Journal of Animal and Veterinary Advances 6 (5): 758-760, 2007

Seroprevalence of *Ehrlichia canis* in Dogs from a Mexico-U.S. Border Desert Region: Pilot Study

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Abstract: A pilot study was conducted to estimate the seroprevalence to *Ehrlichia canis* in dogs in a Mexico-U.S. border desert region. A total of 94 dogs were tested by *Ehrlichia canis* ELISA kit. Information of dogs regarding gender, age, size and intensity of tick infestation was collected and the significance of these class variables was determined by Chi-squared test. An adjusted prevalence of 49.3% (95% I.C. 30.8-54.1%) to *Ehrlichia canis* was obtained using the Rogan-Glanden estimator. Given that ehrlichiosis in dogs is a zoonotic disease and the seroprevalence in this desert region of the Mexicali-U.S border was found to be high, it is necessary to perform a formal study to establish the actual seroprevalence in both dogs and humans and determine the risk factors associated with ehrlichiosis. Since *Ehrlichia canis* is mostly transmitted by ticks *Rhipicephalus sanguineus*, preventive and control measures to erradicate ticks have to be established in order of minimize the risk of infection.

Key words: Seroprevalence, *Ehrlichia canis*, desert region, border, infection, dogs

INTRODUCTION

Canine ehrlichiosis is a zoonotic ricketsial disease (Perez *et al*., 1996) transmited by ticks, meanly *Rhipicephalus sanguineus* known as the brown dog tick. The primary causative agent of canine ehrlichiosis is *Ehrlichia canis*, a small-gram negative, pleomorphic, obligatory intracellular bacteria that presents tropism to mononuclear phagocytes (Mathew *et al*., 1996; McBride *et al*., 1999; Rodriguez-Vivas *et al*., 2005) altough others species of *Ehlichia* present tropism to granulocytes, in both, dogs and humans (Ewing *et al*., 1997).

Ehrlichiosis is characterized by fever, anorexia, depression, lymphadenopathy, thrombocytopenia, hyperglobulinemia, emaciation, bledding, epistaxis and in some cases death (Bakken *et al*., 1996; Panciera *et al*., 2001; Stich *et al*., 2002).

Rhipicephalus sanguineus is probably the most world widespread of all tick species. It is almost exclusively a parasite of dogs that can become established in homes and kennels. It has also been implicated as vector of others diseases (Theis, 1968; Theis and Franti, 1971; Monmouth, 1999).

The aim of this pilot study was to estimate the seroprevalence to *Ehrlichia canis* in dogs in the urban area of Mexicali Baja California, a Mexico-U.S. border desert region.

MATERIALS AND METHODS

Study design and characteristics of the population: A cross-seccional descriptive study was conduced where 10 veterinary clinics and the Animal Control Center participated. The duration of the study was from September to November 2003. A total of 94 dog serum samples were randomly taken, 54 from veterinary clinics and 40 from the Animal Control Center. Mexicali city is situated along the state's northern border with the U.S. state of California and is the northernmost city in Latin America, located at 32°40'0"N, 115°28'0"W, with 855,962 inhabitants (Wikipedia, 2006). Climate is extreme, desert type and the average annual rainfall is 0.63±.43 cm. Climatic conditions data was collected from the United States National Weather Service of the National Oceanic and Atmospheric Administration (http://www.nws.noaa.gov/).

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Data collection: A questionnaire was designed to collect information of the tested dogs and included: 1) General information of dog: Gender (female, male), age (= 1 year, > 2 years), size (small, medium, big) and 2) Intensity of tick infestation: none, low (1-10 ticks), moderate (11-30 ticks), intense (>30 ticks). The outcomes of most of the questions were dichotomous.

Blood collection: Blood samples were collected by certified personal. Briefly, 3 mL of blood were collected by punction of the cephalic vein after proper antisepsis of the area with isopropyl alcohol and placed in tubes Vacutainer®. Each sample was properly labeled and centrifuged at 3500 RPM for 10 min to separate the serum. The serum was transferred into 1mL vials, labeled and stored at -20°C until testing.

Serology: Antibodies against *E. canis* were measured with the kit *Ehrlichia canis* ELISA® Helica Biosystems, Inc. for the detection and semi quantification of canine IgG class which guarantees a 93.8% sensibility and a 92.3% specifity. The Optical Density (OD) at 450 nm was registred, where an OD < 0.5 was considered negative and OD = 0.5 as positive, according to the manufacturer.

