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ORIGINAL ARTICLE

Prevalence, risk factors, and hematologic changes in dogs from Baja California with presence of *Ehrlichia* spp., and coinfection with *Anaplasma* spp.

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ABSTRACT. *Ehrlichia* and *Anaplasma* are obligate intracellular, gram-negative bacteria with tropism for hematopoietic cells, especially leukocytes and platelets. There are several *Ehrlichia* species that infect dogs. *Ehrlichia canis* is transmitted by *Rhipicephalus sanguineus* and replicates within monocytes and macrophages, leading to canine monocytic ehrlichiosis, a disease of worldwide distribution. The clinical signs are varied and non-specific. *Anaplasma* has two species that infect dogs, *Anaplasma phagocytophilum* and *Anaplasma platys*, the second also transmitted by *Rhipicephalus sanguineus*. This study aimed to evaluate the epidemiology and hematologic changes associated with the presence of *Ehrlichia* spp. and *Anaplasma* spp. coinfection in dogs from Baja California. Complete hematological analysis, examination of buffy coat smears, and ELISA tests were performed on blood samples from three veterinary diagnostic laboratories from Mexicali and Tijuana cities in Baja California, Mexico. A total of 5.469 dog samples were analyzed. The overall prevalence of *Ehrlichia* spp., was 4.79%, with a distribution of 6.3% in Mexicali (OR: 2.39 CI: 1.69-3.17) and 2.5% in Tijuana. The peak of infection was found in September. Contact with other dogs and tick infestation were the risk factors associated with infection ($P < 0.05$). There was 96% co-infection only in Tijuana and 0% in Mexicali. Anemia, thrombocytopenia, and hyperproteinemia are associated with *Ehrlichia* spp., and *Ehrlichia/Anaplasma* infection. In view of the foregoing, we have to maintain epidemiologic vigilance, as well as look further into the ticks present in the state and the possibility of transmission of unusual pathogens.

Keywords: *Ehrlichia*, *Anaplasma*, coinfection, prevalence, risk factors, hematologic changes.

INTRODUCTION

There are many pathogens transmitted by vectors that affect dogs. *Ehrlichia* is a genus of obligate intracellular, gram-negative bacteria in the family *Anaplasma*taceae, order Rickettsiales (Ramakant *et al*., 2020), with tropism for hematopoietic cells, especially leukocytes and platelets (Dhavalgi *et al*., 2021). Several *Ehrlichia* species infect dogs, including *E. ewingii*, *E. chaffeensis*, *E. muris*, and *E. canis*. *Ehrlichia ewingii* is found more often in neutrophils and *E. chaffeensis* in monocytic cells, both of which are transmitted by the lone star tick (*Amblyomma americanum*) (Lashnits *et al*., 2019; Xu *et al*., 2023). *Ehrlichia muris* also infects monocytic cells (Feng & Walker, 2004), but is transmitted by black-legged ticks (*Ixodes scapularis*) (Xu *et al*., 2023). *Ehrlichia canis* is transmitted by the brown dog tick (*Rhipicephalus sanguineus*) and replicates within monocytes and macrophages (Rikihisa, 2021), leading to canine monocytic ehrlichiosis (CME), a disease with a worldwide distribution (Christodoulou *et al*., 2023). Canine monocytic ehrlichiosis has been reported in Asia (Ansari-Mood *et al*., 2010; Bhadesiya & Modi, 2015; Kottadamane *et al*., 2017; Mittal *et al*., 2017; Haryanto & Tjahajati 2020), Europe (Pantchev *et al*., 2015; Sainz *et al*., 2015; Piantedosi *et al*., 2017; Jurković *et al*., 2019), and the Americas (Carrade *et al*., 2011; Melo *et al*., 2011; Villeneuve *et al*., 2011; Barrantes-González *et al*., 2016; Pesapane *et al*., 2019).

In Mexico, the seroprevalence of *E. canis* ranges from 33.1% to 74.3% (Sosa-Gutiérrez *et al*., 2013; Salinas-Meléndez *et al*., 2015; Almazán *et al*., 2016; Movilla *et al*., 2016), however, epidemiological studies on this disease in canine populations in the State of Baja California are insufficient. Haro-Álvarez *et al*. (2007) reported a seroprevalence of 21.6% (83/384) in dogs treated at veterinary clinics, including only dogs suspected of having the disease in the city of Mexicali. However, the prevalence of this disease in other cities in the state is unknown. Worldwide, various risk factors have been associated with CME, including age (Pinter *et al*., 2008; Vieira *et al*., 2013; Milanjeet *et al*., 2014), seasonality (Lee *et al*., 2020), presence of ticks in dogs (Yuasa *et al*., 2012; Huerto-Medina & Damasco-Mata 2015; Navarrete *et al*., 2018), and lack of veterinary care (Pérez-Macchi *et al*., 2019), among others.

