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Molecular detection of *Ehrlichia canis* and *Anaplasma phagocytophilum* and hematological changes of infected dogs

Detección molecular de *Ehrlichia canis* y *Anaplasma phagocytophilum* y alteraciones hematológicas de perros infectados

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ABSTRACT

Ehrlichiosis and anaplasmosis are tick-borne diseases caused by bacteria of the genera *Ehrlichia* and *Anaplasma*. Since clinical manifestations are varied and nonspecific, the diagnosis in clinical practice, remains a challenge for veterinarians. Furthermore, the distribution of these infections includes areas where its tick vector, *Rhipicephalus sanguineus* is present. This study was designed to evaluate the prevalence and factors associated with the presence of *Ehrlichia canis* and *Anaplasma phagocytophilum* in dogs from the central area of Tamaulipas. PCR screened 384 canine blood samples obtained from different veterinary clinics and a shelter. The data were analyzed using the Chi-square test (P level <0.05 for statistical significance). The results showed that 103 (26.8%) out of 384 samples were positive for *E. canis*, while *A. phagocytophilum* was not detected. Statistical analysis did not show relationship between *E. canis* and variables like gender, breed, and origin (P>0.05). Nonetheless, there was a statistically significant difference between infected adult dogs (15-84 months) compared to other age groups evaluated (p<0.05). Regarding hematocrit, platelets count, plasma protein, total and differential white blood cells counts, none of these parameters were significantly different (P>0.05).

Keywords: *Ehrlichia canis*, *Anaplasma phagocytophilum,* PCR, hematological findings.

RESUMEN

Las ehrlichiosis y anaplasmosis canina son enfermedades transmitidas por garrapatas, provocadas por bacterias del género *Ehrlichia* y *Anaplasma*. Debido a sus múltiples manifestaciones clínicas, su diagnóstico es un reto para el veterinario. La distribución de estos hemoparásitos incluye áreas donde su principal vector, *Rhipicephalus sanguineus* está presente. Este estudio fue diseñado para determinar la presencia *Ehrlichia canis* y *Anaplasma phagocytophilum,* así como los factores asociados y hallazgos hematológicos comunes en perros de la zona centro de Tamaulipas. Se evaluaron, a través de PCR, 384 muestras de sangre provenientes de animales de diferentes clínicas veterinarias y un refugio. El análisis de datos se realizó con la prueba Chi cuadrada con un nivel de significancia de 0.05. Los resultados muestran que, del total de muestras 103 (26.8%) resultaron positivas a *E. canis*, mientras que para *A. phagocytophilum* no se detectó ningún caso. No se observó asociación significativa con relación al sexo, raza, ni lugar de procedencia (p>0.05), a diferencia de la edad, donde se encontró mayor prevalencia de *E. canis* para adultos (15-84 meses) (p<0.05). En relación con el hematocrito, conteo de plaquetas, proteínas plasmáticas totales, conteo y diferencial leucocitario, no existió diferencias significativas (p>0.05).

Palabras claves: *Ehrlichia canis*, *Anaplasma phagocytophilum,* PCR, valores hematológicos.

INTRODUCTION

Ehrlichiosis and anaplasmosis are diseases of great importance for both veterinary and public health, as they are responsible for diseases such as Monocytic Ehrlichiosis and Human Granulocytic Anaplasmosis (Vieira *et al*., 2013; Farhan 2015; Rodríguez-Vivas *et al*., 2019). Gram-negative obligate intracellular bacteria cause them. They are known as *Ehrlichia* spp and *Anaplasma* spp respectively (Harrus and Waner 2011; Stuen *et al*., 2013). Worldwide, cases have increased considerably in recent years mainly in tropical and subtropical areas where tick vectors (*Ripicephalus sanguineus* and *Ixodes* spp) proliferate (Beugnet and Chalvet-Monfray 2013; Irwin 2014; Little *et al.,* 2014; Battilani *et al*., 2017). Due to the increasing proximity of people to their pets, the likelihood of bites by these ectoparasites is increasing considerably leading to these infections becoming reemerging zoonoses (Bhadesiya and Modi 2015; Ismail and McBride 2017).

In Mexico, canine Ehrlichiosis was reported for the first time in 1996, since then the number of cases has increased considerably (Maggi and Krämer 2019); however, the diagnosis, in many occasions is based on clinical signs without performing laboratory tests that directly or indirectly corroborate its presence. Definitive diagnosis focuses on microscopic techniques; however, these methods have low sensitivity and specificity in patients with low bacteremia, which prevents establishing adequate therapeutics (Harrus and Waner 2011; Allison and Little 2013). In response to this, Polymerase Chain Reaction (PCR) emerges as an important tool to support conventional diagnostic methods (Almazan *et al.,* 2016; Cetinkaya *et al*., 2016; de la Fuente *et al*., 2017).

Tamaulipas state due to its geographical location has ideal characteristics that favor the development of ticks vectoring these diseases (Tinoco-Gracia *et al*., 2009); however, the true magnitude of this problem is unknown. Therefore, the main objective of this research was to determine the presence of *E. canis* and *A. phagocytophilum*, through PCR, in naturally infected dogs in the central zone of Tamaulipas; as well as to evaluate some factors associated with the presence of these diseases.

MATERIAL AND MÉTODOS

Study area

The present work was carried out with blood samples from dogs submitted (during the period March 2020 to March 2021) to the Laboratory of Parasitology and Clinical Analysis of the Faculty of Veterinary Medicine and Zootechnics "Dr. Norberto Treviño Zapata", belonging to the Autonomous University of Tamaulipas. Also, samples from several private veterinary clinics in the capital of Tamaulipas and some surrounding municipalities.

