Revalencia de *ehrlichiosis* en perros en la parroquia rural de guachanama comparando diff quick vs snap*4dx

*Revalence of ehrlichiosis in dogs in the rural parish of Guachanama comparing diff quick vs snap*4dx*

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Palabras claves:

Caninos;
Ehrlichiosis;
Prevalencia.

Resumen

Introducción. En el ámbito de la medicina veterinaria, la elección de métodos diagnósticos para identificar agentes patógenos desempeña un papel crucial en la dirección del tratamiento y el pronóstico de las enfermedades. En entornos de campo, la selección de métodos a menudo se ve influida por desafíos como la dificultad, la falta de equipos y el costo de los exámenes. En este contexto, las pruebas Diff Quick y Snap*4dx emergen como alternativas viables, ya que se caracterizan por su ejecución rápida y facilidad de aplicación en el campo, además de proporcionar un alto nivel de confiabilidad en la determinación diagnóstica. **Objetivo.** El objetivo de esta investigación fue evaluar la prevalencia de la Ehrlichiosis canina en la parroquia rural de Guachanama, mediante la comparación de dos métodos de diagnóstico: Diff Quick y Snap*4dx. **Metodología.** En el marco de la presente investigación, se examinaron 100 muestras de sangre procedentes de caninos en los barrios rurales Limón, Linuma y La Hamaca, ubicados en la parroquia Guachanama del Cantón Paltas. El diagnóstico de la Ehrlichiosis se llevó a cabo mediante la utilización de los métodos Diff Quick y Snap*4dx, posibilitándonos así realizar una comparación diagnóstica entre estos dos enfoques. **Resultados.** Tras obtener los resultados, se evidenció que la eficacia diagnóstica de la técnica con Diff Quick fue inferior, revelando un total de 38 muestras positivas. En contraste, la técnica Snap*4dx mostró un desempeño superior con un total de 40 muestras positivas y 60 negativas. La diferencia fue significativamente más pronunciada en las muestras tomadas de caninos menores a un año, con un total de 12 positivas, en comparación con las muestras de caninos mayores a un año, donde se registraron 20 positivas. En relación con la prevalencia por sectores, se observó que en el barrio La Hamaca, ubicado a una altitud de 2800 metros sobre el nivel del mar, con un rango climático de 18 a 20 grados centígrados, se registró el mayor número de casos positivos, totalizando 19. En el barrio Linuma se identificaron 12 pacientes positivos, mientras que en el barrio Limon, situado a 1100 metros sobre el nivel del mar con un clima cálido, se detectaron 7 casos positivos. **Conclusión.** Basándonos en los resultados obtenidos en el presente estudio, se puede inferir que el diagnóstico de Ehrlichiosis canina a través del método con Diff Quick demostró una eficacia inferior, revelando un total de

38 muestras positivas (38%) y 62 negativas (62%). En contraste, la técnica Snap4dx exhibió un rendimiento superior, con un total de 40 muestras positivas (40%) y 60 negativas (60%). Estos hallazgos destacan la mayor precisión diagnóstica de la técnica Snap4dx en comparación con el método con Diff Quick en el ámbito de la Medicina Veterinaria. **Área del conocimiento:** Medicina Veterinaria

Keywords:
Canines;
Ehrlichiosis;
Prevalence.

