de Fora kennel - Zona da Mata region of Minas Gerais State -, naturally infected with *Anaplasma platys*. Laboratory examinations showed thrombocytopenia, *macroplatelets* and the presence of *A. platys* in the animal's blood smear. The presence of the hemoparasite in the blood sample was confirmed through PCR. The epidemiological importance of the current study lies on the fact that it is the first to report an *A. platys* case identified at the Veterinary Clinical Analysis Laboratory of Federal University of Juiz de Fora (UFJF). The study also highlighted the importance of blood smears to identify this hemoparasite.

**Keywords:** *Anaplasma platys*, thrombocytopenia, *macroplatelets*, hemogram, dog.

**Resumo:** Este estudo descreve o caso clínico de um cão sem raça definida (SRD), naturalmente infectado por *Anaplasma platys*, proveniente do canil municipal de Juiz de Fora (MG), na Zona da Mata Mineira. Os exames laboratoriais demonstraram trombocitopenia, macroplaquetas e a presença de *A. platys* no esfregaço sanguíneo. A presença do hemoparasita na amostra sanguínea foi confirmada por PCR. Este trabalho possui importância epidemiológica porque relata o primeiro caso de *A. platys* no Laboratório de Análises Clínicas Veterinárias da Universidade Federal de Juiz de Fora (UFJF), destacando a importância do esfregaço sanguíneo para identificação de hemoparasitas.

**Palavras-chave:** *Anaplasma platys*, trombocitopenia, macroplaquetas, hemograma, cão.

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## Introduction

Hemoparasitic diseases are very serious illnesses affecting domestic animals; however, their identification is easy, but severe cases still cause the death of infected animals (TORRES et al., 2012). *Anaplasma platys* is one of the main hemoparasites found in dogs in Brazil. It is responsible for infections in blood platelets known as Infectious Canine Cyclic Thrombocytopenia (ICCT) (DUMLER et al., 2001; FERREIRA et al., 2008). This parasitic agent is a gram-negative bacterium (Baker et al., 1987) expressed as basophilic bodies inside blood platelets in blood smears stained with Giemsa or Panotico (MACHADO et al., 2010). These bacteria are transmitted mainly through the bite of tick species *Rhipicephalus sanguineus* (SIMPSON et al., 1991; INOKUMA et al., 2000).

This disease is a cyclic parasitemia that causes cyclic thrombocytopenia, which results in reduced number of blood platelets, even on their disappearance from blood stream. The number of blood platelets returns to normal within approximately four days. These phases (decrease and increase of the platelets) are cyclic (ALMOSNY 2002). Clinical signs, such as vomit, diarrhea, anorexia and hemostasis disorders, can start after the

incubation period - fifteen days, on average (Gasparni et al., 2008; Harrus et al., 1997). However, dogs with ICCT usually show mild clinical signs of the disease, or are asymptomatic (Cardozo et al., 2007), except when they are also infected with *Babesia* sp, *Ehrlichia canis* or with other microorganisms (DANTAS, 2008).

The direct parasitological examinations through the microscopic analysis of blood smears is commonly used to detect *A. platys*, since it is a simple and specific method. However, this method has low sensitivity, mainly to chronic infections, whose parasitemia is low (GREENE, 2006). On the other hand, *A. platys* is the polymerase chain reaction (PCR) mostly used for *A. platys* detection, among diagnostic techniques based on molecular biology. PCR presents high sensibility and specificity, since it is able to detect fragments of parasitic agents, whether they are viable, or not (FARIA et al., 2010).

Frequency of *A. platys* infections in Brazil ranges from 5.1% (16) to 18.8% (Ferreira et al., 2008); this parasite causes thrombocytopenia in dogs living in different regions countrywide. The disease is quite common in inland counties; however, there is lack of studies about this illness in Zona da Mata region - Minas

Gerais State.

