Short Communication

# *Anaplasma platys* in dog and *Rhipicephalus sanguineus* in the city of Salta in Salta Province, Argentina

# *Anaplasma platys* en perro y en *Rhipicephalus sanguineus* en la ciudad de Salta, Provincia Salta, Argentina

https://eqrcode.co/a/dflhf7

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**ABSTRACT:** A dog with 32 specimens of *Rhipicephalus sanguineus* sensu lato were examined at the DNA level to determine the presence of *Borrelia* spp., *Anaplasma platys*, *Ehrlichia* spp., and *Babesia* spp. A partial sequence of *A. platys* gene *gltA* (689 bp) was detected in the dog's blood and three ticks (9.37 %). This is the first detection of *A. platys* in a young dog with symptoms and an animal's *R. sanguineus* s.l. ticks in the city of Salta in northwest Argentina.

**Key words:** *Anaplasma*, hemoparasites ticks, *Canis familiaris*, Argentina.

**RESUMEN:** Se examinó un perro con 32 especímenes de *Rhipicephalus sanguineus* sensu lato a nivel de ADN, para determinar la presencia de *Borrelia* spp., *Anaplasma platys*, *Ehrlichia* spp. y *Babesia* spp. Se detectó una secuencia parcial del gen *gltA* (689 pb) de *A. platys* en la sangre del perro y en tres garrapatas (9,37 %). Esta es la primera detección de *A. platys* en animales jóvenes y enfermos y en *R. sanguineus* s.l y garrapatas en la ciudad de Salta, en el noroeste de Argentina.

**Palabras clave:** *Anaplasma*, hemoparasitos, garrapatas, *Canis familiaris*, Argentina.

## INTRODUCTION

Infectious diseases are the leading cause of death among dogs (1) and a simultaneous occurrence of more than one disease is common, especially in weakened dogs. Although in clinical practice, diagnosis is usually based on the progress of clinical signs and laboratory findings, many illnesses offer generic signs, such as apathy, anorexia and occasionally fever, progressing to respiratory, gastrointestinal, and neurological manifestations (2,3).

Organisms of the Anaplasmataceae family, Rickettsiales order, are Gram-negative bacteria and obligate intracellular parasites. *Anaplasma platys* is the etiological agent of canine cyclic thrombocytopenia (CCT). This agent affects circulating platelets, in which it is possible to observe morulae, particularly in the acute phase of the disease (4). The severity of thrombocytopenia and percentage of parasitized thrombocytes are higher during primary infection, which lasts 10 to 14 days (5). CCT is usually asymptomatic or subclinical and difficult to differentiate from other diseases (6,7). *Anaplasma platys* has been reported to infect other hosts, such as humans (8-11) and cats (12).

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*Anaplasma platys* is predominant in tropical regions, concurring with *Ehrlichia canis* and the distribution of *Rhipicephalus sanguineus* sensu lato (13). Additionally, Brandão *et al*. (14) confirmed the relevance of the degree of infestation in stray dogs, compared to pet dogs, with respect to the degree of infestation by *R. sanguineus* s.l. and the risk of being carriers of *A. platys*. On a related note, Cicuttin *et al*. (15) discussed the vectorial capacity of *R. sanguineus* s.l. confirming the species as a competent transmitter for *Ehrlichia canis* and *A. platys*.

Oscherov *et al*. (16) noted the first detection of Anaplasmataceae family organisms in the Argentine territory when the authors discovered the natural infection in *R. sanguineus* s.l. in the city of Corrientes in northeast Argentina. Later, Eiras *et al*. (17) reported a co-infection with *Hepatozoon canis*, *Babesia vogeli* and *A. platys* in dogs in the province of Buenos Aires, as well. Considering the scarcity of studies on agents of the Anaplasmataceae family in Argentina and the relevance of this subject to South America, the objective of the present study was to report the molecular detection of an animal naturally infected by *A. platys* along with its ticks in Salta, Salta Province, Argentina.