Study population

A non-probabilistic sampling was used. Samples from patients referred with the following inclusion criteria were analyzed: 1) being from Tamaulipas state (central zone), 2) presenting clinical signs related to hemoparasites (fever, diarrhea, uveitis, petechiae, epistaxis, osteoarticular, and respiratory, reproductive and neurological disorders), 3) presenting or having been in contact with ticks, and 4) having the consent of the pet owner. The sample size was 384 animals, which is the minimum sample size obtained from the formula of (n) for infinite population proportions, since there is no canine population census in the area to be evaluated (Wayne and Chad 2013). All dogs were handled according to the official animal welfare standards established by the Bioethics Committee of the Faculty of Veterinary Medicine and Zootechnics of the Autonomous University of Tamaulipas.

Sample collection

A minimum of 3 ml of blood was obtained by venous puncture (cephalic vein), which were rapidly transferred to a tube (BD Vacutainer®) with EDTA K_2 (ethylenediaminetetraacetic acid potassium) anticoagulant. Samples were kept refrigerated (8°C) for no more than 24 hours before processing for hematological evaluation. An aliquot of blood was saved in 1.5 ml vials and was stored at -20°C for subsequent DNA extraction and PCR testing. In all cases, the age, sex, pedigree and season of the year in which the sample was taken from the individuals studied were recorded.

Hematological analysis

The determination of hematological parameters was performed immediately, within 4 hours of blood collection to avoid morphological alterations of cells. The samples were analyzed in an automated equipment (Auto Hematology Analyzer, MINDRAY, BC-2800 Vet; Shenzhen, China). For the determination of plasma proteins, the microhematocrit method was used, using capillary tubes without heparin; which were filled with ¾ parts with blood, sealed and centrifuged (centrifuge KHT-410E Kendal Import S.A.C Gemmy Taiwan) at 11,500 rpm for 5 min. The plasma obtained was placed in a refractometer (American Optical) and total proteins were obtained. The leukocyte differential count was performed manually. The first consisted of assessing and counting in a blood smear (stained with Diff-Quik™) 100 nucleated cells and thus obtaining the percentage count of the different leukocytes: neutrophils, eosinophils, lymphocytes, monocytes and basophils. To determine whether anaemia was present, the hematocrit value was taken into account, which was categorized into 2 groups, with and without the presence of anaemia. The platelet count and total protein were divided into 2 groups, animals with and without thrombocytopenia and with and without the presence of hyperproteinemia, respectively. Total leukocytes as well as their different populations were grouped as normal, high and decreased counts.

Identification of hemoparasites by microscopy

For the search for hemoparasites by microscopy, blood smears were prepared, fixed with methanol for 5 minutes and stained with 10% Giemsa solution for 15 min. Subsequently, multiple random areas of the monolayer and tail of the smear were evaluated under the microscope with the immersion objective (100x); here we looked for the presence of morulae (cytoplasmic aggregates of basophilic color) or any other inclusion body compatible with hemoparasites (Dulmer *et al.,* 2001).

MOLECULAR ANALYSIS

Obtaining nucleic acids

From the stored EDTA blood aliquots, DNA extractions were performed using the commercial DNA extraction and purification kit (Wizard® Genomic DNA Purification-Promega), according to the protocols established by the company. The total DNA extracted was quantified, using a spectrophotometer (NanoDrop2000®, Thermo Scientific, Waltham, MA, USA) and stored at -20 °C until further use in PCR assays.

Polymerase Chain Reaction

For molecular analysis, a region of the GltA gene (used for identification of rickettsiae coding for the enzyme citrate synthase) for *E. canis* and a region of the Msp4 gene (major surface complex) for *A. phagocytophilum* were amplified. The GoTaq® Green Master Mix kit (Promega, Madison, WI USA. Cat. Num: M7122) was used according to protocols established by the company. For this, 21 µl of kit solution, 1 µl of sense primer, 1 µl of antisense primer and 2 µl of DNA from each sample were used to reach a final volume of 25 µl. The samples were then amplified in the thermal cycler (Applied Biosystems™ Num: 2720) with the amplification protocol shown in Table 1. The amplified products were analyzed by 2% agarose gel electrophoresis in 600 ml TAE Buffer, 1X (Promega, Madison, WI USA. Cat. Num: V4271) at 120 V for 40 min using the nucleic acid dye Diamond Nucleic Acid Dye (Promega, Madison, WI USA. Cat.Num: H1181) and subsequently visualized under UV light from the UVP transilluminator (Ultraviolet Products, Inc., California, USA. Cat. Num: TFM-30). DNA fragments of known lengths (E. canis, 200 bp; *A. phagocytophilum*: 980 bp) and a 100 bp DNA Ladder molecular weight marker (Promega, Madison, WI USA. Cat. Num: G210A) were used as a positive control for reference.

