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RESEARCH ARTICLE

PREVALENCE OF *ANAPLASMA PLATYS* IN DOGS FROM IN THE UNITED STATES – MEXICO BORDER REGION, DIAGNOSED BY DIRECT MICROSCOPIC OBSERVATION

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ABSTRACT

The prevalence of the *rickettsia* bacterium *Ehrlichia platys*, now called *Anaplasma platys*, was reviewed and identified in the canine population of Victoria, Tamaulipas, Mexico. A total of 362 venous blood samples were collected from the EDTA tube for a period of one year (February 2, 2016 to February 2, 2017). These samples were analyzed in the laboratory of clinical analysis of the veterinary hospital for small species of the Autonomous University of Tamaulipas where the technique of direct microscopy of blood smears stained by Diff quick (Romanowsky staining) was used as diagnostic method in Where it was sought to visualize the presence of morulae in platelets parasitized by the bacteria. The observation of the samples revealed the presence of corpuscles in platelets compatible with *Anaplasma platys* in 65 of the 362 samples analyzed, giving us a prevalence of 17.95%. It is evident that the presence of this bacterium in the canine population of Victoria, Tamaulipas, is high.

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INTRODUCTION

Anaplasma platys is a gram negative, small, pleomorphic and obligate intracellular bacterium, which may be present in most mammalian animals, both wild and domestic, including man, placing it as an emerging zoonotic infectious disease (Abarca *et al*. (2007).Because the main vector of this bacterium is the brown dog tick (*Rhipicephalus sanguineus*) and the one that most infests dogs (see Annex, Figure 1), exposure to this infection by humans is high (Abarca *et al*., 2007; Abrego *et al*., 2012). The importance of this study is to determine how prevalent we have the *Anaplasma platys* bacteria in the canines of the city and thus to be able to take measures in question of the control of the vector and therefore in the prevention of the disease in both the animals and in the Humans

Bibliographic Review

Within the great variety of species that make up the genus *Anaplasma spp, Anaplasma platys* is found, which was called *Ehrlichia platys*, until in 2001, genomic studies, demonstrated that the gen 16s rRNA of this bacterium was more identical with the genus *Anaplasmataceae* that of the *genus Ehrlichia* (Beaufils *et al*., 2002; Ábrego *et al*., 2012; Tateishi *et al*., 2014). *Anaplasma platys* was first reported by Harvey *et al* (1978) in Florida, USA. Since then, it has been attributed to the etiologic agent of the disease called canine infectious cyclic thrombocytopenia (Abarca *et al*., 2007, IDEXX, 2014 and Alleman, 2016). This bacterium is tropism by the platelets, where they are replicated by binary fission, lodging in a vacuole, until forming colonies of bacteria known as morulae, which come to be composed of 1 to 8 subunits (Beaufils *et al*., 2002, Dolz *et al*., 2013). The disease, as mentioned by its name, is characterized by clot-clotting thrombocytopenia with an interval of 7 to 14 days, and the platelet count may fall to less than 20,000 platelets (200,000-500,000) (Arraga-Alvarado *et al*. , 2003; Albreman *et al*., 2012). The clinical picture can be manifested in three phases: acute phase, subclinical or asymptomatic phase and chronic phase. The most common clinical signs associated with the disease are: lymphadenomegaly, splenomegaly, anorexia, pyrexia, and depression. Among the hematological abnormalities, we found thrombocytopenia, mild to moderate anemia, monocytosis, hypoalbuminemia, hypergammaglobulinemia and sometimes leukopenia (Beaufils *et al*., 2002, Huang *et al*., 2004, Alleman, 2016) . Complications such as thrombocytopenia (immune-mediated thrombocytopenia) and hemorrhagic disorders, which

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are not so common, have been reported, but there have been reports of cases such as those reported by (Beaufils *et al*., 2002). As a vector to the brown tick (*Rhipicephalus sanguineus*), the bacteria *Anaplasma platys* may be present in co-infection with other microorganisms whose vector is the same. Therefore we should consider having a mixed infection. Among the most common co-infections with *Anaplasma platys* reported are A. *platys-Ehrlichia canis*, *A. platys-Babesia canis*, *A. platys-Hepatozoon canis* (see annex, figure 2) (Ábrego *et al*. , 2012; Tateishi *et al*., 2014). In order for the vector to acquire the bacterium by feeding on blood from the infected animal, it must be in the acute stage. In the vector, the bacterium passes from the intestine to the salivary glands, transmitting again, for up to 150 days post-infection (Dolz *et al*., 2013).The epidemiology of *Anaplasma platys* before was not important, since many authors mentioned that there was no contagion to the human, even though it was a non-pathogenic species (Beaufils *et al*., 2002, Dolbre *et al*. 2013). However, the new cases of A. platys were detected in humans diagnosed by PCR (Arraga-Alvarado *et al*., 2014), completely changing the perspective of this bacterium. In the beginning of the study, a new species of zoonosis was introduced (Ferreira *et al*., 2012, Tateishi *et al*., 2014).