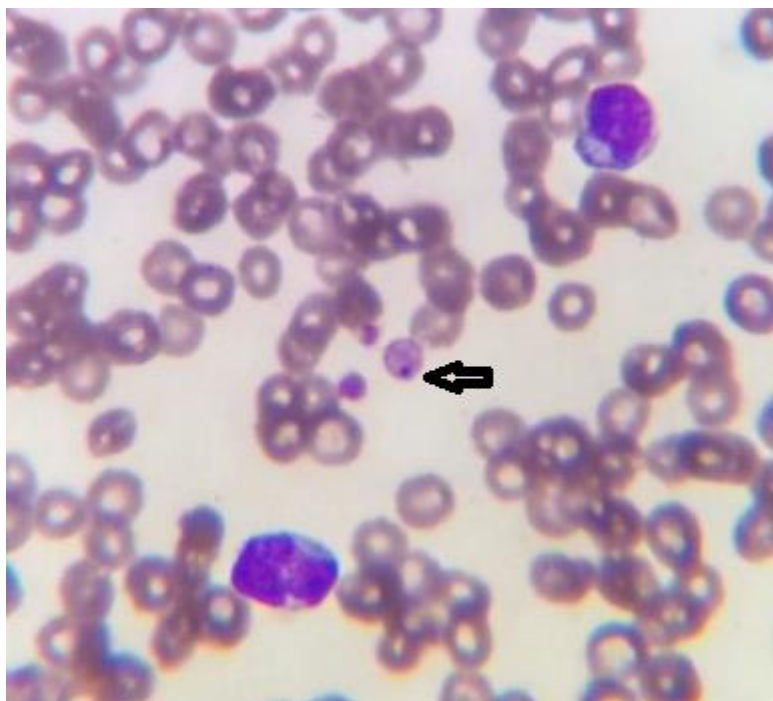
The epidemiologic impact of the current study lies on the fact that it is the first *A. platys* case identified at the Veterinary Clinical Laboratory of Federal University of Juiz de Fora (UFJF), as well as to highlight the important role played by laboratorial examinations in identifying hemoparasites in the herein assessed region.

### Case Report

The reported case regards a young male mixed-breed dog belonging to Juiz de Fora kennel, Minas Gerais State. This dog was about to be subjected to elective sterilization at the Veterinary Clinic of UFJF. His blood samples were collected

through venipuncture for surgical risk assessments and sent to the Veterinary Clinical Pathology Laboratory of the same institution for hemogram examinations and hemoparasitic evaluations.

Parasitemia estimates were calculated based on the blood samples. Blood smears were stained with Diff Quick and subjected to microscopy for macroplatelet observation. Some platelets had inclusions compatible to “*A. platys*” (Figure 1). Erythrogram (Table 1) showed low hemoglobin and hematocrit values, but the number of erythrocytes was within the regular parameters. Leukogram did not identify any changes.



**Figura 1.** Blood smear showing *Anaplasma platys* in macroplatelet.

A complete blood sample (EDTA) was sent to TECSA Laboratory, Belo

Horizonte City, to confirm the infection with “*A. platys*” through the PCR method,

after the tests carried out at the Veterinary Clinical Pathology Laboratory of Federal University of Juiz de Fora. According to

the report provided by this laboratory, the parasite was found in the collected sample.

**Tabela 1** – Hematological parameters of dog infected naturally by *Anaplasma platys*.

Parameters	Results	Reference Values
Erythrocytes	6,1	5,5-8,5 (x 10 <sup>6</sup> /μl) <sup>a</sup>
Hemoglobin	9,5	12-18 (g/dl) <sup>a</sup>
Hematocrit	34,8	37-55 (%) <sup>a</sup>
Plaquetas	106.500	200-500 (x10 <sup>3</sup> ) <sup>a</sup>
Total Leukocytes	13.500	6.000-17.000 (/μl) <sup>a</sup>
Neutrophils	10.164	3.000-11.500 (/μl) <sup>a</sup>
Lymphocytes	2.105	1.000-4.800 (/μl) <sup>a</sup>
Monocytes	842	150-1.350 (/μl) <sup>a</sup>
Eosinophils	389	150-1.250 (/μl) <sup>a</sup>

<sup>a</sup>Weiss & Wardrop (2010).