Anaplasma is an obligate intracellular gram-negative bacterium from the same family and order as *Ehrlichia*, with worldwide distribution (Rar *et al*., 2021). Two species of *Anaplasma* infect dogs, *A. phagocytophilum* and *A. platys*. The first is re-

sponsible for canine granulocytic anaplasmosis (CGA) as it infects granulocytes and is transmitted by *Ixodes* ticks (Carrade *et al*., 2009). In Mexico, it has been reported in various states, but principally in the northern states of the country (Aragón-López *et al*., 2021), however, it has not been reported in Baja California. The second is the causative agent of infectious canine cyclic thrombocytopenia (ICCT) as it infects platelets, is transmitted by *R. sanguineus* (Atif *et al*., 2021) and regarding Mexico, it has been reported by PCR in dogs from Cajeme, Sonora, with 10.58% of prevalence (Aragón-López *et al*., 2021), 31% from the region known as "La Comarca Lagunera" (Almazán *et al*., 2016) and 24.74% in Ciudad Juarez, Chihuahua (Beristain-Ruiz *et al*., 2022). There are also reports of antibodies to *Anaplasma* spp. in several states of Mexico, including Baja California, with a seroprevalence of 32.9% (Bedoya *et al*., 2023).

Considering the limited knowledge about the epidemiology of CME in the region, the objective of this study was to estimate the prevalence, risk factors, and hematological changes associated with CME in dogs with owners in two cities in northwest Mexico and to search for antibodies against *Anaplasma* spp., since both diseases are endemic (Aragón-López *et al*., 2021).

MATERIAL AND METHODS

Data were obtained from 5,469 blood samples from dogs remitted to three veterinary diagnostic laboratories in the cities of Mexicali and Tijuana, Baja California, Mexico, between September 2021 and August 2022. Blood samples (0.5 mL) with EDTA and non-hemolyzed plasma from dogs aged one month of age or older, of any breed, size, and sex were included. Each sample received was processed for complete hematological analysis that included the measurement of hematocrit and total solids and the counts of erythrocytes, leukocytes, and platelets. Blood smears were analyzed to check the leukocyte differential and cell morphology, as well as to search for intracellular morulae of *Ehrlichia* and the presence of structures compatible with *Anaplasma*. Buffy coat smears were performed for every patient to maximize the possibility of finding morulae.

Serology

When no morulae were found, but there was clinical and hematological suspicion of ehrlichiosis, ELISA tests were performed. Some samples were originally sent from veterinary clinics for complete hematological studies and ELISA tests. The ELISA tests utilized (IDEXX® Snap 4DX plus) detects the presence of antibodies against *E. canis/E. ewingii*, *Borrelia burgdorferi*, *Anaplasma platys/A. phagocytophilum* and *Dirofilaria immitis*, with 97.6% sensitivity and 99.0% specificity, as well as IgG antibodies, particularly for *Ehrlichia* spp. and *Anaplasma* spp. (Kaewmongkol *et al*., 2020; Zhang *et al*., 2022).

Cases were considered *Ehrlichia*-positive based on the presence of *Ehrlichia* morulae in the blood/buffy coat smear or by a positive ELISA result. Cases were considered *Anaplasma*-positive only by ELISA tests, as there was no presence in any blood smear.

Data Collection to determine risk factors

In order to determine the risk factors associated with infection, age (1-12 months or >12 months), sex (male or female), breed (mixed or pure), city of origin (Mexicali or Tijuana), presence of ticks (yes or no), street access (yes or no), and contact with other dogs in the house (yes or no) were registered.