Table 1. Sequence of oligonucleotides used for each pathogen, amplification protocol and size of amplified

Organism and target gene	Oligonucleotide sequence (5'-3')	Amplification program	Amplified size (bp)	Reference
E. canis (GltA)	*E. canis* Fw ATAAACACGCTGACTTTACTGTTCC *E canis* Rev GTGATGAGATAGAGCGCAGTACC	95°C for 5 min 94°C for 30 s 60 °C for 30 s 72 °C for 1 min 72 °C for 7 min 35 cycles	200	Stich *et al.*, 2002
A. phagocytophilum (Msp4)	MSP4AP5 ATGAATTACAGAGAATTGCTTGTAGG MSP4AP3 TTAATTGAAAGCAAATCTTGCTCCTATG	94°C for 5 min 94°C for 30 s 50 °C for 30 s 72 °C for 30 s 72 °C for 7 min 35 cycles	849	Yousefi *et al*., 2019

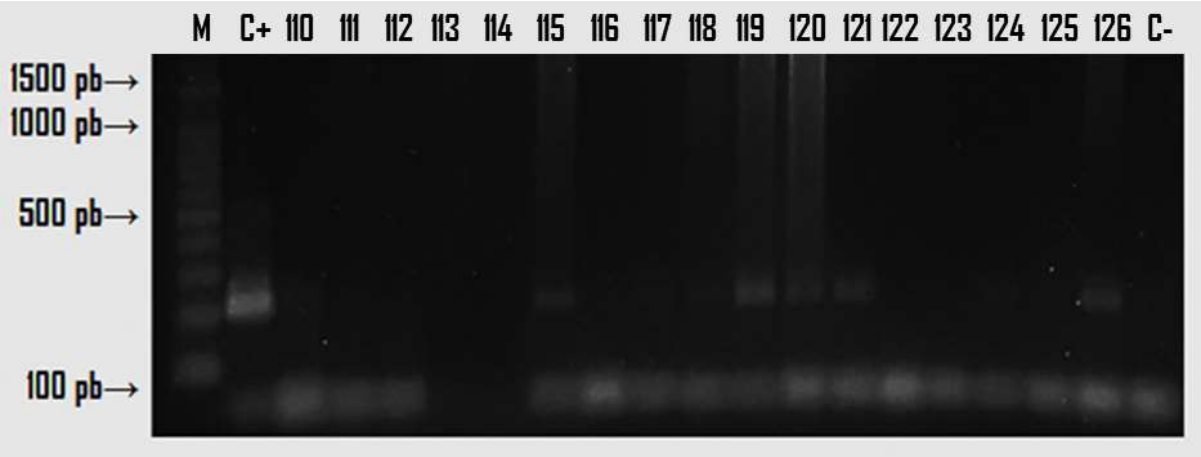
Statistical analysis

Absolute frequencies and percentages of positive cases and hematological findings were represented. The degree of association between the presence of pathogens and the variables evaluated (sex, age, pedigree, time of year and hematological parameters) were analyzed by the Chi-square test of independence with a significance level of 0.05, using the statistical program MedCalc. V. 7.0.

RESULTS

Presence of *E. canis* and *A. phagocytophilum*

From the total number of samples analyzed (384) during the study period, 103 were positive for *E. canis* (frequency of 26.8%) by PCR technique, where the GltA gene was amplified with an expected molecular size of 200 bp, as shown in Figure 1. From the blood, smears evaluated, *E. canis* was identified in only 41 of the samples evaluated (10.7%). Morulae were observed in the cytoplasm of lymphocytes and monocytes as round structures, with a size between 4 to 6 µm in diameter that stained strongly basophilic in color; as shown in Figure 2. On the other hand, none of dogs evaluated by PCR or smear evaluation was positive for *A. phagocytophilum* (Figure 3).



(M) Molecular weight primer, (C+) positive control with 200 bp molecular weight, (115, 117,118, 119, 120, 121, 126) positive samples, (C-) negative control with double distilled water. 2% agarose gel, stained with Diamond.

Figure 1. PCR amplification of *E. canis* in blood samples taken from canines

Characteristics of the dog population

A number of 192 females (50%) and 192 males (50%), ranging in age from 3 months to 20 years, were evaluated. The observed results show that *E. canis* does not distinguish between genders, since within the infected group the percentages of females (29.7) and males (24.0) were not statistically significant ($p>0.05$). When evaluating the relationship between dog age (puppies, adults or seniors) and the percentage of *E. canis* positives, it was determined that there is a significant relationship between both variables, where the adult condition (1 to 7 years) is related to the presence of the disease ($p<0.05$) (Table 2).

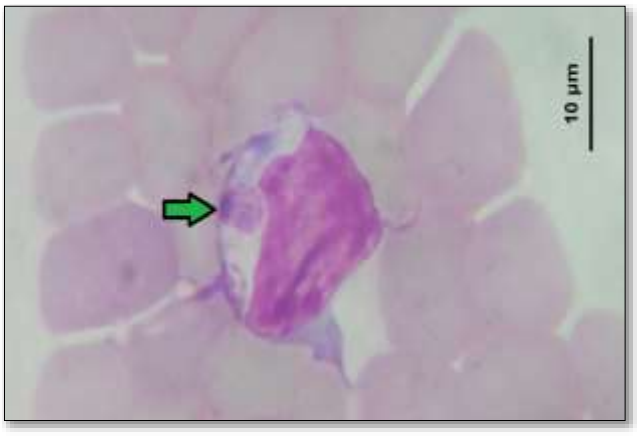
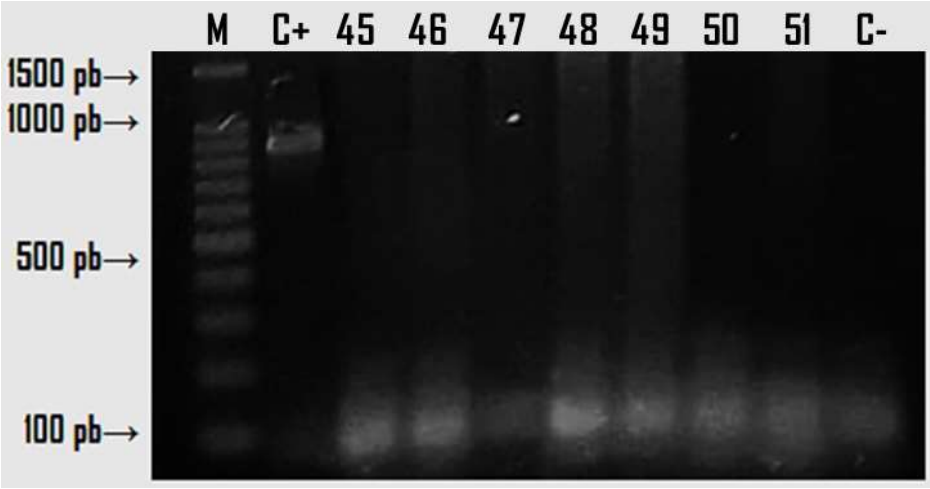