Abstract

Introduction. In the field of veterinary medicine, the choice of diagnostic methods to identify pathogens plays a crucial role in directing the treatment and prognosis of diseases. In field settings, method selection is often influenced by challenges such as difficulty, lack of equipment and cost of testing. In this context, Diff Quick and Snap*4dx tests emerge as viable alternatives, as they are characterized by rapid execution and ease of application in the field, in addition to providing a high level of reliability in diagnostic determination. objective. The objective of this research was to evaluate the prevalence of canine ehrlichiosis in the rural parish of Guachanama, by comparing two diagnostic methods: Diff Quick and Snap*4dx. Methodology. Within the framework of the present investigation, 100 blood samples from canines in the rural neighborhoods of Limon, Linuma and La Hamaca, located in the parish of Guachanama, Canton Paltas, were examined. The diagnosis of Ehrlichiosis was carried out using the Diff Quick and Snap*4dx methods, allowing us to make a diagnostic comparison between these two approaches. Results. After obtaining the results, it was evident that the diagnostic efficacy of the Diff Quick technique was inferior, revealing a total of 38 positive samples. In contrast, the Snap*4dx technique showed superior performance with a total of 40 positive and 60 negative samples. The difference was significantly more pronounced in samples taken from canines younger than one year, with a total of 12 positives, compared to samples from canines older than one year, where 20 positives were recorded. In relation to the prevalence by sectors, it was observed that in the La Hamaca neighborhood, located at an altitude of 2800 meters above sea level, with a climatic range of 18 to 20 degrees Celsius, the highest number of positive cases was recorded, totaling 19. In the Linuma neighborhood, 12 positive patients were identified,

while in the Limon neighborhood, located at 1100 meters above sea level with a warm climate, 7 positive cases were detected. Conclusion. Based on the results obtained in the present study, it can be inferred that the diagnosis of canine Ehrlichiosis through the Diff Quick method showed inferior efficacy, revealing a total of 38 positive (38%) and 62 negative (62%) samples. In contrast, the Snap4dx technique exhibited superior performance, with a total of 40 positive (40%) and 60 negative (60%) samples. These findings highlight the higher diagnostic accuracy of the Snap4dx technique compared to the Diff Quick method in the field of Veterinary Medicine. Area of knowledge: Veterinary Medicine

Introduction.

Canine ehrlichiosis (CE), also called canine tropical pancytopenia, canine typhus, canine hemorrhagic fever and idiopathic hemorrhagic syndrome, among others. (León, Gómez, 2007).

Canine ehrlichiosis is an emerging tick-borne infectious disease caused by Ehrlichia spp. which affects members of the Canidae family. Clara, G. (2016). In tropical countries, infection of dogs with Ehrlichia canis is one of the most common diseases in veterinary practice, the tick vector Rhipicephalus sanguineus is common in rural and urban areas Daniel, M et al (2007). Ticks and tick-borne diseases are considered a major challenge to human and animal health in tropical, subtropical and temperate regions of the worldJean, Z, et al (2020).

E. canis was first identified in Algeria in 1935 (Donatien and Lestoquard). They are small (0.5 μm diameter), Gram-negative, obligate intracellular bacteria, pleomorphic coccoids, which parasitize the cytoplasm of circulating leukocytes (monocytes, macrophages and granulocytes) in groups of organisms called morulae. (Waner et al 2000).

Since 2001, bacteria of the genus Ehrlichia belong to the group Alphaproteobacteria, order Rickettsiales and family Anaplasmataceae (Dumler et al. 2001, Bowman 2011). The order Rickettsiales also includes the family Rickettsiaceae and a biological difference between the two families is that bacteria of the family Anaplasmataceae multiply within membrane-bound vacuoles while members of the family Rickettsiaceae multiply freely in the cytoplasm of the host cell (Rikihisa 2010).

Dog infection occurs when infected ticks ingest blood and their salivary secretions contaminate the feeding site (Procajło et al. 2011). Tick saliva contains a variety of anticoagulant, anti-inflammatory and immunoregulatory molecules that facilitate pathogen acquisition and transmission (Day 2011, Hajdušek et al. 2013).

Among the techniques used for the diagnosis of E. canis are: indirect immunofluorescence (IFI), Anigen Kit for E. canis Ab, ELISA, direct smear, and polymerase chain reaction (PCR), (Carrillo, et, al. 2012).

The patient's history, physical examination, epidemiological data, and checking for the presence of ticks are important to determine the presence of the disease. However, clinical diagnosis is difficult, because the initial signs are generally nonspecific (Barrios, et, al. 2013).