Statistics

The prevalence of the disease was determined by the number of positive cases and the number of patients attended by year. Chi-square (χ^2) estimation, *P*-values and odds ratio with 95% confidence interval were calculated for the association between risk factors and the disease. Furthermore, risk factors with $P < 0.10$ were analyzed using a binomial logistic regression model. The dependent variables were positive *Ehrlichia* cases, and the independent variables were the risk factors described above. To identify statistical differences between hematologic alterations in 1) positive and negative *Ehrlichia* patients; 2) positive for *Ehrlichia* patients by the presence of morulae and positive by ELISA tests; and 3) positive for *Ehrlichia* spp. and positive for *Ehrlichia spp*. and *Anaplasma* spp., Student's t-tests were performed. Probability values less than 0.05 ($P \leq 0.05$) were considered statistically significant. Inferential analysis was performed using Statistix 9® software.

RESULTS

A total of 5,469 dog samples were analyzed. The overall prevalence of *Ehrlichia* spp. was 4.79% (262/5,469), and its distribution among cities was 6.3% (206/3,269) in Mexicali and 2.5% (56/2,200) in Tijuana. Of all the positive cases, 149 were positive for the presence of morulae within mononuclear cells and 105 were positive by ELISA. Eight samples tested positive using both diagnostic methods. Dogs in Mexicali had a 2.57 times higher risk of infection than those in Tijuana. The presence of ticks showed 1.78 times higher likelihood of having the disease, and dogs that had contact with other dogs had a 1.86 times higher risk of being affected by *Ehrlichia* spp. The other evaluated variables were not statistically significant (Table 1).

In the multivariate analysis, the only risk factor associated with *Ehrlichia* infection was the origin of the dog (Table 2), which was 2.31 times more likely to find dogs positive for *Ehrlichia* spp. in Mexicali than in Tijuana. The other factors analyzed were not statistically significant.

The monthly/seasonal tendency of *Ehrlichia* cases was higher in Mexicali (Figure 1). In every city, the number of infected animals peaked in September. In Mexicali, another outbreak occurred in June and July, with both cities having a higher frequency during summer.

In this study, 96% (54/56) of the *Ehrlichia* spp. cases identified in Tijuana showed antibodies against *Anaplasma* spp. by ELISA. Notably, all cases of coinfection were found in Tijuana.

To evaluate the behavior of some hematological indicators between *Ehrlichia* spp., positive and negative cases, we compared the mean values for hematocrit, neutrophils, lymphocytes, platelets, and total solids. The results showed statistically significant differences ($P < 0.05$) between all variables mentioned above, except for neutrophils. However, in cases positive by ELISA, neutrophilia was found, and in every group, we found occasional Döhle bodies and diffuse basophilia (Table 3).

In Table 4, we show the differences found in blood analytes between patients positive for *Ehrlichia* morulae and those positive by ELISA tests. There was statistical difference in lymphocyte and platelet counts.

In Table 5, we present the differences in blood analytes between patients positive for *Ehrlichia* spp. and *Ehrlichia*/*Anaplasma* spp. There was statistical difference in platelets and total solids.

Table 1.
Risk factors associated with the presence of Ehrlichia spp. in dog samples from Baja California.

	N	Positives (%)	OR	95% CI	*P*
City					
Mexicali	3,269	206 (6.3)	2.57	1.91 – 3.47	0.00*
Tijuana	2,200	56 (2.5)			
Access to Street					
Yes	109	8 (7.3)	1.59	0.77 – 3.31	0.21
No	5,360	254 (4.7)			
Presence of ticks					
Yes	290	23 (7.9)	1.78	1.14 – 2.78	0.01*
No	5,179	239 (4.6)			
Contact with other dogs					
Yes	744	57 (7.6)	1.86	1.37 – 2.52	0.00*
No	4,679	200 (4.2)			
Sex					
Female	2,911	141 (4.8)	1.04	0.80 – 1.34	0.78
Male	2,414	113 (4.6)			
Age					
0-12 months	1,062	57 (5.3)	1.16	0.86 – 1.57	0.32
>12 months	4,406	205 (4.6)			

CI: Confidence interval; OR: Odds ratio; P: probability value; * P < 0.05

Table 2.
Risk factors associated with the presence of Ehrlichia spp. in dogs in Baja California. Multivariate analysis.

