Technical note



## Frequency and factors associated with the diagnosis of *Ehrlichia canis* and *Anaplasma* spp. in dogs

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## Abstract:

This study assesses the number of reported cases of canine anaplasmosis and ehrlichiosis in the district of Rímac, Lima, Peru, as well as their association with factors involved in the occurrence of these diseases. In these cases, the presence of anemia and thrombocytopenia is common, which affect normal hematological parameters. All the medical records of the 2018-2021 period of canine patients of the Municipal Veterinary Clinic of Rímac located in the district of Rímac, Lima – Peru, were sampled. The Chisquare statistical test and the contingency coefficient were used to determine the association. All variables were also analyzed using logistic binomial regression. A significance level of 0.05 was used. *Ehrlichia canis* and *Anaplasma* spp. were diagnosed in 4.308 % (224/5,200) of medical records. The Chi-square test was used to evaluate the association with the factors of sex, race, age, and season of the year, concluding that there was an association of the diseases with the age group; at a 95 % confidence interval, it was observed that the frequency of cases of E. canis and Anaplasma spp. was 95.98 % and 1.79 %, respectively, and the co-infection of both pathogens was 2.23 %. The logistic regression model included the effects of live weight and sex on the diagnosis of ehrlichiosis and anaplasmosis, which were significant. There was a significant association between the diagnosis of canine ehrlichiosis and anaplasmosis with age and weight, but there was no effect of breed and season of the year.

Keywords: Anaplasmosis, Anemia, Ehrlichiosis, Medical records, Thrombocytopenia.

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Canine ehrlichiosis, considered one of the most important diseases affecting dogs, has a wide worldwide distribution and is caused by the infectious agent *Ehrlichia canis*, with co-infection with *Anaplasma* spp. (*A. phagocytophilum* and *A. platys*) being common, also transmitted by *Rhipicephalus sanguineus* ticks, which enhances its clinical signs<sup>(1)</sup>, being mostly identified in areas where *E. canis* is endemic<sup>(2,3)</sup>. Infection may be suspected when the dog lives in or travels to an endemic region or has had previous exposure to ticks, with the common diagnostic techniques being hematology, cytology, serology, and isolation, but the definitive diagnosis requires molecular techniques<sup>(4)</sup>. In addition, it is common in warm and temperate climates, such as the summer season, where the vector is active<sup>(5)</sup>. Being on the street, sex, age, German shepherd breed, tick infestation, and not using ectoparasiticides have been mentioned as factors associated with the diagnosis of diseases<sup>(6)</sup>.

Canine ehrlichiosis was first reported in 1982 in Peru, and since then, cases have increased<sup>(7)</sup>. Metropolitan Lima reported a prevalence of canine ehrlichiosis of 4.05 % in the north, 11.5 % in the center, 33.78 % in the south, 43.24 % in the east, and 7.43 % in the west<sup>(8)</sup>. In the districts of Chorrillos, La Molina, and San Juan de Miraflores, 16.5 % of positive cases were reported in 2001<sup>(9)</sup>, another study reported 31.1 % of cases of *E. canis* in Chorrillos in 2019<sup>(10)</sup>, and in 2020 an increase in positive cases of ehrlichiosis was reported, with 59.4 % in the northern zone<sup>(11)</sup>. Current studies conducted in metropolitan Lima reported a total of 29.2 % of positive cases for *Anaplasma platys*<sup>(12)</sup>. Due to the aforementioned information, the objective was to determine the frequency of canine ehrlichiosis and anaplasmosis and the degree of association of the factors of sex, the season of the year, breed, age, and live weight in the Municipal Veterinary Clinic of Rímac (MVCR) in the 2018-2021 period.

To achieve the proposed objectives, a basic, cross-sectional, retrospective, and descriptive study was developed. It has approval from the institutional ethics committee in research on animals and biodiversity of the Scientific University of the South (Code: 399-2021-PRE16). Information from medical records of the MVCR located in the district of Rímac, province of Lima, subregion of Lima-Centro, Peru, was used to carry it out. The climate is subtropical temperate desert with an average annual temperature of 19 °C, ranging between 14 and 30 °C. The average yearly rainfall is less than 15 mm, being more accentuated between July and August. The approximate casuistry per year is 150. The medical records collected were those of canines treated in the period between 2018 and 2021 in the MVCR that have been diagnosed as positive for *E. canis* or *Anaplasma* spp., using the Anigen CaniV-4 kit (BioNote Inc., South Korea), which has a sensitivity and specificity of 97.6 % and 99 % for *E. canis*, while for *Anaplasma* spp., it is 88.5 % and 97.1 %, respectively.

