



Epidemiological status of ehrlichiosis as a high-risk zoonosis in endemic populations

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Abstract

This work was carried out at the animal health veterinary clinic, located on Cuauhtémoc avenue, Colonia La Rivera, in the city of Tuxpan de Rodríguez Cano, Veracruz, Mexico, during the period of October 2019 - March 2020. Aiming to determine the prevalence canine Ehrlichiosis, for which 30 canine *E. canina* serotypes were identified. For this evaluation, the AB GENOME kit was used, which was based on an indirect ELISA test in canines with apparent symptoms of the disease. The data were processed in the SPSS version 10 statistical package and the differences between treatments were detected by Duncan's test, the level of significance was 95% taking into account the variables of sex, age and race. The results indicated 23% of positive cases of the canines exposed to *E. canis* and 7 negative cases with a prevalence of 76.66%. According to the results obtained that were based on the association that existed between the presence of antibodies against *E. canis*, with age, race, sex, it was determined that there is no association between the presence of antibodies against *E. canis* and none of the variables described above.

Keywords: Canine Ehrlichiosis; Canines; AB genome kit; ELISA; Prevalence.

1. Introduction

Canine Ehrlichiosis is a rickettsial disease, the genus Ehrlichia was designated in 1945 and owes its name to Paul Ehrlich [1]. This disease is caused by microorganisms of the genus Ehrlichia spp., having as its main agent *Ehrlichia canis*, a Gram negative intracellular compulsory coccoid or pleomorphic microorganism which has monocytes, macrophages and lymphocytes as target cells. In peripheral blood they are observed in intracytoplasmic bacterial groups called "dense forms" (0.2-0.4ml) and "light forms" (0.8-1.5 ml), the latter being associated with strains of greater pathogenicity [2].

Fisher and Moreina *et al.* (2007) [3], stated that the genus Ehrlichia is made up of gram-negative obligate intracellular bacteria, transmitted by ticks that mainly infect leukocytes (monocytes, macrophages, granulocytes). For several years, the cases of *Ehrlichia canis* have increased worldwide, classifying it as an emerging disease [4-5]. Environmental changes resulting from global warming, the demographic increase of the human population, the transport of pets from one region to another and deforestation are some factors that modify the transmission and spread of this disease (Gutiérrez *et al.*, 2016) [5] [6].

Also known as canine rickettsiosis, canine hemorrhagic fever, sniffer dog disease, canine tick typhus, Nairobi hemorrhagic disorder, and canine tropical pancytopenia [7]. Gutiérrez *et al.* (2013) [8], demonstrated that Ehrlichiosis is a zoonotic disease. In members of the canidae family, it occurs with a high incidence, being infectious and fatal with a febrile nature.

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Currently in the city of Tuxpan; Veracruz, México an increase in dogs with the signs of Ehrlichiosis has been observed, however, as in all emerging diseases, the problem of their accurate and timely diagnosis has made it difficult to establish control measures.

2. Material and Methods

The present work was developed in a small species veterinary clinic, located in the La Rivera neighborhood, during the period October 2019-March 2020 in the city of Tuxpan de Rodríguez Cano; Veracruz Mexico. Geographically located between meridians 20°57'46"N 97°24'01'0. With a tropical climate and an average annual temperature of 24 °C.

In this research, a random study of 30 dogs (*Canis lupus*) of different species, age, breed and indistinct sex was carried out. Dogs that according to the symptoms and clinical history were suspected of the disease were included in the study that consisted of doing the test of the AB Genome *Ehrlichia canis* brand test kit. Test results appear on control and test lines, which use immunochromatography principles. With a specificity of 100% and sensitivity of 98.6%, to achieve the detection of *E. canis*

2.1. The procedure to perform the test was as follows

In this procedure, a blood sample was taken from the cephalic vein of the forelimb, the right vein or the jugular vein with a 3 ml disposable syringe required for the *Ehrlichia canis* test.

A dose was taken with the Test pipette of (2/3) from the tip of the pipette to the control line, three drops were applied in the Test and it took 5 seconds for a complete absorption of the whole blood to be performed. Subsequently 1 drop of Buffer was added (It is a solution whose proton concentration varies when strong acids or bases are added) waiting 5-10 minutes to read the Test.

The interpretation of the test was carried out, which has two white windows marked with the letter C and T. The presence of a red band in section C indicates a Negative result. If the presence of two red bands appears, one in section C and the other in T, this indicates a positive result, which allowed us to make a positive diagnosis for the presence of *Ehrlichia canis*. To collect information on canines with symptoms of the disease, the clinical sheets were used, recording data such as: date, patient name, age, sex, race, temperature, heart rate, respiratory rate, date of deworming and clinical signs.

