



Abanico Veterinario. January-December 2021; 11:1-15. <http://dx.doi.org/10.21929/abavet2021.45>  
Original Article. Received: 23/06/2021. Accepted: 17/12/2021. Published: 30/12/2021. Code: e2021-42.  
[https://www.youtube.com/watch?v=xFBCNm\\_zrmo](https://www.youtube.com/watch?v=xFBCNm_zrmo)

## Molecular detection of *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia rickettsii* in domestic canines from the municipality of Cajeme, Sonora, Mexico

Detección molecular de *Ehrlichia canis*, *Anaplasma platys* y *Rickettsia rickettsii* en caninos domésticos del municipio de Cajeme, Sonora, México

Aragón-López Carlos<sup>1\*</sup> , Luna-Nevárez Pablo<sup>1</sup> , Ortiz-Encinas Veronica<sup>1</sup> ,  
Leyva-Corona Jose<sup>1</sup> , Cantú-Soto Ernesto<sup>2</sup> , Reyna-Granados Javier<sup>1\*\*</sup> 

<sup>1</sup>Instituto Tecnológico de Sonora, Departamento de Ciencias Agronómicas y Veterinarias, Ciudad Obregón, Sonora, México. <sup>2</sup>Instituto Tecnológico de Sonora, Departamento de Biotecnología y Ciencias Alimentarias, Ciudad Obregón, Sonora, México. \*Responsible author: Aragón-López Carlos. \*\*Autor for correspondence: Reyna-Granados Javier. Departamento de Ciencias Agronómicas y Veterinarias, Instituto Tecnológico de Sonora. Antonio Caso 2266, Villa Itson, C.P. 85130, Unidad Obregón, Campus Náinari. Ciudad Obregón, Sonora, México. E-mail: carlos.aragon@itson.edu.mx, pluna@itson.edu.mx, veronica.ortiz@itson.edu.mx, jose.leyva@itson.edu.mx, ernesto.cantu@itson.edu.mx, javier.reyna@itson.edu.mx

### ABSTRACT

Zoonoses are a worldwide problem with an impact on animal health. This group of diseases include Ehrlichiosis, Anaplasmosis and Rickettsiosis, in which their vector is the tick *Rhipicephalus sanguineus*, commonly known as the brown dog tick. Current evidence indicates this microorganism acts as vector of bacterial zoonotic agents including *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia rickettsii*, which have infected a large number of dogs and humans in northern Mexico. This study was conducted in the municipality of Cajeme, Sonora, using blood samples (n=170) from canine. Molecular techniques were used to detect 92 samples positive for *Ehrlichia spp.*, 47 for *Ehrlichia canis*, 18 for *Anaplasma platys* and 2 for *Rickettsia spp.* In addition, co-infection with *Ehrlichia canis* was found in 12 samples positive for *Anaplasma platys*. Sequencing of one positive of each bacterium was performed, obtaining 100% homology in the "GenBank" platform (NCBI). Our results emphasized the importance of the zoonotic impact and co-infection of these diseases. Moreover, this is the first study confirming the molecular identification of the species *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia rickettsii*, as well as their co-infection, in domestic dogs located in the municipality of Cajeme, Sonora.

**Keywords:** zoonoses, Ehrlichiosis vectors, co-infection, molecular techniques.

### RESUMEN

Las zoonosis son un problema mundial con un impacto en la salud animal. Este grupo de enfermedades incluye Ehrlichiosis, Anaplasmosis y Rickettsiosis, en las cuales el vector es la garrapata *Rhipicephalus sanguineus*, comúnmente conocida como garrapata marrón del perro. Recientes evidencias indican que este microorganismo actúa como vector de agentes zoonóticos bacterianos como *Ehrlichia canis*, *Anaplasma platys* y *Rickettsia rickettsii*, que han infectado a un gran número de perros y humanos en el norte de México. Este estudio se realizó en el municipio de Cajeme, Sonora, utilizando muestras de sangre (n = 170) de canino. Se utilizaron técnicas moleculares para detectar 92 muestras positivas para *Ehrlichia spp.*, 47 para *Ehrlichia canis*, 18 para *Anaplasma platys* y 2 para *Rickettsia spp.* Además, se encontró coinfección con *Ehrlichia canis* en 12 muestras positivas para *Anaplasma platys*. Se realizó la



secuenciación de un positivo de cada patógeno, obteniendo 100% de homología en la plataforma "GenBank" (NCBI). Nuestros resultados enfatizaron la importancia del impacto zoonótico y la coinfección de estas enfermedades. Además, este es el primer estudio que confirma la identificación molecular de las especies *Ehrlichia canis*, *Anaplasma platys* y *Rickettsia rickettsii*, así como su coinfección, en perros domésticos ubicados en el municipio de Cajeme, Sonora.