In this context, it is crucial to know the population of dogs and cats in the rural community, as this will allow us to efficiently address various situations, considering that this information, research and actions related to these issues are limited. This research aims to make a contribution to the parish and the neighborhoods under study on animal health. In addition, by determining the prevalence of diseases in these environments, it will provide essential data to make more effective diagnoses in our daily clinic, which is whyThe objective of this research work was to determine the prevalence of Ehrlichiosis canis in dogs from the rural parish of Guachanama.

Methodology.

This study was carried out in three neighborhoods of the rural parish of Guachanama, located in the Paltas Canton of the Province of Loja, Ecuador. A total of 100 blood samples were collected from dogs residing in the rural neighborhoods of Limón, Linuma and La Hamaca, which are located at varying altitudes of 1100, 2000 and 2800 meters above sea level, respectively. The samples were obtained by depilation and disinfection of the area. A 5 ml syringe was used to collect 3 ml of blood sample from the cephalic vein, having as options the femoral and saphenous veins. Both the samples taken from dogs with clinical signs of ehrlichiosis and those without such signs were transported to the VETSALUD Veterinary Clinic. Subsequently, the corresponding diagnostic techniques were carried out.

To perform the Snap4Dx test, the device was placed on a horizontal surface. A drop of blood was carefully added to the sample well, taking care not to spill the contents outside the well. The sample was then allowed to flow through, waiting 30-60 seconds for the device to activate. When the activation circle appeared, the activator was pressed firmly until it was flush with the body of the device. After 8 minutes, the results were read, noting any colour development that indicated a positive sample.

For the Diff Quick technique, a drop of blood sample was placed on a slide. Using another slide, a smear was made and left to dry for a few minutes. Staining was then carried out; initially, we dipped the slide in the fixative five times, repeating the procedure with stains A and B. The slide was then washed with water, left to dry, and observed under a microscope.

Results.

To determine the total prevalence of Ehrlichiosis in dogs, a total of 100 blood samples from three rural neighborhoods of the Paltas Canton were analyzed, which were processed weekly at the Vetsalud veterinary clinic.

To determine the total percentage of Ehrlichiosis, a total of 100 canine samples from 3 rural neighborhoods of the Paltas Canton were analyzed, which were processed at the Vetsalud veterinary clinic.
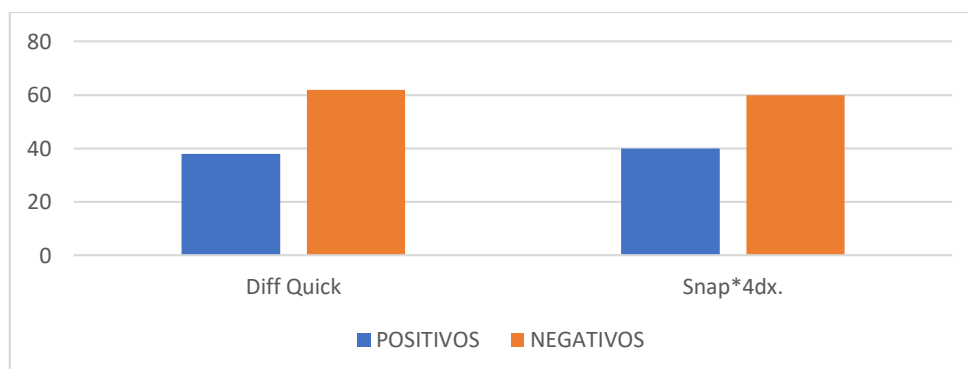
Table 1.

Total percentage of ehrlichiosis in dogs

	DIFF QUICK	SNAP*4DX.
POSITIVES	38	40
NEGATIVES	62	60
TOTAL	100	100

Table 1 shows the total number of dogs sampled, of which 38 canines, representing 38%, are positive for the Diff Quick technique and 60, representing 62%, are negative; while with the Snap*4dx technique, 40 canines, representing 40%, are positive and 60 canines, representing 60%, are negative.