	N	Positives (%)	OR	95% CI	*P*
City					
Mexicali	3,269	206 (6.3)	2.31	1.69 – 3.17	0.00*
Tijuana	2,200	56 (2.5)			
Presence of ticks					
Yes	290	23 (7.9)	1.34	0.85 – 2.10	0.21
No	5,179	239 (4.6)			
Contact with other dogs					
Yes	744	57 (7.6)	1.33	0.96 – 1.83	0.08
No	4,679	200 (4.2)			

CI: Confidence interval; OR: Odds ratio; P: probability value; * P < 0.05

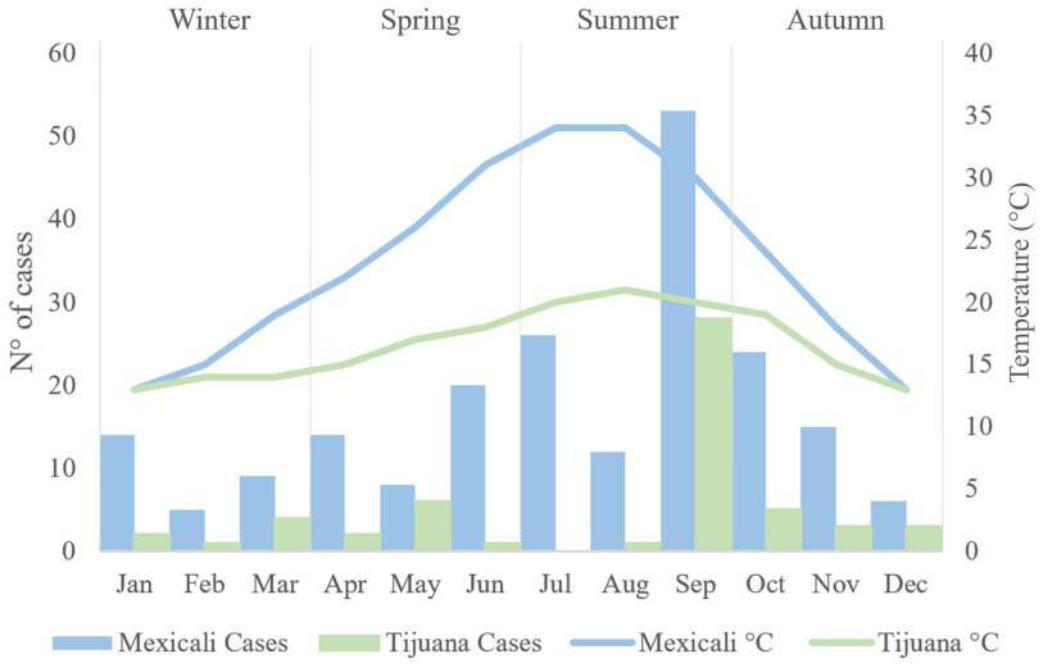

Figure 1.
Monthly and seasonal behavior of positive cases to Ehrlichia spp., in dogs from the cities of Mexicali and Tijuana, Baja California, México. We also show the average temperature in every city.

Table 3.
Hematologic alterations in positive patients by presence of Ehrlichia spp., morulae, or by ELISA test, and negative for Ehrlichia spp.

	Positive cases		Negative cases			
	Average	**Range**	**Average**	**Range**	***P***	**Ref. Int.**
Ht L/L	0.35±0.10	0.08-0.65	0.41±0.10	0.02-0.68	0.00*	0.37-0.55
N x10⁹/L	10.5±16.7	0.0-244.0	10.1±9.2	0.0-150.0	0.57	3.0-11.5
L x10⁹/L	1.8±1.9	0.0-12.0	2.1±1.9	0.0-33.8	0.02*	1.0-4.8
P x10⁹/L	119±118	8-520	279±149	0-1369	0.00*	200-600
TS g/L	73±17	4-120	76±12	16-120	0.00*	60-75

Ht: Hematocrit; N: Neutrophils; L: Lymphocytes; P: Platelets; TS: Total solids; P: probability value; *P < 0.05; Ref. Int.: Reference Interval (Núñez & Bouda, 2007).

Table 4.
Hematologic alterations in positive cases to Ehrlichia spp., in dogs due to the presence of morulae and by ELISA test.