Statistical analysis: Seroprevalence values were calculated by dividing the number of positive sera obtained by the total number of samples analyzed. The adjusted prevalence and its 95% CI were obtained using Rogan-Gladen estimator (Greiner and Gardner, 2000). The significance of the class variables (gender, age, size and intensity of tick infestation) was determined by Chi-squared test (Walker, 1997). All statistical analysis were performed using the Statistical Analysis System for Windows version 9.1.3 (SAS, 2004).

RESULTS AND DISCUSSION

The present pilot study showed an adjusted seroprevalence to *Ehrlichia canis* of 49.3% (95% I.C. 30.8-54.1%) calculated by the Rogan-Gladen estimator (Greiner and Gardner, 2000). Although this study was conducted in autum where a lower tick infestation is expected, the prevalence found was high. Table 1 shows the adjusted prevalence obtained in dogs from veterinary clinics and from the Animal Control Center.

The prevalence in this study (49.3%) was lower than that found in another pilot study done in Mexicali, B.C. in dogs that showed signs of epistaxis and where a 98% (28/30) seroprevalence was obtained by INDX Dip-S-Ticks® ELISA (INDX Integrated Diagnostics Inc., E.U.A) (Romano-Osuna *et al*., 1998). Moreover, the prevalence in this study was higher than that found in a National survey done in Mexico that included 25 states, based in ELISA Snap® 3Dx Assay (IDEXX Laboratories Inc., USA) with 33.1% (793/2395). However, the individual prevalence found for the state of Baja California in the National survey was 70.2% (26/37), higher than the one obtained in the present pilot study, which may be due to biases by including not-healthy animals in the study. Furthermore, a seroprevalence of 44.1% (53/120) was found in Mérida Yucatán, similar to the one obtained in this study (Rodriguez-Vivas *et al*., 2005).

Table 1: Adjusted prevalence* to *Ehrlichia canis* of dogs from Mexicali, an urban area of Mexico-U.S. border desert region

Origin	n	Positives	Adjusted prevalence* (%)	95%I.C. (%)
General	94	40	49.3	30.8-54.1
Veterinary clinics	54	30	64.4	40.1-70.9
Animal control center	40	10	28.9	9.4-40.5

*Estimator de Rogan-Gladen (Greiner and Gardner, 2000)

Table 2: Seroprevalence* to *Ehrlichia canis* in dogs from Mexicali, stratified by origin, gender, age, size and intensity of infestation

	Origin					
	Veterinary clinics			Animal control center		
Class variable[1/]	n	Positive	%	n	Positive	%
Gender						
Male	30	17	56.6[a]	15	2	13.3[a]
Female	24	13	54.1[a]	25	8	32.0[a]
Age						
=1 year old	16	6	37.5[b]	22	3	13.6[b]
>1 year old	38	24	63.1[a]	18	7	38.8[a]
Size						
Small	10	6	60.0[a]	9	2	22.2[a]
Medium	28	12	42.6[a]	23	6	26.9[a]
Big	16	12	75.0[a]	8	2	25.0[a]
Intensity of tick infestation						
None	21	11	52.3[a]	17	3	17.6[a]
Low	26	16	61.5[a]	18	6	33.3[a]
Moderate	5	2	40.0[a]	4	1	25.0[a]
Intense	2	1	50.0[a]	1	0	0.0

* Unadjusted values, [1/] equal letters by class variable within origin, indicate no differences (p>0.05)

Table 2 shows the unadjusted prevalence values stratified by origin, gender, age, size and intensity of tick infestation. In general, not differences (p>0.05) were observed in prevalence values according to gender, size and intensity of tick infestation between dogs from veterinary clinics and those from the Animal Control Center. However, dogs >1 year old showed higher (p<0.05) seroprevalence than those = 1 year old which was expected due to the cronic course of ehrlichiosis.

Since the prevalence found in this pilot study was high, it is necessary to perform a complete study that includes an appropriate sample size, serum sampling all

year long and the evaluation of risk factors so that the appropriate preventive and control measures are established. Also, since ehrlichiosis is a zoonotic desease that may require expensive hospitalization and may cause death it is imperative to know the prevalence in humans, particularly in places like Mexicali, where a high prevalence in dogs has been observed.

CONCLUSION

A high seroprevalence value to *Ehrlichia canis* was observed in this study and because ehrlichiosis is a zoonotic disease, a complete stydy has to be done, where both dogs and humans are included with an appropriate sample size and the evaluation of risk factors. Since *Ehrlichia canis* is mostly transmitted by ticks *Rhipicephalus sanguineus*, preventive and control measures to erradicate ticks have to be established in order of minimize the risk of infection.

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