The reading of the medical records was considered to establish factors associated with the infections mentioned above, achieving the following study variables: number of diagnosed clinical cases (Table 1) and age at diagnosis, categorized into three groups (Table 2). For this grouping, it was considered that dogs at an early age present greater risks of being exposed to the vector than dogs considered elderly because when they complete the vaccination schedule, they begin to have regular walks outside<sup>(13,14)</sup>. Season of the year (Table 3) and breed (Table 4) were also considered.

The information collected was tabulated in the Microsoft Excel 2016 program. The Chisquare statistical test and the contingency coefficient were used to determine a preliminary association between the diagnosis and the associated factors. All variables were analyzed through a logistic binomial regression model (multivariate analysis) using the SPSS v.25 program for Windows, through which regression estimates, odds ratio (OR) 95 % confidence intervals, and significance values were obtained. The dependent variable was the evaluation diagnosis of each animal, and the independent variables were sex, breed, age group, and weight. A significance level of 0.05 was used for all calculations.

After processing and analyzing the information, the cases of *E. canis* and *Anaplasma* spp. represented 4.308 % (224/5,200) of the population, being 95.98 % (215/224) for *E. canis*, 1.79 % (4/224) for *Anaplasma* spp., and 2.23 % (5/224) for the co-infection of both pathogens. The significance of the association between the three groups of cases and sex, together with the contingency coefficient, is shown in Table 1. The ages of the dogs positive for both diseases ranged from 1 mo to 14 yr, with most of them being younger than 2 yr, with 47.76 % (107/224), followed by older than 3 yr, with 32.58 % (73/224) (Table 2). There were more cases of canine ehrlichiosis and anaplasmosis in the autumn season, with 40.18 % (90/224), followed by summer with 33.48 % (75/224) (Table 3). For the breed variable, the majority were crossbred, with 58.04 % (130/224). In crossbred and purebred dogs, canine ehrlichiosis accounted for the majority of cases, with 56.25 % (126/224) and 39.73 % (89/224), respectively, with the Shih Tzu breed standing out (Tables 4 and 5).

Table 1: Frequency of positive cases of canine ehrlichiosis and anaplasmosis associat	ted
with sex, with Chi-square <i>P</i> -value and contingency coefficient in parentheses	

	E. canis	Anaplas ma spp.	<i>Co-infection</i> of <i>E.</i> <i>canis</i> and <i>Anaplasma</i> <b>spp.</b>	Medical records	Percentage of the total	<i>P</i> -value
F	80	4	0	84	37.5	0.072
Μ	135	0	5	140	62.5	(0.174)
Т	215	4	5	224	100	

F= females; M= males; T= total.

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	E canis	Anaplasma	Co-	Medical	Percentage	D voluo
	E. canis	spp.	infection	records	of the total	1 -value
< 2 years	101	2	4	107	46.98	0.003
2-3 years	44	0	0	44	20.47	(0.003)
>3 years	70	2	1	73	32.56	(0.283)
Total	215	4	5	224	100	

**Table 2:** Diagnosis of canine ehrlichiosis and anaplasmosis associated with the age group, with Chi-square *P*-value and contingency coefficient in parentheses

**Table 3:** Diagnosis of canine ehrlichiosis and anaplasmosis associated with season of the year, with Chi-square *P*-value and contingency coefficient in parentheses

	<i>E</i> .	Anaplasma	Co-	Medical	Percentage	<b>P-</b>
	canis	spp.	infection	records	of the total	value
Spring	22	1	1	24	10.71	
Summer	72	2	1	75	33.48	0.051
Autumn	89	1	0	90	40.18	(0.264)
Winter	32	0	3	35	15.63	
Total	215	4	5	224	100	