For the diagnosis of thrombocytic anaplasmosis, both in humans and dogs, it is based on the demonstration under light microscopy, the presence of basophilic inclusions in platelets, in a blood smear stained with a Romanoswky staining (Wright, Giemsa Or Diff Quick). However, this technique is considered low sensitivity. More specific diagnostic tests, such as rapid ELISA tests (IDEXX 4 Dx), direct antibody fluorescence and PCR (Arraga-Alvarado *et al*., 2003; Albrego *et al*., 2012; Alleman *et al*., 2016). As a treatment of choice in dogs doxycycline at doses of 5 mg per kg, Bid, for 4 weeks (IDEXX, 2014), in cases of bacterial resistance or in puppies to avoid adverse effects by doxycycline, can be implemented Of imidocarb dipropionate (2 doses of 5mg / kg with an interval of 14 days between both, subcutaneous route) (Beaufils *et al*., 2002; Rodríguez, 2002). In humans, the dose of doxycycline used was 4.4 mg / kg / day divided into two doses for 21 days (Arraga-Alvarado *et al*., 2014).

METHODOLOGY

The study was carried out in the laboratory of clinical analyzes of the veterinary hospital for small species of the Autonomous University of Tamaulipas.

Geographical location of the State of Tamaulipas,

During a period from February 2, 2016 to February 2, 2017, 362 venous blood samples were processed preserved in EDTA collection tubes. Blood was obtained by puncture of any of these three veins (cephalic, jugular or saphenous). Sampling dogs varied in age, race, and sex. Once the samples were obtained, they worked on the same day, performing the whole procedure for blood counts. The blood smear was made using two slides, one in which a small drop of blood was placed in the sample with the aid of a capillary tube And the other lamella was used to make the spread.

After the blood smear was done, the Diff quick® dye was dyed, passing the lamella for 2 to 3 seconds in the fixative alcohol and 4 to 5 seconds in the remaining two dyes, starting with the acid dye (orange) and Ending with the basic dye (blue). At the end, the surplus of the dye was removed by washing with distilled water under a slow jet. Finally, we waited for the preparation to dry, and once it was dry, a drop of dipping oil was added and we proceeded to observe using a microscope composed of light with the objective of 100x.

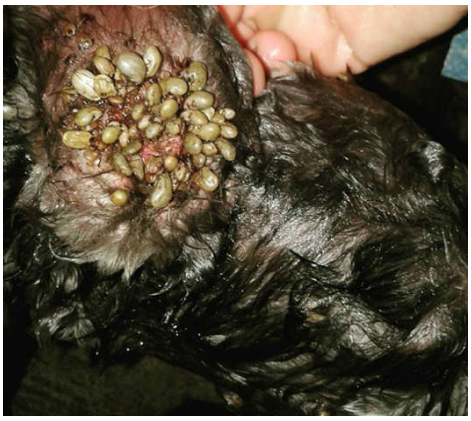

Figure 1. Severe infestation in a domestic canine, by the brown tick (Rhipicephalus sanguineus) Photograph taken by the author

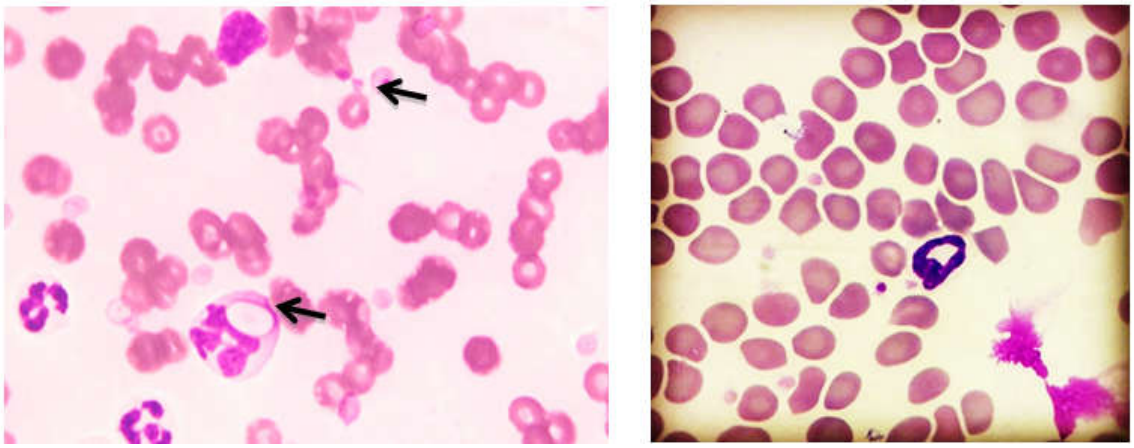

Figure 2. Mixed hemoparasite infection. Anaplasma platys (black arrow) is observed. And you can see Hepatozoon canis (arrow). Photo courtesy of Barron Vargas C A. DVM. Stain Diff quick® Obj. 100x Oil