Figure 1

Total percentage of Ehrlichiosis in dogs

Table 2

Prevalence by age with the Diff Quick technique

AGE	POSITIVES	NEGATIVES
Under one year old	12	45
Older than one year	20	17

As shown in the table, two of the total positive cases, 12 are less than one year old, 20 are older than one year, 45 are negative cases less than one year old, and 17 are negative cases in dogs older than one year.

Figure 2

Prevalence by age with the Snap*4dx technique.
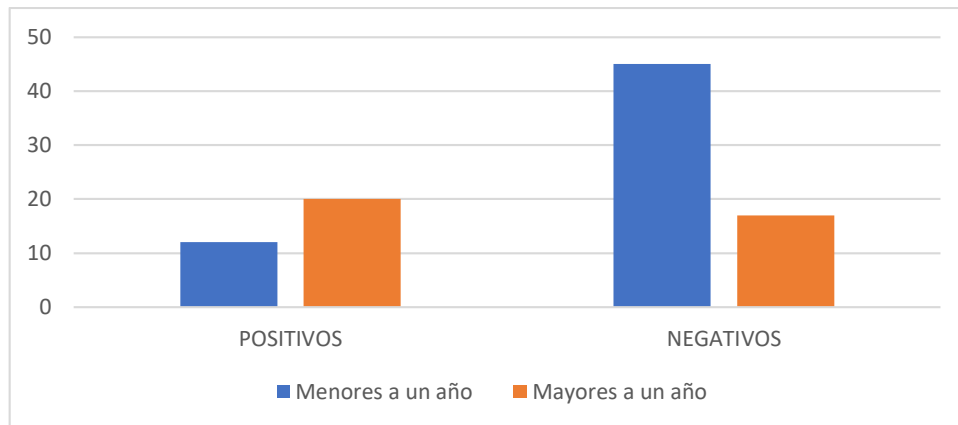
Table 3

Prevalence by age with the snap*4dx technique

	POSITIVES	NEGATIVES
Under one year old	12	45
Older than one year	28	15

As can be seen in table three with the Snap*4dx.s technique, of the total number of positive cases, 12 correspond to samples from dogs less than one year old, 28 positive samples in blood samples from canines older than one year; while 45 negative samples in dogs less than one year old and 15 negative samples in dogs older than one year.

Figure 3

Prevalence by age with the snap*4dx technique
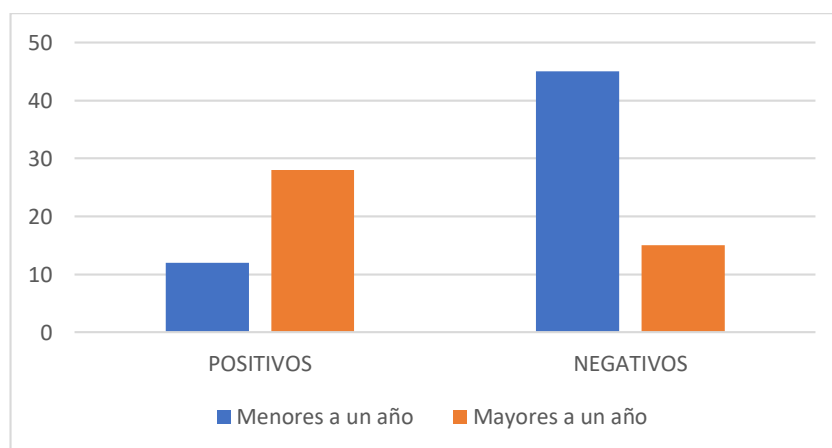
To determine the prevalence of Ehrlichiosis by sectors, a total of 33 blood samples were taken in each of the neighborhoods such as Limón, Linuma and La Hamaca in the Guachanama parish of the Paltas Canton, which were processed at the Vetsalud veterinary clinic.