Figure 2. Lymphocyte in peripheral blood of a canine infected with an *E. canis* morula (arrow). Giemsa stain 10%



(M) Molecular weight primer, (C+) positive control with molecular weight of 849 bp, (45-51) negative samples, (C-) negative control with double distilled water. 2% agarose gel, stained with Diamond.

Figure 3. PCR amplification of *A. phagocytophilum* in blood samples taken from canines

Pedigreed dogs represented 81% (311/384) of the study population and crossbreed constituted 19% (73/384); however, the chi-square test of independence found no significant statistical difference between *E. canis* positive result, in relation to the defined racial groups and crossbreed (p>0.05) (table 2). Similarly, no significant differences were found between the presence of Ehrlichiosis and the year season (Table 2).

Table 2. Frequencies and percentages of *E. canis* positives and negatives grouped by animal characteristic and season of year

Variable	Presence of *E. canis*				Value of p
	Positive		Negative		
	Frequency	%	Frequency	%	
Sex					0.205
Male	42	24.0	146	76.0	
Female	57	29.7	135	70.3	
Age					0.016
Puppy (0-12 months)	16	22.2	56	77.8	
Adult (1 a 7 years)	69	32.5	143	67.5	
Senior (>7 years)	18	18.0	82	82.0	
Breed					0.981
Crossbreed	19	26.0	54	74.0	
Pedigree	84	27.0	227	73.0	
Season of year					0.816
Spring-Summer	71	26.3	199	73.7	
Fall-Winter	32	28.1	82	71.9	

Hematological variables

In relation to laboratory findings for *E. canis* positive dogs, there were no significant differences in those showing anaemia, thrombocytopenia or hyperproteinemia compared to negative animals, many of which presented percentages similar to the infected group ($p>0.05$). On the other hand, significant differences were found for some white blood cell parameters, such as total leukocyte and neutrophil counts ($p<0.05$). However, for these analytes, the greater number of dogs infected with *Ehrlichia* were those that resulted with values within the reference ranges, in comparison with the animals that resulted negative where a large number of dogs with leukocytosis or neutrophilia are shown. For the rest of the hematological parameters evaluated, the statistical test did not find significant differences ($p>0.05$), as shown in Table 3.

DISCUSSION

Nowadays, canine ehrlichiosis and anaplasmosis have gained greater importance worldwide, which is mainly attributed to the fact that their vector (*Rhipicephalus sanguineus*) is considered the tick species with the widest geographical distribution (Aguiar *et al*., 2007; Parola *et al.,* 2013; Cabezas-Cruz *et al*., 2019). In this research work, it was found that of the total number of dogs evaluated (384), 103 were positive for *E. canis* (26.8 %) by PCR technique and only 41 (10.7 %) through blood smear evaluation. This discrepancy of the two methods used is similar to that reported by Happi *et al*., (2018), who out of a total of116 dog samples only 10.3% were positive by microscopy, compared to the PCR technique where 42 positive results were obtained (36.2%). These results were to be expected, since although the diagnosis by microscopic visualization of the typical intracellular inclusions or morulae within the cytoplasm of monocytes or lymphocytes in peripheral blood smears (Figure 3) has been of great importance. This technique has certain disadvantages, such as lack of sensitivity during the early phase of infection, when there is low bacteremia, or when the bacterium multiplies in intracytoplasmic microcolonies in lymphoid organs. It will acquire mechanisms that ensure evasion of the immune response within the host cell (Bai *et al*., 2017; Manasa *et al.* 2017; McClure *et al*., 2017; Tominello *et al*., 2019; Franco-Zetina *et al.,* 2019). In addition, false negatives have been reported in chronic or transient cases, because morulae usually disappear five to eight days after infection, as revealed in experimental studies in dogs and cattle (Gal *et al*., 2008; Stuen *et al*., 2013).

In Mexico, these diseases are frequently underdiagnosed, with few studies that determine their prevalence. In 2009 in Yucatan, a seroprevalence of canine ehrlichiosis of 45% was recorded (Jiménez-Coello *et al*., 2009) and in another investigation involving 28 states of the Mexican Republic. The presence of antibodies against *Anaplasma* spp, *Borreli*a *burgdorferi* and *E. canis*; registering a high prevalence for *E. canis* (55%) and moderate for *Anaplasma* spp (16.4%), for some northeastern states such as Coahuila and Nuevo

León (Movilla *et al.*, 2016). Geographically, the animals that participated in this study belong to the northeastern zone of Mexico; however, if we compare the prevalence obtained in the central zone of Tamaulipas for *E. canis* (26.8%) with these two states, it would be much lower. However, it is important to mention that serological tests were used in this study, which may have the disadvantage of cross-reacting with other closely related microorganisms, overestimating the prevalence results and suggesting the need to carry out studies with molecular techniques that allow more accurate evidence of the type of pathogen involved (Cetinkaya *et al*., 2016).