## MATERIAL AND METHODS

In November 2018, a six-month-old Dachshund was admitted into the Catholic University of Salta Veterinary Hospital to receive clinical care, exhibiting a clinical condition of gastroenteritis. Upon admission, anamnesis was performed, and gastroenteritis, prostration and dehydration were observed. The animal was quickly sent to serotherapy (0.9 % NaCl) for intravenous rehydration.

Blood was collected in a vacuum tube with and without ethylenediaminetetraacetic acid (EDTA) anticoagulant by puncture of the cephalic vein. A drop of blood was used for the rapid test for the detection of *E. canis* antibodies employing a chromatographic membrane (Speed EhrliTM Virbac®), following the manufacturer's operating instructions.

During clinical examination, ectoparasites were collected with tweezers or manually and they preserved in polypropylene tubes containing isopropyl alcohol. The taxonomic identification of ectoparasites was carried out through the dichotomous keys of Barros-Battesti *et al*. (18) for ticks and Linardi and Guimarães (19) for fleas.

Blood DNA extraction was carried out with a DNeasy Blood & Tissue Kit® (Qiagen, Hilden, NRW, Germany), according to the manufacturer's instruction. The DNA extraction of ectoparasites was performed by the phenol chloroform technique described by McIntosh *et al*. (20). The extracted DNA was submitted to PCR to detect the DNA of *Borrelia* spp., *A. platys*, *Ehrlichia* spp. and *Babesia* spp., using primers with their respective protocols, as shown in Table 1.

The products amplified by PCR were purified based on ExoSAP-IT® (Affymetrix USB®) according to the manufacturer's recommendation. After purification, DNA was sequenced using a capillary-type Sanger platform on a DNA analyzer ABI 3730 (Applied Biosystems, Life Technologies®). The resulting sequences were compared to those published utilizing the platform, NCBI Nucleotide BLAST.

According to clinical examination, the animal exhibited depletion, loss of appetite and dehydration levels of approximately 10 %. It also evidenced eyeball retraction, pale mucosae, dyspnea, 32°C (89.6ºF) temperature, diarrhea, and generalized petechiae. The tutor reported that the animal had already been assisted by other veterinary clinics, being under treatment with Ranitidine, Tramadol and Sucralfate.

The vaccine protocol for distemper and parvovirus viruses was incomplete. Six hours after service arrival, the animal died. No technique for diagnosing viral disease was attempted despite the fact that the presumptive diagnosis was gastroenteritis caused by the distemper virus.

## RESULTS AND DISCUSSION

Thirty-three ectoparasites were collected from the animal and identified according to morphology: 32 ticks (7 larvae, 23 nymphs and 2 adults) of the species *R. sanguineus* s.l. and 1 flea of the species *Ctenocephalides canis*.

PCR was applied to analyze the extracted DNA of all ectoparasites along with blood

**Table 1.** List of the primers used for PCR to detect the DNA of *Borrelia* spp., *A. platys*, *Ehrlichia* spp., and *Babesia* spp., from dog's blood and ticks from Salta City, Argentina./ *Lista de los cebadores utilizados para la PCR para detectar el ADN de Borrelia spp., A. platys, Ehrlichia spp. y Babesia spp., a partir de sangre de perro y garrapatas de la ciudad de Salta, Argentina*.