Table 4

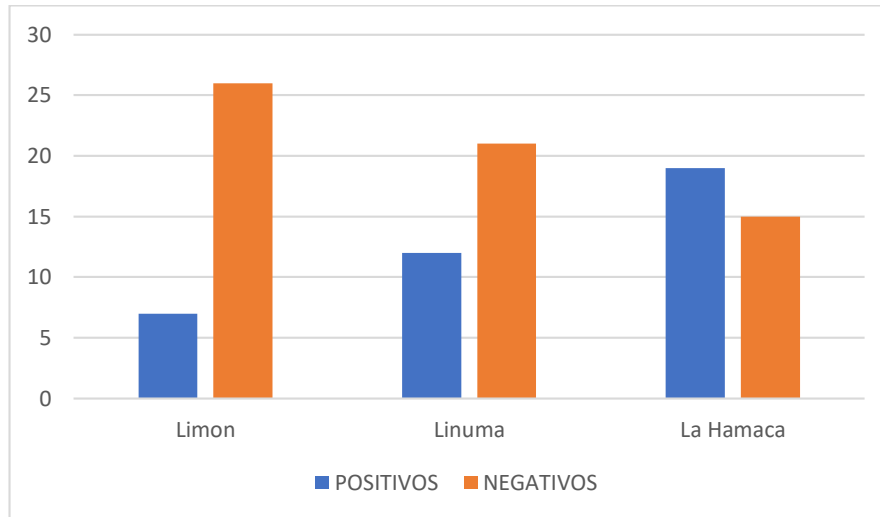Prevalence by sector with the Diff Quick technique

NEIGHBORHOODS	POSITIVES	NEGATIVES
Lemon	7	26
Linuma	12	21
The Hammock	19	15
TOTAL	38	62

As shown in table four, using the Diff Quick technique, of the 33 samples taken in each of the neighborhoods, the positive results are 7 in the Limón neighborhood, 12 in the Linuma neighborhood, and 19 positive samples in the La Hamaca neighborhood; as for the negative samples, we have 26 in the Limón neighborhood, 21 negative samples in the Linuma neighborhood, and 15 negative samples in the La Hamaca neighborhood.

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Figure 4

Prevalence by sector with the Diff Quick technique

Prevalence by Snap*4dx technique sectors.

In the same way, the 33 samples were evaluated with the Snap*4dx technique in each of the neighborhoods such as Limon, Linuma and La Hamaca in the Guachanama parish of the Paltas Canton, which were processed in the Vetsalud veterinary clinic.
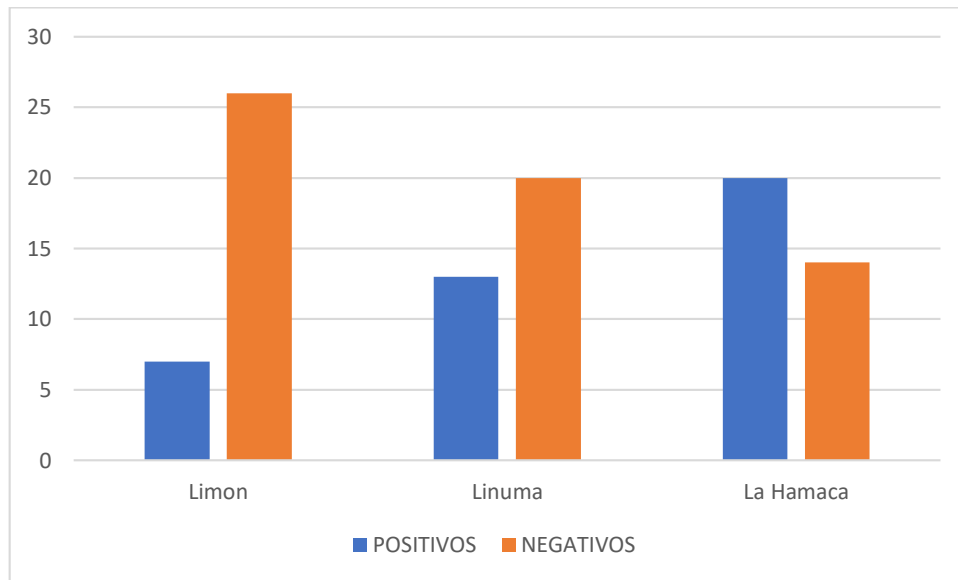
Table 5

Prevalence by Snap*4dx technique sectors.