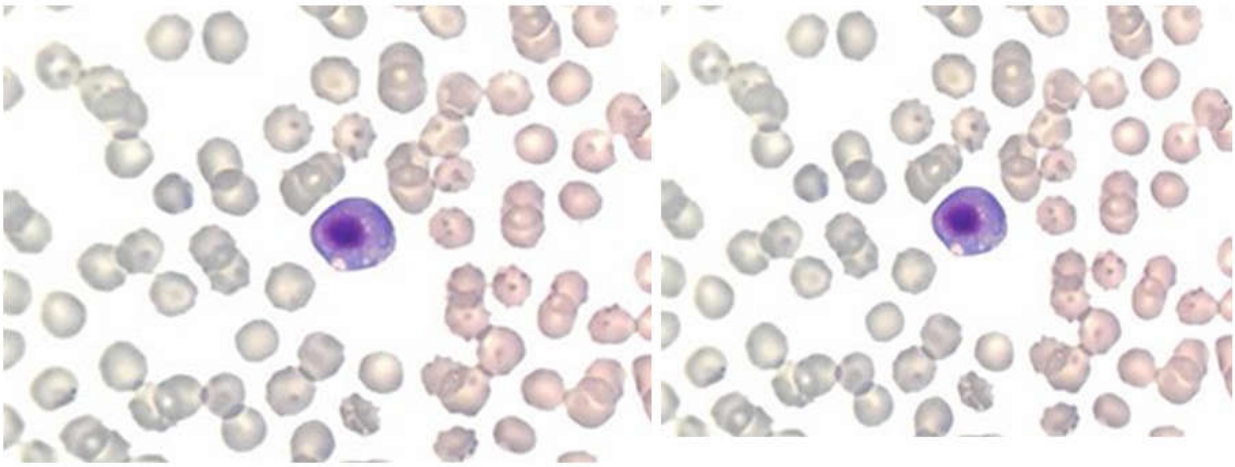

Figure 3. Macroplot infected with an Anaplasma platys morula (black arrow). Photograph taken by the author. Stain Diff quick® Obj. 100x Oil

About 30 smear fields were observed in which the platelets were located in search of basophilic cytoplasmic inclusions (Figure 3 and 4), (positivity criterion). If nothing was observed in 30 fields it was given as negative. Basic descriptive statistics were used for the statistical analysis.

Prevalence = total number of positive samples x 100

N ° total of analyzed samples

RESULTS AND DISCUSSION

The study was carried out according to the method described, obtaining a total of 362 canine blood samples. Of the 362 samples, 65 were positive for *Anaplasma platys*.Of the 65 positive, 27 were females and 38 males. The Chihuahuan breed is the most affected (18), followed by the Creoles (12) and the Pit Bull (8).The prevalence results from the study are shown in Total; 65 = Prevalence(%)=17.95. The study showed us a prevalence of 17.95%, considered high (above 10%). However, it is very similar to that obtained by Abarca *et al*. (2007) in a sample in Chile where it obtained a prevalence of 20%, similarly (Ferreira *et al*., 2012) in Brazil with a prevalence of 19.4% and Huang *et al*., 2004) in Venezuela with a prevalence of 16%.

The technique used has a low sensitivity. However, the confidence index of this study was considered acceptable, because (Martínez-Álvarez, 2014) conducted a seroprevalence study in canines of Ciudad Victoria, Tamaulipas, using the IDEXX® rapid ELISA 4Dx test for the Detection of antigen against *Dirofilariaimmitis* and antibodies to *Ehrlichia spp, Anaplasma spp. And Borrelia spp*. Obtaining a prevalence for *Anaplasma spp.* Of 22.68%, which when compared with our prevalence obtained differs only 4.73%. Although the study of Martínez-Álvarez, 2014, measured antibodies and this the presence of the bacterium (antigen), the prevalences agree without much error, and the data obtained in this study are feasible**.**

Conclusion

It was possible to measure the prevalence of *Anaplasma platys* in the canine population of Victoria, Tamps, Mexico. This value (17.95%) gives us a warning signal, which we have to interpret, since once its zoonotic potential is proven, it poses a risk to public health, together with the possibility of a mixed infection by several rickettsia, Increasing the risk of human infection. However, efforts have to be made to study and control the vector and to make diagnostic tests, such as the ELISA test, available to state laboratories for rapid and accurate diagnosis. With the results obtained it is concluded that canine *Anaplasmosis* is in an emerging infectious disease status.

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