Table 3. Frequencies and percentages of *E. canis* positives and negatives grouped in red series and platelets

Variable	Presence of *E. canis*				Value of p
	Positive		Negative		
	Frequency	%	Frequency	%	
Hematocrit					0.280
Anaemia (< 0.37 L/L)	49	24.3	153	75.7	
Without anaemia (≥0.37 L/L)	54	29.7	128	70.3	
Plasma Proteins					0.739
Without hyperproteinemia (<75 g/L)	45	25.7	130	74.3	
With hyperproteinemia (>75 g/L)	58	27.8	209	72.2	
Platelets					0.946
Thrombocytopenia (<180X10^9/L)	6	28.6	15	71.4	
Without thrombocytopenia (≥180X10^9/L)	97	26.7	266	73.3	
Leukocytes					0.005
Leukopenia (<6x10^9/L)	3	15.8	16	84.2	
Normal (6-17x 10^9/L)	71	33.3	142	66.7	
Leukocytosis (>17x10^9/L)	29	19.1	123	80.9	
Monocytes					0.060
Without Monocytosis (≤1.4x10^9/L)	31	21.1	116	78.9	
Monocytosis (>1.4x10^9/L)	72	30.4	165	69.6	
Lymphocytes					0.235
Lymphocytosis (>4.8x10^9/L)	12	18.5	53	81.5	
Normal (1.0-4.8x10^9/L)	72	28.1	184	71.9	
Lymphopenia (<1.0x10^9/L)	19	30.2	44	69.8	
Segmented Neutrophils					0.004
Neutropenia (<3.0 x10^9/L)	30	18.4	133	81.6	
Normal (3.0-11.5x10^9/L)	70	33.8	137	66.2	
Neutrophilia (>11.5x10^9/L)	3	21.4	11	78.6	
Eosinophils					0.575
Without eosinophilia (<0.9x10^9/L)	90	26.2	253	73.8	
With eosinophilia (>0.9x10^9/L)	13	31.7	28	68.3	

In 2019, a molecular detection study of *E. canis* was conducted in rural areas of Yucatan, finding a 29.26% prevalence (Ojeda-Chi et al., 2019), which is close to that reported in this work (26.8%); but much higher compared to the prevalence found in dogs evaluated in the Comarca Lagunera (4%) (Almazán *et al*., 2016).

As for *A. phagocytophilum* infections, they have been increasingly diagnosed in companion and farm animals' worldwide (McMahan *et al*., 2016). In Mexico, *A. phagocytophilum*, has been detected in opossums and dogs in Campeche state, with a prevalence of 3 and 27%, respectively (Rojero *et al*., 2017); however, in this work none of the dogs tested were positive by PCR or blood smear. This is not surprising, since *Ixodes* spp. and *Dermacentor* spp. ticks, infrequent in the study area, have been recognized as the most important vectors in the transmission cycle of this bacterium, which could have contributed to its null presence (Tinoco-García *et al.,* 2009; Guzmán-Cornejo *et al*., 2016; Rodríguez-Vivas *et al*. 2019).
The results observed in this research show that *E. canis* has no predilection between gender, since within the infected group the percentages of females (29.7) and males (24.0) were not statistically significant (p>0.005). This same variable has been studied by several authors (Nuñez, 2003; Rodríguez-Vivas *et al*., 2005), finding similar results. However, this disagrees with what has been reported by other researchers, where they argue that females, especially during estrus, pregnancy or parturition, favor the risk of contracting *E. canis* infections (Salazar *et al*., 2014; Abdelfattah *et al.,* 2021).

In relation to hematological findings associated with the presence of canine ehrlichiosis and anaplasmosis, it has been reported that these alterations will depend on the disease stage (Afusat *et al.,* 2020). During the acute stage, the presence of anaemia is common, which is usually mild to moderate (usually normocytic, normochromic, non-regenerative) (Eberts *et al.,* 2011).
In this work, the presence of anaemia was not significantly related to any of the diseases. Thrombocytopenia has been a hematological finding that has traditionally been associated with canine ehrlichiosis (Piratae *et al.,* 2019). However, in this study the presence of thrombocytopenia (<200,000) had no association with *E. canis* positive animals. Several studies have reported an association between platelet count and the presence of *E. canis*, particularly in animals with platelet cell counts below 100 $X10^9$/L (Bulla *et al*., 2004; Tngsahuan *et al.,* 2020). Although in the study many animals were reported with the presence of anaemia and hyperproteinemia, there is no significant statistical association when compared with animals that tested negative. This may be due to the possible presence of other hemoparasites such as *Ehrlichia ewingii* or *Anaplasma platys* that can produce degrees of anaemia and hyperproteinemia similar to those reported in dogs infected with *E. canis* (Piratae *et al.,* 2019).

On the other hand, it is possible that many of the *E. canis*-positive individuals with unaltered hematological results had been in the subclinical phase of the disease. The latter would be of great importance since if the disease is not detected during this phase it could progress to a chronic stage, producing severe irreversible damage such as thrombocytopenia, leukopenia and severe non-regenerative anaemia resulting from bone marrow suppression (Little *et al*., 2014).