**Palabras clave:** zoonosis, vectores de Ehrlichiosis, coinfección, técnicas moleculares.

## INTRODUCTION

Zoonoses involve several diseases that represent a significant worldwide problem affecting human and animal health (García *et al.*, 2013). Such diseases include Ehrlichiosis, Anaplasmosis and Rickettsiosis which are caused by a gram-negative bacteria characterized by intracellular obligated growth (genus *Rickettsia*, family *Rickettsiaceae*; genus *Ehrlichia* and *Anaplasma*, family *Anaplasmataceae*). These are mainly transmitted by ectoparasites, including *Rhipicephalus sanguineus*, the brown dog's tick, which affect ground vertebrates (Parola *et al.*, 2009; Alvarez, 2017). Several studies have demonstrated these ectoparasites act as vectors from zoonotic agents including *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia rickettsii* (Gaunt *et al.*, 2010).

*E. canis* causes a zoonotic disease in dogs, cats and rodents; then, the human is an accidental victim after being stung by the *Rhipicephalus* tick hosted in these animals. After the infection, the microorganism is incubated during 1-2 weeks and enters into the blood and lymphatic vessels; then, it travels to the spleen, liver and lymph nodes to be multiplied by binary fission for spreading to other body organs (Castro *et al.*, 2004).

*E. canis* is considered as a bacterium of cosmopolitan distribution. In Mexico, it was first described in 1996 and classified as endemic because it has been reported in the whole country, but mainly in northwest states such as Sonora and Sinaloa. In Sonora, *E. canis* is considered as a health emergency and a growing public health issue. Since 2002, more than 600 cases have been reported, the vast majority in municipalities in the north of the state (Álvarez, 2017; Sosa *et al.*, 2013).

Clinical signs of the acute phase of the disease are hematologic alterations, leukopenia, thrombocytopenia and mild to moderate anemia; the chronic phase is characterized by thrombocytopenia, epistaxis, nephropathy, dyspnea, hepatomegaly, splenomegaly or lymphadenopathy, inflammatory or hemorrhagic meningitis, among others (Ismail *et al.*, 2010).

*A. platys* is distributed worldwide and it is also transmitted by the ticks *R. sanguineus*. It was first described in 1978 in dogs from United States (Sánchez & Tesouro, 2001). This bacteria belongs to the *Anaplasma* genus (Ábrego *et al.*, 2009). It causes canine infectious cyclic thrombocytopenia. The disease may present with fever, anorexia, petechiae, uveitis, generalized lymphadenopathy, leukopenia, moderate anemia and especially thrombocytopenia, occurring in episodes of 3-4 days at intervals of 7-21 days, eventually leading to chronic thrombocytopenia with slow recovery (Cicutin *et al.* 2014).



Currently, *A. platys* has been detected with low incidence in the states of Coahuila, Durango and Sonora (Almazán *et al.*, 2016; Murrieta *et al.*, 2017). As zoonotic disease, Arraga *et al.* (2014) reported two women in Venezuela who were exposed to *R. sanguineus*. Intraplatelet inclusion bodies suggestive of *A. platys* were subsequently observed in smears and *A. platys* DNA was amplified and sequenced from whole blood, although treatment with doxycycline did not alleviate their symptoms. These cases provide additional support for *A. platys* as a tick-borne zoonotic pathogen, most likely of low pathogenicity; however, the cause of *A. platys* disease in humans has not been confirmed. *R. rickettsii* is the main agent of spotted fever in the Americas; cases have been reported in the U.S., Canada, Mexico, Costa Rica, Panama, Colombia, Brazil and Argentina (López *et al.*, 2007; Herrero *et al.*, 2010; Lebruna *et al.*, 2011), with a high mortality rate due to late detection of the pathogen (Oteo *et al.*, 2014). It is considered that Mexico has the ideal conditions for the transmission cycle of the disease, commonly associated with living conditions (poverty) since most of the cases presented have been detected in marginalized and rural areas of Mexico (Peniche *et al.*, 2015). Recent studies demonstrated the presence of *Rickettsia spp.* in ticks from the state of Sonora by PCR technique (Foley *et al.*, 2019).