**Table 4:** Diagnosis of canine ehrlichiosis and anaplasmosis associated with breed, with

 Chi-square and contingency coefficient in parentheses

en square and contingency coefficient in parentices						
	E. Anaplasma Co- Total Percentage P-					
	canis	spp.	infection	cases	of the total	value
Crossbred	126	3	1	130	58.04	0.774
Purebred	89	1	4	94	41.96	(0.074)
Total	215	4	5	224	100	

		Ananlasma	E. canis and	
Breed	E. canis	Anapiasma	Anaplasma	Total cases
		շիհ.	spp.	
American bully	2	0	0	2
Bichon	3	0	0	3
Bull terrier	3	0	0	3
English bulldog	3	0	0	3
Chihuahua	2	0	0	2
Chow chow	2	0	0	2
Cocker	8	0	0	8
Dobermann	1	0	0	1
Argentine Dogo	1	0	0	1
Golden	7	0	0	7
Labrador	4	0	1	5
Maltese	2	0	0	2
Shepherd	1	0	0	1
German Shepherd	2	0	0	2
Pekingese	1	0	0	1
Peruvian hairless dog	1	0	1	2
Pit Bull	8	0	0	8
Poodle	7	0	0	7
Pug	1	0	0	1
Rottweiler	1	1	0	2
Samoyed	3	0	0	3
Schnauzer	8	0	0	8
Shar-pei	1	0	0	1
Shih Tzu	12	0	0	12
Siberian	3	0	2	5
Dachshund	1	0	0	1
Yorkshire terrier	1	0	0	1
Crossbred	126	3	1	130

**Table 5:** Diagnosis of positive cases of canine ehrlichiosis and anaplasmosis according to breed at the Municipal Veterinary Clinic of Rímac

Regarding the results of the complete blood count recorded in the medical records and the result of multinomial regression: regression coefficient ( $\beta$ ), odds ratio at a 95 % confidence interval, it was found that the risk of thrombocytopenia and anemia with thrombocytopenia in females is 0.28 and 0.41 times less likely than males, respectively. On the other hand, the risk of thrombocytopenia for each kilogram gained in weight is 1.172 times more likely (Table 6).

		-	OR 95% confidence interva		
	β (SE)	Odds ratio	Lower	Upper	
Normal vs Anemia					
Intercept	0.402 (0.746)				
Female	-0.401 (0.453)	0.67	0.275	1.629	
Male		Refe	rence		
Crossbred	0.306 (0.465)	1.359	0.546	3.377	
Purebred	,	Refe	rence		
Age group G1	0.640 (0.856)	1.896	0.354	10.157	
Age group G2	-2.168 (1.459)	0.114	0.007	1.998	
Age group G3		Refe	rence		
Weight	0.03 (0.049)	1.03	0.936	1.134	
8	(,				
Age group G1 x Weight	-0.122 (0.074)	0.885	0.766	1.023	
Age group G2 x Weight	0.206 (0.140)	1.228	0.934	1.616	
Age group G3 x Weight		Refe	rence		
Normal vs Thrombocyto	penia				
Intercept	-1.183 (1.830)				
Female	-1.274 (0.532)*	0.28	0.099	0.794	
Male	1127 (01002)	Refe	rence	0.77	
Crossbred	-0.148 (0.517)	0.863	0.313	2.378	
Purebred		Refe	rence		
Age group G1	1.932 (1.154)	6.9	0.719	66.204	
Age group G2	1.401 (1.571)	4.06	0.187	88.199	
Age group G3	~ /	Refe	rence		
Weight	0 158 (0 056)**	1 170	1 051	1 207	
vv eigin	0.138 (0.030)***	1.1/2	1.031	1.307	
Age group G1 x Weight	-0.138 (0.073)	0.871	0.755	1.005	
Age group G2 x Weight	0.047 (1.141)	1.048	0.796	1.381	
Age group G3 x Weight		Refe	rence		
Normal vs A AND T					
Intercept	0.254 (0.762)				
-					

**Table 6:** Multinomial regression result: regression coefficient (β), odds ratio, and 95 % confidence interval

Female Male	-0.892 (0.427)*	0.41 Refe	0.178 erence	0.946
Crossbred Purebred	0.406 (0.433)	1.502 Refe	0.642 erence	3.51
Age group G1 Age group G2 Age group G3	1.011 (0.843) -1.022 (1.414)	2.748 0.360 Refe	0.527 0.023 erence	14.340 5.752
Weight	0.040 (0.050)	1.041	0.943	1.149
Age group G1 x Weight Age group G2 x Weight Age group G3 x Weight	-0.016 (0.062) 0.155 (0.139)	0.984 1.167 Refe	0.872 0.889 erence	1.111 1.533