	Morulae (+)		ELISA (+)			
	Average	Range	Average	Range	*P*	Ref. Int.
Ht L/L	0.36±0.08	0.08-0.56	0.34±0.11	0.08-0.65	0.06	0.37-0.55
N x10⁹/L	9.7±8.6	0.0-78.3	11.7±22.0	0.0-244.0	0.33	3.0-11.5
L x10⁹/L	2.22±2.11	0.1-11.8	1.41±1.49	0.0-12.0	0.00*	1.0-4.8
P x10⁹/L	195±115	28-520	37±40	8-334	0.00*	200-600
TS g/L	4±120	17-72	30±120	18-74	0.43	60-75

Ht: Hematocrit; N: Neutrophils; L: Lymphocytes; P: Platelets; TS: Total solids; P: probability value; *P < 0.05; Ref. Int.: Reference Interval (Núñez & Bouda, 2007).

Table 5.
Hematologic alterations in patients positive to Ehrlichia spp., and positive to Ehrlichia/Anaplasma spp.

	Ehrlichia (+)		*Ehrlichia and Anaplasma* (+)			
	Average	Range	Average	Range	*P*	Ref. Int.
Ht L/L	0.35±0.10	0.08-0.65	0.36±0.09	0.14-0.54	0.70	0.37-0.55
N x10⁹/L	10.8±18.3	0.0-244.0	9.4±8.3	0.9-50.2	0.55	3.0-11.5
L x10⁹/L	1.9±2.0	0.0-12.0	1.4±1.3	0.1-6.0	0.09	1.0-4.8
P x10⁹/L	136±127	8-520	62±48	10-334	0.00*	200-600
TS g/L	72±17	4-120	79±18	50-120	0.00*	60-75

Ht: Hematocrit; N: Neutrophils; L: Lymphocytes; P: Platelets; TS: Total solids; P: probability value; *P < 0.05; Ref. Int.: Reference Interval (Núñez & Bouda 2007).

DISCUSSION

This study represents the first report on the prevalence and distribution of *Ehrlichia* spp. by analyzing a large number of dogs in two cities in the State of Baja California, Mexico.

The only available publications on prevalence rates in the state were conducted by Núñez (2003), who reported a seroprevalence of 70.2% in Baja California. It is important to note that this study only analyzed 37 dogs that attended veterinary clinics with or without compatible signs of the disease, and the specific city where the samples were collected was not specified. Additionally, Haro-Álvarez *et al.* (2007) reported a prevalence of 21.6% in Mexicali. One possible explanation for the difference in prevalence (21.6% vs. 6.3%) could be the rickettsiosis outbreak that occurred in Mexicali in 2009, when several human deaths occurred, leading to increased awareness among pet owners and the Health Department regarding the importance of implementing preventive medicine programs to combat ticks in the municipality. This included programs for junkyard clearance in neighborhoods with a high prevalence and fumigation of homes and pets. Furthermore, the Institute of Research in Veterinary Sciences collaborated through a university rickettsiosis program to provide informative talks to educate the population on these topics.

Recently, Backus *et al.* (2022) conducted a study in four locations in the area (San Diego, Imperial, Tijuana, and Mexicali) from October 2021 to May 2022, analyzing 63 animals in Mexicali and 78 in Tijuana, founding seroprevalences for *Ehrlichia* spp., of 49.2% and 39.7%, respectively. However, the forementioned study is not comparable with ours, since they analyzed abandoned dogs that were kept in confinement, whereas we analyzed samples from owned dogs. Therefore, in the study

by Backus *et al.* (2022), the possibility of transmission and disease was greater than in our study.

In the present study, the prevalence found in Mexicali was 6.3% (206/3269), which is similar to the 7.6% reported in southern Italy (Ebani, 2019) and the 10.0% reported in Iran (Abdous *et al.*, 2024). These results, in the case of Mexicali, are consistent with the fact that vector mortality increases as temperature increases and relative humidity decreases (Tian *et al.*, 2023), as the average temperature in these cities varies from 23.7°C, 14.8°C and 19.6°C respectively, and the mean annual precipitation varies from 0.3, 1.7 and 0.6 inches respectively (Weather Spark, 2024a). In the case of Tijuana, the prevalence observed in our study was 2.5% (56/2200), which is similar to the reported rates of 2.43% in the north central region of Mexico (Aguascalientes, Guanajuato, and Queretaro) (Movilla *et al.*, 2016). Tijuana and these areas have similar temperatures, averaging around 12.7-15.0°C, however, while Tijuana's mean annual precipitation is 0.7 inches, in the other locations vary between 1.3-1.6 inches (Weather Spark, 2024b).