These pathogens could be present previously in the tick, causing simultaneous co-infections in the host (i.e. more than one agent can be transmitted), which will be causing the disease at the same time. This can result in the manifestation of clinical signs, in a more severe and non-specific way, being a disadvantage for the clinical diagnosis by human and veterinary physicians attending these cases (Alleman & Wamsley, 2008; Mutz *et al.*, 2009).

Considering the zoonotic importance of these diseases, and that no previous studies have been reported in the municipality of Cajeme, Sonora, the presence of vectors of *E. canis*, *A. platys* and *R. rickettsii* in domestic dogs in the municipality is suggestive of the presence of these zoonotic diseases. Therefore, the objective was the molecular identification of the species *E. canis*, *A. platys* and *R. rickettsii* to determine their co-infection in domestic dogs in the municipality of Cajeme, Sonora.

## MATERIAL AND METHODS

### Type of study

A descriptive observational study was conducted to detect the presence of *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia rickettsii*.

### Study area

The present work was carried out from blood samples of dogs sent to the Laboratory of Molecular Biology of Veterinary Medicine and Zootechnics of the Technological Institute



of Sonora, coming from private veterinary clinics in the town of Cajeme in the state of Sonora, Mexico.

### **Experimental sampling**

We used 170 canine whole blood samples with EDTA anticoagulant from a commercial veterinary diagnostic laboratory located in Ciudad Obregon, Sonora. Only canines with presumptive clinical diagnosis or positive for *E. canis*, *A. platys* and *R. rickettsii* were selected based on the symptomatology presented and complementary analyses performed, such as blood smears and hemograms. The samples were transported to the Veterinary Molecular Biology Laboratory at ITSON and in all cases were kept at 4°C until their final processing, for a period of no more than 24 hours.

### **Blood sample processing**

The samples were thawed and centrifuged for 15 minutes at 3,500 rpm in order to obtain 200 µl of leukoplatelet layer and start the extraction of the genetic material.

### **Extraction of bacterial DNA**

For DNA extraction from blood samples, the commercial DNeasy Blood and Tissue Kit (QIAGEN®) were used following the manufacturer's instructions. The quantity, quality and purity of DNA were measured in an automatic spectrophotometer BioSpect-nano (Shimadzu), and the integrity was observed in 1.5% agarose gel stained with 1.5 µl ethidium bromide.

### **Synthesis of positive controls**

The BLASTn GenBank® tool of the NCBI (National Center for Biotechnology Information) database was used to download the sequences obtained from the alignments. Such alignments were performed using the specific primers of the bacteria *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia rickettsii*, in FASTA format. We used 50 bp upstream from the first forward and 50 bp downstream from the first reverse where the oligonucleotides were aligned. The sequences were entered into the IDT (Integrated DNA Technologies) platform for the synthesis of the gBlocks™ gene fragments, facilitating standardization for PCR detection of each of the microorganisms under study.

### **Amplification by PCR**

The DNA samples were analyzed by polymerase chain reaction (PCR) using the primer sets described in Table 1. Initially, PCR assays targeted the genera *Ehrlichia spp.*, *Anaplasma spp.* and *Rickettsia spp.* Samples that tested positive for each genus were subjected to their second PCR with primers specific for *E. canis*, *A. platys* and *R. rickettsii*. For the reactions was used the GoTaq® Flexi DNA Polymerase PCR preloaded kit (Promega) containing Green GoTaq®, which serves as reaction buffer and gel loading



solution, allowing to load reactions directly for fast and efficient analysis. Reactions were run in a final volume of 25  $\mu$ l, starting with the manufacturer's concentrations: 1X Green GoTaq Buffer 5x, 1.5mM MgCl<sub>2</sub>, 0.2 mM for each dNTP, 0.4  $\mu$ M of each primer, 1.25u of GoTaq DNA Polymerase, 2  $\mu$ l of DNA and nuclease-free H<sub>2</sub>O to 25  $\mu$ L. The product was identified on 1.5% agarose gel and ethidium bromide, considering positive bands with the size of each agent.

