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Full Length Research Paper

Seroprevalence of *Ehrlichia canis* in dogs from Monterrey, Mexico

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Infection by *Ehrlichia canis* in dogs causes the worldwide tick-borne disease called canine monocytic ehrlichiosis (CME), and the presence of *E. canis* has been serologically demonstrated in all continents, with prevalence ranging from 0.2 to 80%. In southern Mexico, a prevalence of 44% was found, whereas in the northwest part of the country it varies from 21 to 49%. In the present study, a commercial kit for the detection of antibodies against *E.* canis was used in 391 dogs from the city of Monterrey, which is located at northeast of Mexico. A total of 54 samples were positive, giving a prevalence of 13%. According to sex, prevalence was 14% for males and 13% for females. Positive animals varied in age from 21 to 132 months old and only 10 of them presented ticks. As in the southern and northwest parts of Mexico, CME is present in northeast region, although with a lower prevalence.

Key words: *Ehrlichia canis*, dogs, serology, Mexico.

INTRODUCTION

The dog can be infected by different species of *Ehrlichia*, and *Ehrlichia canis* is the most important species; it is transmitted by *Rhipicephalus sanguineus* (Pusterla et al., 1998), although the American dog tick, *Dermacentor variabilis*, has also been shown to be a vector transmitter of this disease (Johnson et al., 1998).

E. canis is the primary causal agent of Canine Monocytic Ehrlichiosis (CME), a worldwide tick-borne disease (Kamani et al., 2013; Stich et al., 2008); it is an obligate intracellular gram-negative bacterium that multiply in eukaryotic cells, like monocytes and macrophages, developing leucopenia and thrombocytopenia (Stich et al. 2008).

CME can be divided in an acute phase, beginning from 8 to 20 days after infection, involving anemia, anorexia, ataxia, conjunctivitis, depression, fever, leucopenia, ocular discharge, thrombocytopenia and vomiting that end with a partial recovery of the dog, followed by an months-to-years subclinical phase. The chronic phase can be mild or severe with recurrent clinical and hematologic signs like pancytopenia, hemorrhage, monocytosis, lynphocytosis and weight loss (Stich et al., 2008). It is thought that *E. canis* is the only agent responsible for the development of CME. It has been

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suggested that this bacteria could be the causal agent in the human granulicytic ehrlichiosis and it has even been successfully isolated from human patients with symptoms similar to those caused by other infections by *E. chafeensis* and *E. ewingii* (Perez et al., 2006; Nicholson et al., 2010).

Serologic evidence of previous studies around the world indicated that *E. canis* is present among dogs throughout all continents, where serologic studies have found a prevalence ranging from 30 to 80% in some countries of Africa (Azzag et al., 2015; Inokuma et al., 2006; Eoghain and Raoult, 2004; Ndip et al., 2005; Davoust et al., 2006), whereas in some Asian countries it was of 0.2 to 30% (Inokuma et al., 1999; Rajamanickam et al., 1985; Stich et al., 2008).

In Europe, a prevalence ranging from 2 to 50% have been found (Solano-Gallego et al., 2006; Cocco et al., 2003; Pusterla et al., 1998; Sainz et al., 1995). A study realized in the USA detected most often *Ehrlichia* antibodies in dogs in the Southeast, with 1.3% of samples testing positive, whereas other regions showed lower numbers ranging from 0.3 to 0.6%. (Bowman et al., 2009). Different results were found in Oklahoma, where the prevalence of *E. canis* was 10.8% by serology and 3.1% by the polymerase chain reaction (PCR) method (Murphy et al., 1998). Among dogs belonging to the U.S.A. military forces, seropositivity to *E. canis* ranged from 8% in cold zones (above 45° latitude) to 24% in temperate places (between 40 and 45° latitude); a 13% prevalence was found in tropical zones (below 40° latitude) (Keefe et al., 1982).

Several studies on *E. canis* prevalence have been realized in Brazil. Melo et al. (2011) reported a prevalence of 74.4% in urban and of 67.5% in rural dogs (overall frequency of 70.9%), whereas Witter et al. (2013) informed a seroprevalence of 70.1%; in this last study the frequency of *E. canis* infection was of 23.3% by PCR. On the other hand, also in Brazil a prevalence of *E. canis* of 41.5% by IFA and of 9.4% was found in cats (Braga et al., 2014).

In Grenade, 43.8% of dogs tested were positive for *E. canis* (Yabsley et al., 2008). In Mexico, studies performed in the southern area found 44% of seropositive dogs to *E. canis* with ELISA testing (Rodriguez-Vivaz et al., 2005), 36% prevalence by PCR and 45% in dogs located at animal shelters (Pat-Nah et al., 2015), whereas at the northwest region a prevalence of 49% was found (Tinoco-Gracia et al., 2007). In another study (Haro-Álvarez et al., 2007), a 21.6% prevalence, with 40% of the dog population in contact with E. canis, have been reported.

Although much have been said about the presence of this disease in Mexico, currently there are no reports of it in the northeast region; therefore, the goal of the present study was to estimate the seroprevalence of ehrlichiosis in dogs from the city of Monterrey, located in this part of Mexico.