Regarding the evaluation of the white series, it is observed that despite the existence of significant differences between negative and positive cases to *E. canis* for total leukocyte, neutrophil and monocyte counts; the results were not as expected, since the negative dogs resulted with more alterations in these cells (either increased or decreased), compared to the positive ones. These findings are in agreement with the results obtained by Asgarali and colaboradores (2012), who reported that dogs with Ehrlichiosis manifested neutrophil and monocyte levels within reference ranges; in contrast to negative animals, which had a significant increase in these cells. A possible explanation for why many of the positive dogs showed no alterations in the white series is that these animals may have been in the subclinical phase of the disease, where most of them are asymptomatic and do not present significant hematological alterations (de Castro *et al*., 2004).

CONCLUSIONS

The present study showed that the hematological alterations evaluated in dogs with suspicious signs of *Ehrlichia canis* were not specific, since a large number of these animals were not infected. On the other hand, many of the dogs that did test positive remained without apparent changes in their blood counts, which is of great relevance, since these individuals, if not diagnosed in time, could be reservoirs for other hosts including humans. In addition, the veterinary clinician should consider that these diseases could present a subclinical picture without signs or with the presence of co-infections that produce similar signs, which would hinder their diagnosis and therefore the adequate treatment. Further research is suggested that includes the detection of other species of hemoparasites in the region, due to their importance as potentially zoonotic agents.

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CITED LITERATURE

ABDELFATTAH S, Alanazi AD, Alireza S, Domenico O. 2021. Seroprevalence and associated risk factors for vector-borne pathogens in dogs from Egypt. *Parasit Vectors*. 14:175. https://doi.org/10.1186/s13071-021-04670-0

AFUSAT JJ, Obokparo GO, Oluwafemi AA, Latifat AA. 2020. Haematology and serum chemistry in clinical canine ehrlichiosis. *Vom Journal of Veterinary Science.* 15(1):61-66. https://www.researchgate.net/publication/346785733

AGUIAR DM, Cavalcante GT, Pinter A, Gennari SM, Camargo LMA, Labruna MB. 2007. Prevalence of *Ehrlichia canis* (Rickettsiales: Anaplasmataceae) in dogs and *Rhipicephalus sanguineus* (Acari: Ixodidae) ticks from Brazil. *Journal of Medical Entomology.* 44:126-132. https://doi.org/10.1093/jmedent/41.5.126

ALLISON RW, Little SE. 2013. Diagnosis of rickettsial diseases in dogs and cats. *Veterinary Clinical Pathology*. 42:127-144. https://doi.org/10.1111/vcp.12040

ALMAZÁN C, Gonzalez-Alvarez VH, de Mera IGF, Cabezas-Cruz A, Rodríguez-Martínez R, de la Fuente J. 2016. Molecular identification and characterization of *Anaplasma platys* and *Ehrlichia canis* in dogs in Mexico. *Ticks and Tick-borne Diseases*. 7:276-283. https://doi:10.1016/j.ttbdis.2015.11.002

ASGARALI Z, Pargass I, Adam J, Mutani A, Ezeokoli. 2012. Haematological parameters in stray dogs seropositive and seronegative to *Ehrlichia canis* in North Trinidad. *Ticks and Tick Borne Diseases*. 3(4): 207-211. https://doi.org/10.1016/j.ttbdis.2012.03.006

BAI L, Goel P, Jhambh R, Kumar P, Joshi V G. 2017. Molecular prevalence and haemato-biochemical profile of canine monocytic ehrlichiosis in dogs in and around Hisar, Haryana, India. *J Parasit Dis*. 41(3): 647-54. https://doi.org/10.1007/s12639- 016-0860-8

BATTILANI M, De Arcangeli S, Balboni A, Dondi F. 2017. Genetic diversity and molecular epidemiology of Anaplasma. *Infection Genetics and Evolution*. 49:195-211. https://doi.org/10.1016/j.meegid.2017

BEUGNET F, Chalvet-Monfray K. 2013. Impact of climate change in the epidemiology of vector-borne diseases in domestic carnivores. *Comp Immunol Microbiol Infect Dis*. 36:559–566. https://doi.org/10.1016/j.cimid.2013.07.003

BHADESIYA CM, Modi DV. 2015. Correlation of epidemiology of *Rhipicephalous sanguineus* and canine ehrlichiosis in nine different localities of middle Gujarat. *International Agricultural Science and Veterinary Medicine*. 3(1): 2320-3730. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.740.420&rep=rep1&type=pdf

BULLA C, Kiomi R, Pessoa J, Trinca LA, Souza R, Wiedmeyer CE. 2004. The relationship between the degree of thrombocytopenia and infection with *Ehrlichia canis* in an endemic area. *Veterinary Research*. 35:141-146. https://doi.org/10.1051/VETRES:2003038

CABEZAS-CRUZ A, Allain E, Ahmad AS, Saeed MA, Rashid I, Ashraf K, Estrada-Peña A. 2019. Low genetic diversity of *Ehrlichia canis* associated with high co-infection rates in *Rhipicephalus sanguineus* (sl*). Parasites & Vectors*. 12:12. https://doi.org/10.1186/s13071-018-3194-9

CETINKAYA H, Matur E, Akyazi I, Ekiz EE, Aydin L, Toparlak M. 2016. Serological and molecular investigation of *Ehrlichia* spp. and *Anaplasma* spp. in ticks and blood of dogs, in the Thrace Region of Turkey. *Ticks and Tickborne Diseases*. 7:706-714. https://doi.org/10.1016/j.ttbdis.2016.02.021