Note:  $R^2 = 0.219$  (Cox and Snell), 0.237 (Nagelkerke); Final model=  $\beta$  + Sex + Breed + Age Group + Weight + Age Group \* Weight; Ji<sup>2</sup>= 55.508; \* *P*<0.05; \*\* *P*< 0.01; Normal: it considers canines that have no alterations in the complete blood count. G1= under 2 yr of age; G2= 2 to 3 yr; G3= over 3 yr old.

In 2009, cases of canine ehrlichiosis with a history of origin were reported in multiple districts of Lima, obtaining 4.05 % in the north, 11.5 % in the center, 33.78 % in the south, 43.24 % in the east, and 7.43 % in the west<sup>(8)</sup>; in the districts of northern Lima, there was a frequency of 36.7 % for *E. canis* in  $2017^{(15)}$ , increasing to 59.4 % in  $2020^{(11)}$ ; in Callao, the overall seroprevalence for canine ehrlichiosis was 57.5 % in Ventanilla<sup>(16)</sup>; for the districts of Chorrillos, La Molina, and San Juan de Miraflores, 16.5 % of antibodies against *E. canis* were reported for the first time with the ELISA technique<sup>(9)</sup>. In Chorrillos, the prevalence of *E. canis* was 31.1 % in 2019<sup>(10)</sup>; in San Juan de Lurigancho, it was 46.44 % in  $2016^{(17)}$ , increasing to 47.5 % in  $2017^{(14)}$ ; in Lima, at the Cayetano Heredia University, 45.5 % of canine ehrlichiosis and 10.6 % of canine anaplasmosis were obtained. In 2015, positive cases of canine anaplasmosis were 29.2 % in Lima<sup>(12)</sup>. Having these reference data to compare the frequency of 4.308 % of cases of canine ehrlichiosis and anaplasmosis in the district of Rímac, it is suggested that they are less frequent than in other districts, probably due to different inclusion criteria or the use of other variables included in the analysis or even the use of more precise molecular techniques<sup>(12)</sup>. Nonetheless, there may also be a low prevalence, as found in the districts of the northern zone or the central zone mentioned above<sup>(8)</sup>, considering that the district of Rímac is close or even adjacent to these areas.

Paiva and Giset<sup>(18)</sup> mention that for the vector to complete its biological cycle, it must have optimal conditions of climate and humidity, ideally high temperatures of 30 °C and humidity of 20 % to 93 %, otherwise the cycle can extend for several months, which is why the tick-borne disease is considered to be present in tropical and subtropical regions<sup>(3)</sup>, such as Lima, which has an arid subtropical climate with annual temperatures ranging from 19.5 °C to  $20.3^{(19)}$ , with the highest temperature recorded in February with an average of 26.5 °C<sup>(7)</sup>. This study recorded a higher frequency of cases of canine ehrlichiosis and anaplasmosis in autumn, with 40.18 % (Table 3); this does not necessarily indicate that there is a greater probability of contagion in autumn, as another study indicates higher cases in summer, with 64.6 % of canine ehrlichiosis<sup>(14)</sup>; it is known that canine ehrlichiosis can be present throughout the year, probably due to climate change and temperature variation in Peru, or the incubation period to present clinical signs according to the canine's immune system<sup>(20)</sup>; on the other hand, because the vector is sensitive to cold, its presence decreases in winter<sup>(21)</sup>, but after winter rest, some of the different stages of its biological cycle survive and simultaneously infect the susceptible animal, mainly in spring and autumn, reaching its maximum multiplication in summer<sup>(14)</sup>.