NEIGHBORHOODS	POSITIVES	NEGATIVES
Lemon	7	26
Linuma	13	20
The Hammock	20	14
TOTAL	40	60

As we can see in figure five four with the Snap*4dx technique, of the 33 samples taken in each of the neighborhoods, the positive results are 7 in the Limon neighborhood, 13 in the Linuma neighborhood, and 20 positive samples in the La Hamaca neighborhood; as for the negative samples, we have 26 in the Limon neighborhood, 20 negative samples in the Linuma neighborhood, and 14 negative samples in the La Hamaca neighborhood.

Figure 5

Prevalence by Snap*4dx technique sectors.

The prevalence of Ehrlichiosis according to sex was carried out by classifying both males and females taking into account the information taken and attached in the registration sheet in order to determine the prevalence in each sex.

Table 6

Prevalence of Ehrlichiosis according to sex

SEX	TOTAL,OF DOGS EXAMINED	POSITIVES	NEGATIVES
Males	56	25	31
Females	44	20	24
TOTAL	100	45	55

As observed in Table 6, of the total of 56 (56%) males sampled, 25 (25%) were positive and 21 (21%) negative; of 44 (44%) strands sampled, 31 (31%) were positive and 24 (24%) negative strands.

Figure 6

Prevalence of Ehrlichiosis according to sex
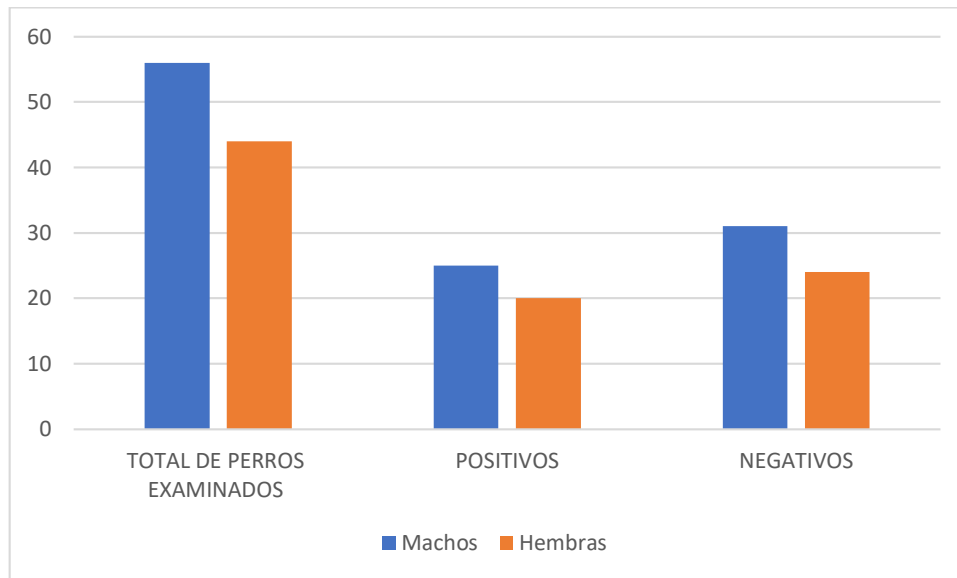
Discussion.