DE CASTRO MB, Machado RZ, de Aquino LP, Alessi AC, Costa MT. 2004. Experimental acute canine monocytic ehrlichiosis: clinicopathological and immunopathological findings. *Vet Parasitol*. 119:73-86. https://doi.org/10.1016/J.VETPAR.2003.10.012

DE LA FUENTE J, Antunes S, Bonnet S, Cabezas-Cruz A, Domingos AG, Estrada-Peña A, *et al*. 2017. Tick-pathogen interactions and vector competence: identification of molecular drivers for tick-borne diseases. *Frontiers in cellular and infection microbiology.* 7:114. https://doi.org/10.3389/fcimb.2017.00114

DUMLER JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, Rurangirwa FR. 2001. 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*. 51(6):2145-2165*.* https://doi.org/10.1099/00207713-51-6-2145

EBERTS MD, Vissotto de Paiva Diniz PP, Beall MJ, Stillman BA, Chandrashekar R, Breitschwerdt EB. 2011. Typical and atypical manifestations of *Anaplasma phagocytophilum* infection in dogs. *Journal of the American Animal Hospital Association*. 47:86-94. https://doi.org/10.5326/JAAHA-MS-5578

FARHAN AA. 2015. *Anaplasma marginale* and *Anaplasma phagocytophilum*: Rickettsiales pathogens of veterinary and public health significance. *Parasitol Res.* 114(11):3941-57. https://doi.org/10.1007/s00436-015-4698-2

FRANCO-ZETINA M, Adame-Gallegos J, Dzul-Rosado K. 2019. Effectivity of diagnostic methods for the detection of human and canine monocytic ehrlichiosis. *Rev. Chilena Infectol*. 36(5):650–655. https://doi.org/10.4067/S0716-10182019000500650

GAL A, Loeb E, Yisaschar-Mekuzas Y, Baneth G. 2008. Detection of Ehrlichia canis by PCR in different tissues obtained during necropsy from dogs surveyed for naturally occurring canine monocytic ehrlichiosis. *Veterinary Journal*. 175:212-217. https://doi.org/10.1016/J.TVJL.2007.01.013

GUZMÁN-CORNEJO C, Robbins RG, Guglielmone AA, Montiel-Parra G, Rivas G, Pérez TM. 2016. The Dermacentor (Acari, Ixodida, Ixodidae) of Mexico: hosts, geographical distribution and new records. *ZooKeys.* 569:1-22. https://doi.org/10.3897/zookeys.569.7221

HAPPI AN, Toepp AJ, Ugwu CA, Petersen CA, Sykes JE. 2018. Detection and identification of blood-borne infections in dogs in Nigeria using light microscopy and the polymerase chain reaction. *Vet Parasitol Reg Stud Reports*. 11: 55-60. https://doi.org/10.1016/j.vprsr.2017.12.002

HARRUS S, Waner T. 2011. Diagnosis of canine monocytotropic ehrlichiosis (*Ehrlichia canis*): an overview. *The Veterinary Journal*. 187:292-296. https://doi.org/10.1016/j.tvjl.2010.02.001

IRWIN PJ. 2014. It shouldn't happen to a dog … or a veterinarian: clinical paradigms for canine vector-borne diseases. *Trends Parasitol*ogy 30:104–12. https://doi.org/10.1016/j.pt.2013.12.001

ISMAIL N, McBride JW. 2017. Tick-borne emerging infections: ehrlichiosis and anaplasmosis. *Clinics in Laboratory Medicine*. 37:317-340. https://doi.org/10.1016/j.cll.2017.01.006

JIMÉNEZ-COELLO M, Pérez-Osorio C, Vado-Solís I, Rodríguez-Buenfil JC, Ortega-Pacheco A. 2009. Serological survey of *Ehrlichia canis* in stray dogs from Yucatan, Mexico, using two different diagnostic tests. *Vector Borne Zoonotic Diseases*. 9:209-212. https://doi.org/10.1089/vbz.2008.0039

LITTLE E, Beall MJ, Bowman DD, Chandrashekar R, Stamaris J. 2014. Canine infection with *Dirofilaria immitis*, *Borrelia burgdorferi*, *Anaplasma* spp., and *Ehrlichia* spp. in the United States, 2010-2012. *Parasites & Vectors*. 7:1-9. https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-7-257

MAGGI RG, Krämer F. 2019. A review on the occurrence of companion vector-borne diseases in pet animals in Latin America. *Parasitic Vectors*. 12:145. doi: 10.1186/s13071-019-3407-x

MANASA RK, Pritpal SD, Lachhman DS, Baljinder KB, and Sanjeev KU. 2017. Clinical and hematobiochemical response in canine monocytic ehrlichiosis seropositive dogs of Punjab. *Veterinary World*. 10(2): 255–261. https://doi.org/10.14202/vetworld.2017.255-261

MCCLURE EE, Chávez ASO, Shaw DK, Carlyon JA, Ganta RR, Noh SM, Wood DO, Ba voil PMA, Brayton KA, Martinez JJ, McBride JW, Valdivia RH, Munderloh UG, Pedra JHF. 2017. Engineering of obligate intracellular bacteria: progress, challenges and paradigms. *Nat Rev Microbiol*. 15:544–558. https://doi.org/10.1038/nrmicro.2017.59

MCMAHAN CS, Wang D, Beall MJ, Bowman DD, Little SE, Pithua PO, Julia L, Sharp JL, Stich RW, Yabsley MJ, Lund RB. 2016. Factors associated with *Anaplasma* spp. seroprevalence among dogs in the United States. *Parasites & Vectors*. 9:169. https://doi.org/10.1186/s13071-016-1431-7