The data were processed in the SPSS version 10 statistical package and the differences between treatments were detected by Duncan's test. The level of significance was 95%. The variables to take into account were race, sex and age.

3. Results and Discussion

The pathogenesis of ehrlichiosis includes an incubation period of 8 to 20 days, followed by an acute, subclinical, and sometimes chronic phase. During the acute phase, the parasite enters the blood and lymphatic streams and is localized in the macrophages of the reticulo-endothelial system of the spleen, liver and lymph nodes, where it replicates by binary fission. From there, the infected mononuclear cells spread the rickettsia to other organs of the body. Dogs from endemic regions, traveling to or from endemic areas should be considered as potential candidates to become ill. The distribution of ehrlichiosis is related to the distribution of the *Rhipicephalus sanguineus* vector and its occurrence has been described in four continents including Asia, Africa, Europe and America [9].

Table 2 Distribution of canines positive and negative to canine Ehrlichiosis according to the AB Genome Test

Cases		Number	%
Prevalence	Positives	23	76.66
	Negativos	7	23.33
Total		30	100

A wide variety of studies have described that clinical signs can be due to many factors, including differences in pathogenicity between strains of ehrlichia, concomitant infections with other tick-borne diseases, and the immune status of the dog. There is no sexual or age predilection in infection with *E. canis* and all races can be infected [10].

In the identification of canine serotypes to ehrlichiosis, assisted in the animal health veterinary clinic, using the Kit AB genome test. It can be seen in Table 2, the distribution of the 30 canines that were subjected to the test and with it the determination of the number of positives (23) and negatives (7) to canine Ehrlichiosis, observing a prevalence of 76.66%. The classification of positive results by sex (Table 3) is important in the study, since it is possible to know if females or males are predisposing,

Table 3 Distribution of canines positive for canine ehrlichiosis evaluated according to sex

<i>Ehrlichia canis</i>	SEX				Total
	Male		Female		
	Number	%	Number	%	
	17	56.66	13	43.33	

The results are classified according to age; Taking into account gender and the range of 0 - 2 years, 3 - 5 years and older than 6 years, in this way it was analyzed if there was any predisposition to the disease (Table 4).

Table 4 Distribution of positive canines evaluated Canine ehrlichiosis according to age

<i>Ehrlichia canis</i>	EDAD Y SEXO											
	< 2 years				3-5 years				> 6 years			
	M		H		M		H		M		H	
	N°	%	MN°	%	NN°	%	MN°	%	NN°	%	N°	%
	5	221.73	77	330.43	44	117.39	77	330.43	22	88.69	11	4.34

M: Male; H: Remake; N° Sample number

The results considered the pure breed and mestizo, in this way it was analyzed if there is any predisposition between them (Table 5).

Table 5 Distribution of positive canines evaluated Canine ehrlichiosis taking into account the breed

<i>Ehrlichia canis</i>	Race				Total
	Pure		Mongrel		
	N°	%	N°	%	
	16	53.33	14	46.66	30

In the identification of canine serotypes to ehrlichiosis, assisted in the animal health veterinary clinic, using the KIT AB GENOME test. It was observed that the 30 canines that were subjected to the test the number of positives 23 and negatives 7 to canine Ehrlichiosis. Observing a prevalence of 76.66%. The signs of *Ehrlichia canis* are varied and nonspecific because it affects circulating monocytes in the blood and therefore affects systemically in the body, thus producing multisystemic signs in the animal. The hematological analysis test currently used is through the AB Genome Test kit test, it has been the most complete and easy test to detect this disease and give an accurate diagnosis.

It is essential to start the tests aimed at the diagnosis of this disease, which was made clear in this research that *E. canis* does not have a predilection for the age, race or sex of the animal [11]. The highest specific prevalence was observed in females with (43.33%) data that contrasts with the study by Cartagena Yarce et al., (2015) [12], in which females presented a prevalence of infection of (25.9%), this difference could be attributed to the size of the population evaluated.

Signs found epidemiologically worldwide [1], [4] and those reported with the literature [13], coincide with those found in this study. When evaluating the relationship between the age of the canine, race and sex categorized by groups and in the diagnosis of *E. canis* it was determined that there is a significant relationship between both variables, therefore in our study including (race, age and sex) they did not show differences as significant regarding the presence of *E. canis*; that is to say, racially the animals have the same probabilities of being infected [4].

According to the authors Sainz *et al.* (2015) [14], Ehrlichiosis became more important in the Vietnam War, when military dogs became infected with the disease. It is widely distributed worldwide, which has been described in Venezuela, the United States, in the Asian continent (China, Japan, Taiwan, Israel), Australia and Europe mainly; Spain, Greece, France, Germany and Italy.