In this research, a comparison was carried out between two diagnostic methods: Diff Quick and Snap4dx, with the aim of determining which of them is more effective. When analyzing the samples and reviewing the results, it was observed that the diagnostic technique using Diff Quick turned out to be less efficient, revealing a total of 38 positive samples and 62 negative ones. On the other hand, the Snap4dx technique showed a slightly higher performance, with a total of 40 positive samples and 60 negative ones.

These results could be in line with the observations of Wagner, T. et al (19987), who, when studying clinically healthy dogs, stated that the high incidence of the disease in the area could be attributed to the subtropical climate, which provides a favorable habitat for ticks, especially the Rhipicephalus genus, the main vector present in tropical and temperate areas worldwide. These findings highlight the importance of considering climatic and geographical conditions when evaluating the efficacy of diagnostic methods in this specific region.

The data collected on the prevalence of the disease in relation to sex reveal that, of the 56 males sampled (56%), 25 (25%) were positive and 21 (21%) were negative. Regarding the 44 females sampled (44%), 31 (31%) were positive and 24 (24%) were negative. These results agree with the statement of León and Gómez (2007), who indicated that the disease manifests itself independently of age, sex and race, thus coinciding with the findings of our study.

Regarding the age variable in our study, we observed that 12 positive cases were recorded in dogs under one year of age, 20 positive cases in dogs over one year of age, 45 negative cases in dogs under one year of age, and 17 negative samples in dogs over one year of age. These results agree with the statements of León and Gómez (2007) and Sainz et al. (2000), who maintain that there is no significant relationship between the presentation of the disease and the age of the canines.

The prevalence of canine ehrlichiosis in the different neighborhoods reveals a significant pattern, with the La Hamaca neighborhood presenting the highest prevalence. 20 positive samples were identified in this sector, which represents 20% of the canine population studied in that area. This finding raises questions about possible environmental, geographic or management factors that could be contributing to this disparity in prevalence compared to other neighborhoods evaluated.

A previous study conducted by Caraguay (2015) on dogs from rural neighborhoods in Catamayo Canton supports the idea that the prevalence found in neighborhoods may be influenced more by extrinsic factors than by factors dependent on the animal. For example, the neighborhoods of La Vega and Monterrey, with 81.25% of positive cases each, have canines destined to care for farms and to accompany their owners in the daily workday. The proximity of these neighborhoods to sugarcane plantations and the close interaction with animals of other species could be exacerbating the situation, according to the study. These findings suggest the need to further explore the contextual elements that could explain the differences in prevalence observed between the different neighborhoods.

Conclusions.

- Based on the findings of this research, it is concluded that the canine Ehrlichiosis diagnostic method using Quick Diff showed lower efficacy, yielding a total of 38 positive samples (38%) and 62 negative samples (62%). In contrast, the Snap4dx technique exhibited superior performance, recording a total of 40 positive samples (40%) and 60 negative samples (60%). These results highlight the greater efficacy of the Snap4dx technique compared to the Quick Diff method in the field of canine Ehrlichiosis diagnosis.

- The results of this study also reveal variations in the prevalence of canine Ehrlichiosis based on the sex of the canines examined. The prevalence in males was found to be 25%, while in females it was slightly higher, reaching 31%. This finding suggests the possibility of a disparity in the susceptibility of the genders to Ehrlichia infection, which could have significant implications for the development of preventative strategies and control protocols in canine populations. Further research is essential to further our understanding of the

underlying factors contributing to these sex-based prevalence disparities and to assess their impact on the overall health of the canine population.

- The analysis of the prevalence of canine Ehrlichiosis at the neighborhood level highlights a significant pattern, with the La Hamaca neighborhood standing out as having a higher prevalence. 20 positive samples were identified in this neighborhood, representing 20% of the canine population studied in this sector. This finding points to a more marked concentration of positive cases in La Hamaca compared to other neighborhoods evaluated, raising questions about possible environmental, geographic or management factors that could contribute to this disparity in prevalence.

Conflict of interest

The authors certify that there are no conflicts of interest in this work.

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Indexaciones