MOVILLA R, García C, Siebert S, Roura X. 2016. Countrywide serological evaluation of canine prevalence for *Anaplasma* spp., *Borrelia burgdorferi* (sensu lato), *Dirofilaria immitis* and *Ehrlichia canis* in Mexico. *Parasites & Vectors*. 9:421. https://doi.org/10.1186/s13071-016-1686-z

NÚÑEZ OL. 2003. Estudio de la seroprevalencia de *Ehrlichia canis* en México. *Asociación Méxicana de Médicos Veterinarios Especialistas en Pequeñas Especies*. 14:83-85. https://www.researchgate.net/publication/291003826

OJEDA-CHI MM, Rodriguez-Vivas RI, Esteve-Gasent MD, Pérez de León A, Modarelli JJ, Villegas-Perez SL. 2019. *Ehrlichia canis* in dogs of Mexico: Prevalence, incidence, co–infection and factors associated. *Comparative Immunology, Microbiology and Infectious Diseases*. 67:101351. https://doi.org/10.1016/j.cimid.2019.101351

PAROLA P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. 2013. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev*. 26:657–702. https://doi.org/10.1128/CMR.00032-13

PIRATAE S, Senawong P, Chalermchat P, Harnarsa W, Sae-chue B. 2019. Molecular evidence of *Ehrlichia canis* and *Anaplasma platys* and the association of infections with hematological responses in naturally infected dogs in Kalasin. *Thailand. Veterinary World*. 12:131. https://doi.org/10.14202/vetworld.2019.131-135

RODRÍGUEZ-VIVAS RI, Albornoz REF, Bolio GME. 2005. *Ehrlichia canis* in dogs in Yucatan, Mexico: seroprevalence, prevalence of infection and associated factors. *Veterinary Parasitology*. 127:75-79. https://doi.org/10.1016/J.VETPAR.2004.08.022

RODRÍGUEZ-VIVAS RI, Ojeda-Chi MM, Bolio-González ME, Rosado-Aguilar JA. 2019. Las garrapatas como vectores de enfermedades zoonóticas en México. *Bioagrociencias*. 12(1):19-26. https://www.revista.ccba.uady.mx/ojs/index.php/BAC/article/view/2993

ROJERO-VÁZQUEZ R, Gordillo-Pérez G, Weber M. 2017. Infection of *Anaplasma phagocytophilum* and *Ehrlichia* spp. in opossums and dogs in Campeche, Mexico: the role of tick infestation. *Frontiers in Ecology Evolution*. 5:161. https://doi.org/10.3389/fevo.2017.00161

SALAZAR H, Edwin F, Buriticá EF, Echeverry DF, Barbosa I. 2014. Seroprevalencia de Ehrlichia canis y su relación con algunos parámetros clínicos y hematológicos en caninos admitidos en clínicas veterinarias de la ciudad de Ibagué (Colombia). *Revista Colombiana de Ciencia Animal*. 7 (1):56-63.
http://revistas.ut.edu.co/index.php/ciencianimal/article/view/542

STICH RW, Rikihisa Y, Ewing SA, Needham GR, Grover DL, Jittapalapong S. 2002. Detection of *Ehrlichia canis* in canine carrier blood and in individual experimentally infected ticks with a p30-based PCR assay. *Journal of Clinical Microbiology*. 40:540-546. https://doi.org/10.1128/JCM.40.2.540-546.2002

STUEN S, Granquist EG. Silaghi C. 2013. *Anaplasma phagocytophilum* a widespread multi-host pathogen with highly adaptive strategies. *Frontiers in Cellular and Infection Microbiology*. 3:31. https://doi.org/10.3389/fcimb.2013.00031

TINOCO-GRACIA L, Quiroz-Romero H, Quintero-Martinez MT, Renteria-Evangelista TB, Gonzalez-Medina Y, Barreras-Serrano A, *et al*. 2009. Prevalence of *Rhipicephalus sanguineus* ticks on dogs in a region on the Mexico-USA border. *Veterinary Record*. 164:59-61. https://doi.org/10.1136/vr.164.2.59

TOMINELLO TR, Oliveira ERA, Hussain SS, Wells AEJ, Golden B, Ismail N. 2019. Emerging roles of autophagy and inflammasome in ehrlichiosis. *Front Immunol*. 10:1011. https://doi.org/10.3389/fimmu.2019.01011

TNGSAHUAN S, Chethanond U, Wasiksiri S, Saechan V, Thongtako W, Musikacharoen T. 2020. Hematological profile of blood parasitic infected dogs in Southern Thailand. *Vet World.* 13(11): 2388-2394. https://doi.org/10.14202/vetworld.2020.2388-2394

VIEIRA RFDC, Vieira TSWJ, Nascimento, DDAG, Martins TF, Krawczak FS, Labruna MB, Vidotto O. 2013. Serological survey of Ehrlichia species in dogs, horses, and humans: zoonotic scenery in a rural settlement from southern Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*. 55:335-340. https://doi.org/10.1590/S0036-46652013000500007

WAYNE WD, Chad LC. 2013. *Biostatistics: A Foundation for Analysis in the Health Sciences*. 10th Edition Wiley. Pp. 191. ISBN 978-1-118-30279-8.

YOUSEFI A, Chaechi Nosrati MR, Golmohammadi A, Azami S. 2019. Molecular Detection of *Anaplasma Phagocytophilum* as a Zoonotic Agent in Owned and Stray Dogs in Tehran, Iran. *Archives of Razi Institute*. 74:33-38. https://doi.org/10.22092/ARI.2018.114893.1142