## Discussion

The tick species *Rhipicephalus sanguineus* is the vector of animal rickettsioses caused by *E. canis* and *A. platys* (INOKUMA et al., 2000). According to Gasparni et al. (2008), inoculation with blood from an infected animal can also transmit these parasites. Therefore, infection was most likely caused by tick bites, since the animal was not subjected to blood transfusion. The animal came from a kennel where the control of ectoparasites is permanent; however, many animals are already infected with these parasites when they get there.

The hemogram of the herein reported animal showed thrombocytopenia (106,500 platelets). Although, according to Eddlestone et al., (2007), it is quite difficult to diagnose this disease, given the small number of platelets, it was possible to observe inclusions compatible to *A. platys* inside platelets in the blood smear subjected to microscopic analysis. Morulae of this hemoparasite were observed in the macroplatelets, since, according to CORRÊA et al. (2011), platelets infected with *A. platys* look like macroplatelets.

Physical exams did not show any

clinical sign of the disease in the animal, because, according to Cardozo et al. (2007), dogs infected with *Anaplasma platys* often show mild clinical signs of it, or are even asymptomatic. Thus, based on the lack of ICCT clinical signs, the animal was in the subclinical phase of the disease, when one can observe thrombocytopenia, megakaryocytic hyperplasia and rare inclusions compatible to *A. platys* inside platelets in the blood smear. Erythrogram and leukogram values remain normal at this phase (Almosny 2002), as observed in hemogram of the herein assessed dog.

Based on Chang et al., (1996), PCR is useful to detect *A. platys* in the early stages of the infection and in its chronic phase. Therefore, given the suspicion that the animal was in the subclinical phase of anaplasmosis, the decision was made to carry out PCR to confirm the infection.

### Conclusion

Although canine anaplasmosis is a disease without remarkable signs, or responsible for high death rates, it is important to describe the first case of *A. platys* diagnosed at Veterinary Clinical Pathology Laboratory of Federal University of Juiz de Fora Federal through microscopy and confirmed through PCR. The case report shows the diagnostic reliability of microscopic examinations applied to blood smears. It also highlights

the epidemiological importance of the disease in the assessed region.

### References

1. ALMOSNY, N.R.P. Hemoparasitoses em Pequenos Animais Domésticos e como Zoonoses. 1.ed. Rio de Janeiro: L.F.Livros, 2002. 135p.
2. BAKER, D.C.; SIMPSON, M.; GAUNT, S.D.; CORSTVET, R.E. Acute *Ehrlichia platys* infection in the dog. *Veterinary Pathology*, v.24, p.449-453, 1987.
3. CARDOZO, G.P.; OLIVEIRA, L.P.; ZISSOU, V.G.; DONINI, I.A.N.; ROBERTO, P.G.; MARINS, M. Analysis of the 16S rRNA gene of *Anaplasma platys* detected in dogs from Brazil. *Brazilian Journal of Microbiology*, v.38, p.478-479, 2007.
4. CHANG, W.L.; PAN, M.J. Specific amplification of *Ehrlichia platys* DNA from blood specimens by two-step PCR. *Journal of Clinical Microbiology*, v.34, p.3142-3146, 1996.
5. CORREA, E.S.; PALUDO, G.R.; SCALON, M.C.; MACHADO, J.A.; LIMA, A.C.Q.; PINTO, A.T.B.; THIEBAUT, J.T.L.; ALBERNAZ, A.P. Investigaç o molecular de *Ehrlichia* spp. e *Anaplasma platys* em felinos dom sticos: altera es cl nicas, hematol gicas e bioqu micas. *Pesquisa Veterin ria Brasileira*, v.31, n.10, p.899-909, 2011.
6. DANTAS-TORRES, F. Canine vector-borne diseases in Brazil. *Parasites & Vectors*, 1(1), 25, 2008.
7. DUMLER, J.S.; BARBET, A.F.; BEKKER, C.P.; DASCH, G.A.; PALMER, G.H.; RAY, S.C.; RIKIHISA, Y.; RURANGIRWA, F.R. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *International Journal of*