**Table 1. Primers used for agent detection in canine blood samples**

Agent	Primers (5'-3')	Gen	pb	Primer Tm	ID sequence	Reference
<i>Ehrlichia</i> spp.	ECC-AGAACGAACGCTGGCGGCAAGCC ECB-CGTATTACCGCGGCTGCTGGC	16S	478	61 °C	MH020203.1	Dawson <i>et al.</i> (1996)
<i>Rickettsia</i> spp.	CS78- GCAAGTATCGGTGAGGATGTAAT Cs323- GCTTCCTTAAAATTCAATAAATCAGGAT	<i>gltA</i>	401	48 °C	MG717529.1	Labruna <i>et al.</i> (2004)
<i>Ehrlichia canis</i>	HE- TATAGGTACCGTCATTATCTTCCCTAT ECA- CAATTATTTATAGCCTCTGGCTATAGGAA	16S	389	57.4 °C	KX818219.1	Murphy <i>et al.</i> (1998)
Anaplasma platys	pla- HS475- AAGGCGAAAGAAGCAGTCTTA pla-HS1198- CATAGTCTGAAGTGGAGGAC	<i>groEl</i>	724	58 °C	EU516386.1	Inokuma <i>et al.</i> , 2002
<i>Rickettsia rickettsii</i>	Rr190.70p- ATGGCGAATATTTCTCCAAAA Rr190.602n- AGTGCAGCATTCGCTCCCCCT	<i>ompA</i>	530	48 °C	U55822.1	Regnery <i>et al.</i> , 1991

### Sequencing and in silico analyses of amplified fragments

A PCR product from each microorganism that was positive, it was also purified with the commercial kit Wizard® SV Gel and PCR Clean-Up System (Promega) to corroborate that the amplified fragment belongs to the regions of interest. Amplicons were then sequenced using the Sanger process performed at Langebio Cimvestav Lab Irapuato unit. The fragments generated with nucleotide sequences representative of each bacterium were analyzed with the Snappene Viewer program, and subjected to the BLASTn algorithm to evaluate the percentage of homology of each agent with the sequences available in the GenBank database.

## RESULTS

It was observed by electrophoresis that all the extracted DNA showed a good integrity of the molecules and yielded an average quantification by spectrophotometry of 248.32 ng/ $\mu$ l in the leukoplatelet layer samples. A purity value of 1.85 measured through the ratio 260-280 indicate the DNA as "pure", using samples from whole blood with EDTA.

For the PCR technique, a concentration of 0.5 ng/ $\mu$ l of each gblocks was used as positive controls, obtaining the corresponding base pairs of each agent, achieving good efficiency and speed for molecular standardization.



The number of positive cases and frequency of *Ehrlichia spp.*, *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia spp.* obtained from molecular analysis of canine blood samples (n=170) are described in Table 2. Of the 92 samples that tested positive for *Ehrlichia spp.* 12 co-infections (Table 3) of *Ehrlichia canis* with *Anaplasma platys* (Genus EA) were found (13%).

**Tabla 2. Number of cases and frequency of *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia rickettsi* in dogs from Cajeme, Sonora, by PCR (n = 170)**

Agente	PCR	
	Positives	Frequency
<i>Ehrlichia spp</i>	92	54%
<i>Ehrlichia canis</i>	47	28%
<i>Anaplasma platys</i>	18	11%
<i>Rickettsia spp.</i>	2	0.8%

**Table 3. Co-infections and frequency in positive dogs to the genus EA**

Co-infecciones	PCR
( <i>E. canis</i> / <i>A. platys</i> )	Positives 92
Positives	12
Frequency	13%

In addition, sequencing of 1 sample of each positive for the different species of microorganisms analyzed was performed, obtaining pure chromatograms analyzed with the SnapGene Viewer program. The analysis of the sequences in the BLASTn program (National Center for Biotechnology Information) detected a similarity between 99 and 100% with the previously reported sequences.

## DISCUSSION

Microorganisms of the order Rickettsiales have zoonotic conditions called Rickettsiosis, Ehrlichiosis and Anaplasmosis that are due to several pathogens of veterinary importance that have gained ground not only in our region but also worldwide (Rodríguez *et al.* 2016), this is mainly due to its vector *Rhipicephalus sanguineus*, being the most commonly reported tick species and with the greatest geographic distribution (Sosa *et al.*, 2016; Cabezas-Cruz *et al.*, 2019). These pathogens are increasing due to the activity of human beings that has generated radical changes in the environment, favoring vector-borne diseases (Suthers, 2004) and increasing their prevalence in late spring and summer. Due to the fact that these ectoparasites increase their activity in high ambient temperatures during these seasons of the year, inoculating the mentioned hemoparasites more quickly (Parola *et al.*, 2009), necessitating the standardization of precise techniques for the



detection of these zoonotic microorganisms of the *Rickettsiales* order in Tropicales and Subtropicales regions.