MATERIALS AND METHODS

Blood samples were obtained from 391 dogs of different breeds in the city of Monterrey, using as inclusion factor only animals with fixed address, age over 6 months. It was decided to sample only one animal per house in case of having more than one dog. The examination of the dogs started with physical evaluation followed by blood sampling. All dogs showed no symptoms of any disease.

This study was carried out during 2014 in the city of Monterrey, Nuevo Leon, located in the northeast of Mexico, with a territorial extension of 451.30 square kilometers. Location coordinates are 25°40´17´´ N, 100° 18´31´´ W. Altitude is 530 m above sea level. The climate of the region has an average of 21°C, but because of annual thermal oscillation of 18°C, with important contrast among seasons. In summertime, temperatures above 30°C are common with an average in July and August of 34°C. In Winter, cold air arrive constantly to the region, often accompanied of humidity from the coast, making the temperature descend drastically, and every year at least two to three days are recorded with 0°C or less. The average annual precipitation is of 600 ml spread mainly in summer, with September as the rainiest month. The city was divided in quadrants in accordance with its cartographic plan. From this map, the 15 most urbanized quadrants were chosen, since the others belonged to non-well developed neighborhoods and little human population. Sampling was performed according to the dog population density and owner cooperation, and only one animal per city block and only one animal per house. To determine the sample size, calculations were made in basis of the population's representative sample (infinite), with precision level of 5%, confidence level of 95% and a power of statistical test of 80% in order to ensure reliability of the results and that they could be translated to the population under study. Sample size was determined using Epidat 3.1. For the *in vitro* diagnosis for detection of antibodies against *E. canis* in the samples, a commercial kit canine SNAP*4Dx (IDEXX labs, Inc. USA) was used. Before starting the procedure, samples must be at room temperature. The sera, either fresh or refrigerated, were utilized after no more than a week from the sampling. Sensitivity and specificity of the kit for the disease are reported with a minimum of 98.8% and 100%, respectively, and detects antibodies generated against peptides from the proteins p30 and p30-1 of *Ehrlichia*. (O'Connor et al., 2004, 2006).

RESULTS AND DISCUSSION

For the present work, 391 blood samples were taken from dogs located in the city of Monterrey, Mexico; antibodies against *E. canis* were found in 54 samples, resulting in a prevalence of 13.8%. Regarding to sex, animal's samples comprised 173 males and 218 females of which 25 males and 29 females were positive, giving a prevalence of 14.5 and 13.3% respectively (Table 1).

Positive animals varied in age from 21 to 132 months old; and according to size, 19 were small, 27 medium and eight large. Only 10 positive animals presented ticks (*Rhipicephalus sanguineus*). The distribution of positive animals by breed is presented in Table 2; the biggest percentage of positive dogs was for mixed-breed.

Comparing the frequencies found in the present work to other studies on the subject can be difficult due to the wide range of prevalence reported according to the continent in which such studies were performed (from 0.2 to 80% in Africa, Asia and Europe), as can be seen in the

Table 1. Distribution of positive animals for *Ehrlichia canis* by sex.

Sex	Number of dogs sampled	Number of positive dogs	%
Female	218	29	13.3
Male	173	25	14.5
Total	391	54	13.8

Table 2. Number of positive animals to *Ehrlichia canis* according to breed.

Breed	Number of positive animals
Basset Hound	1
Boxer	3
Bull Terrier	3
Chihuahua	2
Cocker Spaniel	2
Collie	1
Mixed breed	16
Doberman	1
French poodle	5
Great Dane	2
Maltese	4
Labrador	4
Schnauzer	2
German shepherd	1
Shar Pei	2
Shih tzu	4
Westhighland	1

Introduction section. However, when we compare our work with studies done in the U.S.A., we find that a very similar prevalence (10.8%) was found in Oklahoma by serology, although in this same paper the prevalence was 3.1% by PCR (Murphy et al., 1998). This low prevalence of *E. canis* by PCR in the U.S.A. is confirmed by other work that informed 1.3% in the Southwest and 0.3 to 0.6% in other areas of that country (Bowman et al., 2009). On the other hand, a very large prevalence of *E. canis* in dogs has been informed in both Grenade (Yabsley et al., 2008) and south Mexico (Rodriguez-Vivaz et al., 2005); in the first, the prevalence was of 43.8% and in the second of 44%. Other studies in Mexico concluded that the prevalence of *E. canis* is high, ranging from 40 to 49% in both the northwest and south part of the country (Haro-Álvarez et al., 2007; Pat-Nah et al., 2015; Tinoco-Gracia et al., 2007). These results are in disagreement with the ones presented in our work. Therefore, a wide range of results regarding the prevalence of *E. canis* in dogs exist in the literature. One possible explanation to this disagreement could be the diagnostic method. Work in this subject indicate that the IFA method may be better than ELISA (Jimenez-Coello et al., 2009); using the IFA method these authors found a 8.7% prevalence, which is closer to the results informed in the present work; the previously mentioned work also indicates that the sampling method can also have an influence in the results. We think that both the sampling method and the technique used in the present study give an accurate view of the actual prevalence of *E. canis* infection in dogs located in the northeast region of Mexico; the prevalence we found is close to the ones reported in the U.S.A. and in the work done by Jimenez-Coello et al. (2009) in Mexico, but much lower to the prevalence informed in both the south and northwest areas of Mexico, as well as in other parts of the world as mentioned above.

Conflict of interests

The authors did not declare any conflict of interest.

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