**Systematic and Evolutionary Microbiology**, v.51, p.2145-2165, 2001.

8. EDDLESTONE, S.M.; GAUNT, S.D.; NEER, T.M.; BOUDREAUX, C.M.; GILL, A.; HASCHKE, A.; CORSTVET, R.E. PCR detection of *Anaplasma platys* in blood and tissue of dogs during acute phase of experimental infection. **Experimental Parasitology**, v.115, p.205-210, 2007.

9. FARIA, J.L.; DAGNONE, A.S.; MUNHOZ, T.D.; JOAO, C.F.; PEREIRA, W.A.; MACHADO, R.Z.; TINUCCI-COSTA, M.. *Ehrlichia canis* morulae and DNA detection in whole blood and spleen aspiration samples. **Revista Brasileira de Parasitologia Veterinária**, v.19, n.2, p.98-102, 2010.

10. FERREIRA RF, CERQUEIRA AMF, PEREIRA AM, VELHO PB, AZEVEDO R, RODRIGUES IL, ALMOSNY NRP. Avaliação da ocorrência de reação cruzada em cães PCR-positivos para *Anaplasma platys* testados em ELISA comercial para detecção de anticorpos de *Anaplasma phagocytophilum*. **Revista Brasileira de Parasitologia Veterinária**, v.17, n.1, p.5-8, 2008.

11. GASPARNI, M.R.; COELHO, A.L.M.; JOJIMA, F.S.; VIDOTTO, M.C.; VIDOTTO, O. Ocorrência de *Ehrlichia canis* e *Anaplasma platys* em cães de uma população hospitalar em Londrina, Paraná. In: Program & Resumos do XV Congresso Brasileiro de Parasitologia Veterinária, II Seminário de Parasitologia Veterinária dos países do Mercosul, 2008, Curitiba. XV Congresso Brasileiro de Parasitologia Veterinária. Curitiba, (2008)

12. HARRUS, S.; AROCH, I.; LAVY, E.; BARK, H. Clinical manifestations of infectious canine cyclic thrombocytopenia. **Veterinary Record**, v.141, p.247-250, 1997.

13. INOKUMA, H.; RAOULT, D.; BROUQUI, P. Detection of *Ehrlichia platys* DNA in brown dog ticks (*Rhipicephalus sanguineus*) in Okinawa Island, Japan. **Journal of Clinical Microbiology**, v.38, p.11, p.4219-4221, 2000.

14. GREENE, C.E. Infectious Diseases of the dog and cat. 4<sup>a</sup> ed, St Louis: Elsevier, 2011, 1296p.

15. SIMPSON, R.M.; GAUNT, S.D.; HAIR, J.A.; KOCAN, K.M.; HENK, W.G.; CASEY, H.W. Evaluation of *Rhipicephalus sanguineus* as a potential biologic vector of *Ehrlichia platys*. **American Journal of Veterinary Research**, v.52, n.9, p.1537-1541, 1991.

16. TORRES, F.; CHOMEL, B.B.; OTRANTO, D. Ticks and tick-borne diseases: a One Health perspective. **Trends in Parasitology**, v.28, n.10, p.437-446, 2012.

17. WEISS, D.J.; WARDROP, K.J. Schalm's veterinary hematology. 6th ed. Ames: Lippincott Williams & Wilkins, 2010, 1206p.