Currently using gBlocks gene fragments as positive controls used in this study have become popular as standards and synthetic positives for the detection of bacterial microorganisms. Currently similar data were reported in Barcelona where this type of fragments were used for detection of *E. coli*, *E. faecalis* and *Legionella pneumiphilia* (Cardenas, 2018); also, in Australia such fragments were used as standards in the multiplex PCR detection of *Taenia spp.* (Ng-Nguyen *et al.*, 2017) Therefore, the use of gBlocks for PCR standardization is recommended as it increased the speed of the bioassay in detection due to the lack of availability of biological isolates.

The study was the first work using PCR to detect the species *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia rickettsi* in blood samples from dogs in the municipality of Cajeme and in Sonora, allowing to know the expanded geographical distribution of the three pathogens in the state. The percentage of 54% to at least one pathogenic agent in our study agrees with investigations performed using molecular tools in some South and Central American countries, where Brazil reported 69% (Tanikawa *et al.*, 2013), Nicaragua 80% (Wei *et al.*, 2014), Panama 70.6% (Santamaria *et al.*, 2014), Costa Rica 45% (Rojas *et al.*, 2015) and El Salvador 60% (Miranda *et al.*, 2018). Our prevalence percentage differed when was compared with high percentages of other countries, probably because they worked with dogs without owners and being more in contact with the vector of the diseases as it is associated with living conditions in marginalized areas (Peniche *et al.*, 2015). Prevalence to hemoparasites have been reported in some other states of Mexico, where Sinaloa reports 74.3% (Sosa-Gutiérrez *et al.*, 2013), Coahuila and Durango 41% (Almazán *et al.*, 2016), Yucatán 69% (Díaz *et al.*, 2016), Chihuahua 40% (Escárcega *et al.*, 2018) and Sonora 33% (Murrieta *et al.*, 2017). As early mentioned, Cajeme yielded 57% of infection, positioning Sonora at this time as one of the main states with the highest incidence in tropical and subtropical regions where parasites and the vector are present.

Regarding the percentage of presence of each hemoparasite, our study evidenced the higher prevalence of *E. canis* reported in Mexico (28%). Other studies reported lower results. In the municipality of San Luis Rio Colorado, 8% of infection for *E. canis* was found in blood samples from 235 dogs (Murrieta *et al.*, 2017); also, in the State of Coahuila and Durango 10% was found in a population of 100 healthy dogs infested with ticks (Almazán *et al.*, 2016). In Yucatan, 36% was determined in a population of 50 dogs (10 domestic dogs and 40 in an animal control center), where all positive dogs were from samples collected from the animal shelter, representing a prevalence of 45% for this sampling site (Path *et al.*, 2015). There are also findings in other countries such as Buenos Aires, Argentina, where the results were similarly lower in a population of 223 dogs, obtaining 6.7% of positives to *E. Canis* (Cicuttin, 2016), Uruguay is one of the few countries which reported the presence of other hemoparasites, but not the presence of *E. Canis* in a population of 191 dogs (Carvalho, 2017). In contrast to Brazil, the percentage in our study was lower because the worked with 472 dogs in the Brazilian northeast detecting 34.5% of positive dogs (Silva *et al.*, 2010), Colombia represents the largest area with high



prevalence reviewed on *E. canis*, mainly the municipalities of Palmira (92.8%) and Cartago (90%) (Rojas *et al.*, 2013). However, it is worth mentioning that this work was performed on stray dogs unlike our study.

The presence of *Anaplasma platys* (11%) in our study, turned out to be higher than those reported in Buenos Aires, Argentina of 223 dogs, being positive for *A. platys* 7.2% (Cicuttin, 2016). In Uruguay, out of 191 dogs, 4.2% were positive (Carvalho, 2017). Also in San Luis Rio Colorado, Sonora in 235 canine samples were positive for 18% (Murrieta *et al.*, 2017). Other publications show similar values to Costa Rica with 10% (Wei *et al.*, 2014), Nicaragua 13% (Rojas *et al.*, 2014), Cuba 16% (Silva *et al.*, 2016) and El Salvador with 17% (Murrieta, 2017), but proved to be lower with respect to Panama with 21.3%. In Brazil, in a population of 100 dogs, a higher prevalence was obtained for *Anaplasma platys* with 21% and 9% for *Ehrlichia*, being one of the few studies that differs in our results, since, in our work and most of the research conducted in Central and South America, they report a lower incidence of *A. platys* than *E. canis*.