Although the presence of the vector in the regional area is necessary for the transmission of the disease, its transmission in an iatrogenic way would also be feasible compared to carrying out blood transfusions from infected animals [4]. It is considered a seasonal disease associated with the higher prevalence of the vector in hot and humid periods [6].

The disease is transmitted by the brown dog tick, *Rhipicephalus sanguineus*, and also by blood transfusions from an affected dog to a susceptible dog [15]. Ramírez (2013) [10] describe this disease in three phases. It is common to find ticks on the dog. Clinical signs can be mild and nonspecific, although in some cases they can be severe and life threatening. After an incubation period of 8 to 20 days, this phase begins and lasts for 2 to 4 weeks. It is characterized by hematological alterations: thrombocytopenia, leukopenia and variable mild anemia. Other alterations that may occur are weight loss, anorexia, lethargy, hyperthermia, (41° C), lymphadenomegaly, serous or purulent nasal exudate, hemorrhage, dyspnea. Due to the short incubation period, an obvious infestation of ticks may be found in some of these animals, if they have not yet been eliminated. In most cases, this phase resolves spontaneously and the next phase begins.

Thrombocytopenia is the most common and consistent hematologic finding in acute canine ehrlichiosis. A concurrent and significant increase in mean platelet volume is also usually seen, reflecting active thrombopoiesis. Leukopenia and moderate anemia are common in the acute phase of the disease. Moderate thrombocytopenia is a common finding in the subclinical phase of the disease. There may be a decrease in the number of neutrophils. Red blood cell parameters are not normally affected at this stage of the disease. Severe thrombocytopenia, leukopenia, and anemia most commonly present during the chronic phase of canine ehrlichiosis. Severe pancytopenia is the hallmark of the severe chronic phase and occurs as a result of a suppressed hypocellular bone marrow [9].

The clinical symptoms of canine ehrlichiosis are very varied and nonspecific, so it can be confused with a large number of pathologies. However, it must fundamentally be differentiated from multiple myeloma, lymphoma, chronic lymphocytic leukemia, and systemic lupus erythematosus. In a dog with chronic weight loss, splenomegaly, generalized lymphadenopathy, pancytopenia, bone marrow plasmacytosis, and monoclonal gammopathy, the only way to differentiate canine ehrlichiosis from multiple myeloma is to obtain a positive serology. This is also the only way to distinguish ehrlichiosis from chronic lymphocytic leukemia in an animal with weight loss, mild lymphadenopathy, hepatosplenomegaly, lymphocytosis, and monoclonal gammopathy [13].

A history of tick infestation together with the presentation of symptoms characterized by fever, apathy, adynamia, weight loss, lymphadenopathy, anorexia, pale mucous membranes, often accompanied by bleeding, conjunctivitis, rhinorrhea, locomotor disorders, dermatitis, etc. they constitute solid pillars on which to base a clinical diagnosis of Ehrlichiosis [9].

If, in addition, routine blood tests show the existence of marked hypoproteinemia and thrombocytopenia, associated with anemia and / or leukopenia, the clinical data pointing to ehrlichiosis are even more evocative. Even so, the qualifier that we could apply to the diagnosis of ehrlichiosis would be no more than presumptive, which suggests the need for confirmation. For this, in ehrlichiosis, as in all diseases, we have direct and indirect diagnostic laboratory methods [10].

Icen *et al.* (2010) [1], coincide that the animals that spend more hours of the day in the open, are more exposed to temperatures with intervals of 25 °C to 40 °C, which facilitates the presence of vector ticks variety *Rhipicephalus sanguineus*, which carry out the molt between its stages facilitating the presence of this disease. In relation to sex, females presented a higher prevalence, this could be due to situations where the immune system is in low response to the antigen, such as heat or pregnancy. It is worth mentioning that it has not yet been confirmed, so it is expected to continue with more studies of this disease in the city of Tuxpan; Veracruz, México and other tropical areas and with a greater number of animals.

4. Conclusion

Canine ehrlichiosis, which was analyzed in the study animals (n = 30), proved to be a disease whose presenting symptoms are nonspecific. The high prevalence of *Ehrlichia canis* (76.66%) were positive animals in the city of Tuxpan de Rodríguez Cano; Veracruz, this could be due to the climatic environment and its animal sanitation conditions. The detailed analysis of the anamnesis, and the correct interpretation of the blood tests led us to the correct diagnosis of this disease.

Various studies were analyzed to search for predisposing factors for ehrlichiosis such as: age, race and sex, of which it was evidenced that *E. canis* has no predilection for any of these. On the other hand, factors such as: high temperature and humidity, contact with ticks, the animal's health status and malnutrition are more closely related to the animal's immune status.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

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