| Primers | Gene | Organism | Nucleotide sequences (5'-3') | Expected Amplicon length | References |
|---|---|---|---|---|---|
| BorFlaF1 | *fla*B | *Borrelia* spp. | TACATCAGCTATTAATGCTTCAAGAA | 740 pb | Blanco *et al*. (21) |
| BorFlaR1 | | | GCAATCATWGCCATTGCRGATTG | | |
| BorFlaF2 | | | CTGATGATGCTGCTGGWATGG | | |
| BorFlaR2 | | | TCATCTGTCATTRTWGCATCTT | | |
| BT-F3 | 18S rRNA | Ordem Piroplasmida | TGGGGGGAGTATGGTCGCAAG | 650 pb | Seo *et al*.(22) |
| BT-R3 | | | CTCCTTCCTTTAAGTGATAAG | | |
| DSB-330 | *Dsb* | *Ehrlichia* spp. | GATGATGCTTGAAGATATSAAACAAAT | 349 bp | Almeida *et al*. (23) |
| DSB-380 | | | ATTTTTAGRGATTTTCCAATACTTGG | | |
| DSB-720 | | | CTATTTTACTTCTTAAAGTTGATAWATC | | |
| Platys689F | *glt*A | *Anaplasma platys* | ATGCTGTTTTGATGTGCGGG | 689 bp | This study |
| Platys69R | | | CCGCACGGTCGCTGTT | | da Silva *et al*. (24) |

samples. Although an evaluation by the PCR technique to determine the presence of agents transmitted by ticks showed a negative result for *Ehrlichia* spp., the quick testing using Speed EhrliTM® had presented positive results for the *E. canis* species. Furthermore, when the materials were tested for *A. platys* gene *gltA*, both the animal's blood and 3 ticks (3/32) amplified the sequence. The partial sequences of the *gltA* gene for *A. platys* were submitted to the GenBank under the accession numbers, MN725733 (Blood) and MN725734 (Ticks). Those sequences were compared with others deposited in the GenBank and yielded an identity of 100 %, 99.68 % and 99.56 % with *A. platys* (KP903286), (KR011928) and (KP903289), respectively.

The present study refers to a dog infected with *A. platys* exhibiting classic symptoms of CCT (Petechiae) as represented by anorexia, fever, anemia, apathy, and petechiae. To date, few occurrences of clinical infection by *A. platys* in dogs in Argentina have been reported (13,15,25). This is the first report of *A. platys* causing symptoms in a dog in the Argentine territory; that said, it is a likely case of co-infection with the distemper virus, which may have led to the animal's death. The literature offers no records regarding gastroenteritis caused by *A. platys*.

In the present study, serology was positive for *E. canis* though negative by PCR. Considering the animal's young age, it can be concluded that maternal antibodies were still present as acute infection increased the chance of detection by molecular techniques as demonstrated by Almeida *et al*. (26). However, it was not possible to discard cross-reactions between *A. platys* and *E. canis* as the manufacturer did not mention tests demonstrating the absence of that possibility.

In the present study, *A. platys* was also detected in three *R. sanguineus* s.l. ticks out of the total number of specimens found parasitizing the animal under consideration. The presence of *A. platys* in Argentina is a ubiquitous phenomenon in both tropical and temperate lineages of *R. sanguineus* s.l (15). Additionally, the presence of *A. platys* in ticks collected from positive dogs indicates their ability to ingest the bacterium during a blood meal, possibly in relation to the level of rickettsia of dogs as evidenced by Breitschwerdt *et al*. (27).

Furthermore, ticks belonging to this group are competent vectors for many other pathogens to dogs, including phylogenetically related bacteria, such as *E. canis* (7,8). Otherwise, the failure in demonstrating the vector competence of *R. sanguineus* for *A. platys* in the study of Simpson *et al*.(25) may be related to the tick strain or species used by the researchers.

This is the first molecular record on the presence of *A. platys* in association with

distemper in the northwest region of Argentina. This result indicates that CCT caused by *A. platys*, together with the results generated by Cicuttin *et al*. (15), may be endemic in the region. As several studies have demonstrated, even though *A. platys* is considered less pathogenic than other agents of the Anaplasmataceae family, its importance in terms of zoonoses should not be underestimated (8,10,11).

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**Ethical Statement:** All procedures involving animals were evaluated and approved by the Ethical Principles of Animal Experimentation established by the Committee of Ethical of Animal Experimentation of the Universidad Catholica de Salta, Argentina.

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