The molecular diagnosis in our research of hemoparasites related to *Ehrlichia* and *Anaplasma* has been easy to identify, unlike the bacterium *Rickettsia rickettsii* with the PCR technique from blood samples in canines. Our prevalence percentage to this agent was lower (0.8%) compared to *E. canis* and *A. platys*, this may be because it is typically mentioned that low numbers of *rickettsiae* circulate in the blood in the absence of advanced disease or fulminant infection (CDC, 2017; Tinoco *et al.*, 2018).

Due to this we found in the literature different investigations, mostly directed to the diagnosis of *Rickettsia* spp. and *R. rickettsii* in *R. sanguineus* ticks in dogs from different regions, as is the case of Yucatan, selected 28 dogs where 106 *Rhipicephalus sanguineus* ticks were collected with a 26% incidence (Peniche *et al.*, 2015) In Matamoros, Coahuila, the endpoint PCR technique was performed for the analysis of 100 ticks (*Rhipicephalus sanguineus*) giving as positive to *Rickettsia* spp 4% of the samples analyzed (Castillo *et al.*, 2015). In Mexicali Baja California, Mexico, dog ticks belonging to the morphology of *Rhipicephalus sanguineus* were analyzed by means of PCR, resulting in positive samples with 100% homology to *R. rickettsii* (Foley *et al.*, 2019). Currently Sonora leads the entities with the most reports of Rickettsiasis in the country, being Cajeme one of the main cities with more cases of death, despite being a data associated with public health, it is important to know the incidence of *R. rickettsii* in animals (PSS, 2014), giving greater importance to our work, because not only *Rickettsia* spp. but *R. rickettsii* was also detected, with 100% homology to the genus with the *gltA* gene and the species with the *OmpA* gene in blood samples from 2 dogs (n = 170), these genes are the most used for the detection of the bacteria that cause spotted fever.

The results obtained from the three agents, increases the alert and the incidence of cases associated with *Rickettsiales* in the state of Sonora transmitted by the brown tick *Rhipicephalus sanguineus*. It is currently the most important vector of *Rickettsiales* in Mexico (Labruna, 2009), constituting a public health problem, since *R. rickettsii*, *E. canis* and *A. platys* are considered zoonotic disease by the CDC in 2017 and that over the years have acquired greater territory of infection. As mentioned above, these pathogens can be





present in the tick, causing simultaneous co-infections in the host, i.e. more than one single agent can be transmitted, as demonstrated in the present investigation, finding 13% of co-infection to the agents *E. canis* and *A. platys*.

The presence of co-infection with these bacteria in our study is not a strange finding, since they are the most common in Latin America. In our study we have similar percentages obtained in Panama, which report a co-infection between *E. canis* and *A. platys* of 7.5% (Santamaría *et al.*, 2014), El Salvador with 4.5% (Miranda *et al.*, 2018) and San Luis Rio Colorado, Sonora with 12.2% (Murrieta *et al.*, 2017), mentioning that the latter is very similar to our co-infection results and is from the same state.

It is essential to consider that the remaining 46% of the negative samples in the study to genus and species, may be due to the absence of the pathogens or the presence of other diseases, because in the investigation all blood samples came from presumptive dogs or manifested clinical symptoms, although it may also be due to the presence in undetectable low quantities of the pathological agents.

Finally, it is important to highlight that the differences found between the samples positive to genus and negative to *E. canis*, *A. platys* and *R. rickettsii* species are probably due to the fact that the primers used in the first genus PCR (ECC/ECB) also amplify other species, which could indicate infection by other *Ehrlichia* species (*E. ewingii* or others) or other species of the *Anaplasmataceae* family (*A. phagocytophilum* or others).

## CONCLUSIONS

The percentage of haemoparasites in domestic dogs reported in this study, currently positions Sonora as one of the main states with the highest frequency in tropical and subtropical regions. The three agents were detected by molecular techniques in the blood of suspected dogs from the city of Cajeme, Sonora, identifying *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia rickettsii* with frequencies of 28%, 11% and 0.8%, respectively. In addition, these three agents were confirmed by sequencing. Regarding co-infections, only *Ehrlichia canis* and *Anaplasma platys* were detected simultaneously with 13% of frequency.

To our knowledge, this is the first molecular identification investigation in our state, confirming the presence of *Ehrlichia canis*, *Anaplasma platys* and *Rickettsia rickettsii* starting from whole blood of canines. However, further studies are suggested to explore the frequency of these infectious agents in other regions from the state on Sonora.

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