However, our results are very different from those reported by Díaz-Medina *et al.* (2016) in Yucatan, who found a prevalence of 69.2%. Furthermore, these results were obtained using nested PCR in a completely different climate that included an average temperature of 26°C and a relative humidity of 83%, which are favorable conditions for the vectors, promoting their longevity and feeding activity (Abdous *et al.*, 2024). Similar conditions are present in Matto Grosso, Brazil, where Melo *et al.* (2011) reported a 70.9% seroprevalence in dogs from urban and rural areas. Another result was from Ceylan *et al.* (2021), who found a seroprevalence of 19.8% in Turkey, where, although it is not as warm as Yucatan or Brazil, there is a lot of rainfall annually.

It is very relevant that in Baja California, the climatic conditions for the vector life cycle are better in Tijuana (16.6°C and 0.7 inches of rain) (Weather Spark, 2024a) than in Mexicali (23.7°C and 0.3 inches of rain) (Weather Spark 2024b); however, the prevalence of infection in dogs is higher in Mexicali (6.3% versus 2.5%), and outbreaks even occur in the hottest and driest months of the year; therefore, this could be due to heat stress in dogs, as it leads to immunosuppression and thus, the presence of the disease in both the chronic and acute phases (Price *et al.*, 1987; Procajlo *et al.*, 2011). In this regard, we should not underestimate climate change and possible adaptations in vectors, as ecological studies are necessary to understand the natural history of the disease and vector behavior in these extreme climates.

The presence of ticks and cohabitation or contact with other dogs are significant risk factors in animals with the disease, as they are more likely to come into contact with other dogs carrying the vector or places where the vector may be abundant (Yuasa *et al.*, 2012; Huerto-Medina & Dámaso-Mata, 2015; Navarrete *et al.*, 2018). In our univariate analysis, we observed that this risk factor is significant, but this association was not demonstrated in the multivariate analysis, indicating that other conditions in the city of Mexicali play an important role in the presentation of *Ehrlichia*.

The sex and age of the animal did not show statistical significance and can be infected indiscriminately, as reported in other studies (Milanjeet *et al.*, 2014; Navarrete *et al.*, 2018). This could be explained by the presence of ticks and contact with other dogs. If contact occurs at any age or sex, the disease is considered present.

Regarding hematological analyses, we found anemia in every positive group but the positive by morulae group. Mild anemia was observed in every group, but it was significantly different between the negative and positive patients for *Ehrlichia*, which is different from the results obtained by Merino-Charrez *et al.* (2021), who found no statistical difference in anemias between positive and negative *Ehrlichia* patients. This could be explained by the time of infection and the moment of the test, as Gaunt *et al.* (2010) reported that anemia is present in *E. canis* and *E. canis/A. platys*-positive patients from 10-20 days post infection and until 70 days post-infection, after which the hematocrit could be normal again.

In the case of platelets, thrombocytopenia was observed in the mean values of every positive group (table 3-5) being more severe in patients positive by ELISA, probably because of the chronicity of the disease (Gaunt *et al.*, 2010) and *Ehrlichia/Anaplasma* coinfection, since rickettsial organisms commonly produce thrombocytopenia (Chapman *et al.*, 2023) being, in fact, one relevant finding in CME as well as CGA (Khatat *et al.*, 2021) and ICCT (da Silva *et al.*, 2016), since these agents can lead to immune-mediated platelet destruction, consumption due to hemorrhage, and decreased production (Lara *et al.*, 2020). Possible explanations for this behavior include myelosuppressive activity, vasculitis, and immune-mediated destruction in affected individuals, resulting in decreased erythroid and megakaryocytic production in the bone marrow (Martínez *et al.*, 2015; Ybañez *et al.*, 2016).

Few studies have reported that *E. canis* can affect the myeloid cell line, resulting in leukopenia (Martínez *et al.*, 2015; Ybañez *et al.*, 2016; Piratae *et al.*, 2019; Asgarali *et al.*, 2012); however, this effect was not observed. Nevertheless, lymphocytes were significantly lower in positive patients than in negative ones (Table 3), ELISA-positive than in morulae-positive patients (Table 4), and positive for *Ehrlichia/Anaplasma* than in *Ehrlichia*-positive patients (Table 5), possibly as a response to endogenous corticosteroids due to stress (Boes & Durham., 2017), although lymphopenia was found in *Ehrlichia* patients (Bhadesiya & Modi, 2015; Villaescusa *et al.*, 2012; Quorollo *et al.*, 2019) as part of the redistribution during the acute phase response (Long & Vodzak, 2018). It is important to mention that all morulae were found within lymphocytes and monocytes, which is relevant because, although there are reports of *E. canis* morulae within neutrophils (Moura *et al.*, 2019), *E. canis* and *E. chaffeensis* use to produce morulae within mononuclear cells (Aziz *et al.*, 2023). However, *E. chaffeensis* is transmitted by *A. americanum* (Pasternak & Palli 2023), a tick only present in central and eastern Mexico