It is known that the infection of both diseases does not distinguish the host by sex, age, or breed<sup>(22,23)</sup>; nevertheless, another author considers females more susceptible to contracting the disease during the estrus season due to exposure to males that do not always have control against ectoparasites<sup>(9)</sup>. Previous studies by Rodríguez *et al*<sup>(24)</sup> and Zambrano<sup>(25)</sup> found more cases of anaplasmosis and ehrlichiosis in males. Infection may be related to the degree of immune response and the presence of the vector<sup>(18,26)</sup>. These studies coincide with those of the present study since 62.5 % of the dogs positive for *E. canis* and/or *Anaplasma* spp. were males (Table 1); these results may probably be influenced by the number of male, crossbred, and medium-sized dogs, 56.6 %, 54.1 %, and 42.2 %, respectively, according to the study by Arauco *et al*<sup>(27)</sup>.

It has been reported that one of the risk factors associated with canine ehrlichiosis disease is early age, indicating more cases in dogs under 1 yr of  $age^{(16,28)}$ , over 1 yr of  $age^{(29)}$ , followed by 6 to 11 mo of  $age^{(30)}$ ; in addition, it has been reported that most dogs affected with *E. canis* and *A. platys* are between 13 and 24 mo old<sup>(31)</sup>; between 2 and 4 yr old<sup>(8,11,32)</sup>, older than 4 yr<sup>(33)</sup>, 2 to 6, and 6 yr old or older<sup>(10)</sup>; on the other hand, Villaverde<sup>(13)</sup> mentions that the median age of dogs with antibodies positive for *Ehrlichia* spp. is 24 mo, coinciding with the results of this study since the age group that had the highest number of cases was that of less than 2 yr, with 47.76 % (Table 2). These results suggest that, at the end of the vaccination schedule, canines at an early age are more exposed to the vector since owners consider that they are fully protected against pathogens<sup>(7,14)</sup>.

It is known that all breeds have the same probability of infection<sup>(22)</sup>; however, the German Shepherd breed seems to have a greater predisposition to develop the clinical form<sup>(8)</sup>, as does the Springer Spaniel<sup>(22)</sup>; in contrast, in this study, the Shih Tzu breed stood out among breeds (Table 5). In this study, the majority of cases of canine anaplasmosis and/or ehrlichiosis were obtained in crossbred dogs, with 58.04 % (Table 4), coinciding with Coello *et al*<sup>(34)</sup>, who indicate that cases of anaplasmosis predominate in crossbred dogs, and what was reported by Lorsirigool and Pumipuntu<sup>(35)</sup>, Villaverde<sup>(13)</sup>, and Cusicanqui and Zuñiga<sup>(11)</sup>, where dogs infected with *E. canis* are mostly crossbred, being common in dogs that have never used an ectoparasiticide or have used it intermittently. The number of dogs positive for these diseases did not allow to identify significant differences, or there were no differences, as has already been shown in other studies<sup>(6)</sup>.

These diseases often alter hematological values, and a complete blood count is essential for diagnosis since thrombocytopenia is considered a factor associated with the disease during all phases<sup>(5,36)</sup>, appearing in 80 % of cases and may be accompanied by regenerative or non-regenerative anemia<sup>(20)</sup>. These results were found in most of the medical records of dogs affected by E. canis and/or Anaplasma spp., obtaining, according to the odds ratio, a lower probability of presenting thrombocytopenia, and anemia with thrombocytopenia in females compared to males (Table 6); this may be associated with the fact that some canines may be undergoing the subclinical stage of the disease or may be incubating the agent without presenting relevant symptomatology or hematological findings. On the other hand, the variation in the kilograms of weight of each canine described in the medical records corresponds to different sizes, breeds, and ages, obtaining a greater probability of presenting thrombocytopenia for each kilogram increase (Table 6); in addition, considering that a different physiological behavior has been described between young and adult dogs for erythrocyte and leukocyte values<sup>(37)</sup>, it has been mentioned that adult dogs positive for the disease have lower values of the red, white and platelet series, and puppies show a lower mean of hemoglobin and red blood cells<sup>(11)</sup>, suggesting that the findings of this study are likely influenced by age between puppy or adult. Finally, this work allows us to conclude that the frequency of cases of E. canis and Anaplasma spp. was 4.308 %. Of this sample, the canines diagnosed with E. canis were 95.98 %, with Anaplasma spp., they were 1.79 %, and the co-infection of both was 2.23 %. There was a significant association between the diagnosis of canine ehrlichiosis and anaplasmosis with age, sex (OR), and weight (OR), but there was no association with the factors of breed and season of the year.

## **Conflict of interest**

The authors declare that they have no conflict of interest.

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