(Guzmán-Cornejo *et al*., 2011), and the USA (Rochlin *et al*., 2022), whereas *R. sanguineus*, the vector for *E. canis*, is a tick with worldwide distribution and is endemic to Baja California (Sánchez-Montes *et al*., 2021); therefore, it is very likely that the *Ehrlichia* described here is *E. canis*.

For the neutrophils, we observed a slight increase in the positive by ELISA cases, but there was no statistical difference in any positive or negative group of patients; however, it is important to note that we did not find *Ehrlichia* or *Anaplasma* morulae within any neutrophil, which reduces the possibility of infection by *E. ewingii* or *A. phagocitophylum*, which mostly infects this cellular line (da Silva *et al*., 2016; Quorollo *et al*., 2019). Another important feature we observed was toxic changes in some neutrophils, such as occasional Döhle bodies sometimes with focal basophilia, both findings related to the presence of infectious agents and chronic inflammation (Núñez & Bouda, 2007; Harvey, 2011). Gofton *et al*. (2018) reported the presence of Döhle bodies in positive and negative *Ehrlichia*-like bacteria from platypus blood; however, further studies are necessary.

The total solids use to be increased in *Ehrlichia* infections (Nimsuphan *et al*., 2020), however, we found that the mean value in negative cases barely increased, while in positive cases, the total solids were in range, although a statistically difference was present. In contrast, in the co-infected patients, we found an increase that was significantly different from the *Ehrlichia* positive group, which was expected and comparable to that reported by Saeng-Chuto *et al*. (2016).

It is important to note that, out of all positive cases for *Ehrlichia* spp., 59.92% (157/262) were identified with morulae, indicating that the patients were experiencing acute disease. Our results show that proportionally, there are more animals in the acute phase, which makes them prone to rapidly disseminating the disease to more vectors and animals in contact with them. This is in accordance with the findings of Mylonakis *et al*. (2003) in their study of dogs with clinical signs of acute disease that were positive for the disease. They demonstrated that using the buffy coat in blood smears had a sensitivity of 66% (33/50) for detecting morulae. Therefore, it has been shown that searching for morulae is useful in cases of acute and subclinical courses, as we could detect the disease within the first two weeks of infection, in contrast to ELISA tests that require a certain amount of antibodies in the blood, which are typically achieved around day 24 (Gaunt *et al*., 2010). Another alternative is the use of PCR, which may not be available or cost effective.

Regarding co-infection between *Ehrlichia* spp. and *Anaplasma* spp., in 96% of Tijuana cases, there are several reports of this co-infection in different ecosystems. Ceylan *et al*. (2021) report 6.5% co-infection with these two agents in Turkey; Beristain-Ruiz *et al*. (2022) reported a co-infection rate of 9.27 % in Chihuahua; Bedoya *et al*. (2023) found an 11.9% prevalence by analyzing blood samples from dogs in 22 Mexican states; Aragón-López *et al*. (2021) reported a prevalence of 13.04% in Sonora; and Lara *et al*. (2020) reported 19% co-infection in dogs from veterinary clinics in the Lesser Antilles. As we can observe, in all these cases, a much lower percentage than ours is present, therefore, it is very important to identify the tick species present in the city as well as maintain epidemiologic vigilance of these two rickettsial agents, since they alone can produce severe illness (Piratae *et al*., 2016) and this coinfection can result in a bad forecast.

Competing Interests Statement

The authors declare that they have no competing interests.

Ethics statement

All samples were collected using the standard collection method, without harming the dogs. The research was conducted with the approval of the Ethics Committee of the Veterinary Sciences Research Institute from the Autonomous University of Baja California, registered as CEE-IP-201-72024-1. The personal data provided by the owners of the pets sampled in this study were handled in accordance with the Federal Law for the Protection of Personal Data in Possession of Private Parties (DOF 05-07-2010).

Author contribution

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