FACTORS ASSOCIATED WITH *Ehrlichia canis* INFECTION IN DOGS INFESTED WITH TICKS FROM HUANUCO, PERU

Edward Huerto-Medina[1,a], Bernardo Damaso-Mata[2,b]

ABSTRACT

The aim of the study was to determine the frequency and associated factors of *Ehrlichia canis* infection in dogs. Blood samples from 150 dogs infested with ticks in 10 veterinary clinics in the city of Huanuco in Peru were collected. The dogs were randomly selected without regard to breed, age or sex. *Ehrlichia canis* antibodies were detected by chromatographic immunoassay.51.3% of dogs were infected with *Ehrlichia canis*. In the multivariate analysis, factors associated with the presence of *Ehrlichia canis* were: poor health of the dog (p = 0.049), a higher average of tick infestation (p = 0.018), and adult dogs (p = 0.038). The frequency of *Ehrlichia canis* in dogs of this city is high. Control of the brown dog tick (Rhipicephalus sanguineus) vector of *Ehrlichia canis* is recommended.

Key words: Ehrlichia canis; Rhipicephalus sanguineus; Prevalence; Immunoenzyme techniques (source: MeSH NLM).

INTRODUCTION

Canine ehrlichiosis is a disease of domestic and wild canines and occurs worldwide. It is caused by *Ehrlichia canis* and is also called “tracker dog disease,” “tropical canine pancytopenia,” “canine hemorrhagic fever,” and “canine typhus” [(1)]. *E. canis* is a gram-negative pleomorphic microorganism of the Rickettsiaceae family and infects circulating monocytes within their cytoplasm in aggregates known as morula. *E. canis* is transmitted by the brown dog tick, *Rhipicephalus sanguineus*, which occurs in the transstadial form but not in the transovarial form [(2)]. The infection in the animal disseminates through the blood or the lymph system within infected mononuclear cells, thus reaching other organ systems [(3)]. The majority of cases occurs in endemic areas in spring and summer months, when ticks are most active. Because the transmission of ehrlichiosis is mechanical and nonbiological, transfusions with infected blood can result in high rates of infection [(2)].

The brown dog tick, *R. sanguineus*, is widespread in Peru [(4)]. Some studies of *E. canis* have shown increased susceptibility of the German shepherd dogs to this disease, as compared to other breeds [(5)]. Although *E. canis* was identified for the first time in Algeria in 1935, it received broad attention in 1987 when *E. chaffeensis*, a closely related microorganism (shares 98.2% homology with ribosomal DNA [16S rDNA] of *E. canis*), was identified as the cause of human monocytic ehrlichiosis [(2)]. In recent years, a new species of monocytic *Ehrlichia* was isolated and characterized in Venezuela; this organism may be a subspecies of *E. canis*; consequently, human ehrlichiosis is considered a disease of zoonotic importance by the Pan-American Health Organization (PAHO). In 2006, by PCR analysis of the rRNA 16S

[1] Faculty of Veterinary Medicine. *Universidad Nacional Hermilio Valdizán.* Huanuco, Peru.
[2] Faculty of Medicine. *Universidad Nacional Hermilio Valdizán.* Huanuco, Peru.
[a] Veterinarian, master’s degree in veterinary science, [b] internist, doctorate in medicine.

This paper forms part of the dissertation to obtain the master’s degree in veterinary sciences. Graduate school, Universidad Nacional Herminio Valdizán.
Received: 2/5/2015 Approved: 7/8/2015

Citation: Huerto-Medina E, Damaso-Mata B. Factors associated with *Ehrlichia canis* infection in dogs infested with ticks from Huanuco, Peru. Rev Peru Med Exp Salud Publica. 2015;32(4):756-60.

gene, researchers found that 30% of seropositive patients have symptoms consistent with ehrlichiosis; this was the first report of *E. canis* infection in humans [6].

Ehrlichiosis has been reported as an emerging zoonotic disease, and multiple cases have been described worldwide. In Peru, ehrlichiosis has been detected in dogs (canine monocytic ehrlichiosis) since 1982 [7], and the number of reported cases has increased since then.

In 2001, 16.5% seroprevalence was discovered in metropolitan Lima in the districts of Chorrillos, La Molina, and San Juan de Miraflores [8]. In 2005, a dog with canine granulocytic ehrlichiosis (CGE) was identified in the district of La Molina. In addition, seroprevalence of up to 76% was reported in 2006 for Sullana, Piura [9].

This disease is highly likely to develop in our environment; therefore, serological detection of antibodies to *E. canis* in dogs should be useful in the fields of veterinary medicine and public health. The objective of this study was to determine the frequency of and factors associated with canine ehrlichiosis in the province of Huanuco, Peru.

THE STUDY

This was a descriptive cross-sectional study. The city of Huanuco is located in north-central Peru. According to the National Institute of Statistics and Informatics, in 2012, this city had an estimated population of 301,396. It is located 1800 m above the sea level, in the valley formed by the Huallaga River. With the average temperature of 24ºC, the lowest temperature is observed in winter, during the months of July and August (21ºC during the day and 17ºC at night). The highest temperatures are in spring, during November and December (30ºC during the day): endemic conditions for tick infestation. In the rural area, people have a habit of keeping multiple dogs per household, and the majority of these owners do not treat their dogs for ticks. In addition, there are many stray dogs, making it easier for the *R. sanguineus* tick to reproduce. Therefore, the presence of appropriate vectors for known ticks as well as host reservoirs largely determine where *E. canis* occurs (it is primarily transmitted by *R. sanguineus)* [11]. The estimated canine population is 57,850 (according to The Office of Environmental Health of the Huanuco Health Network; 2012).

The selection of the sample was not probabilistic (via casual or accidental sampling); the dogs were included consecutively as they were brought to the clinics. We used a confidence level of 95%, an estimate of 6%, and prevalence of 16.5% [8]. The statistical sample consisted of 150 dogs.

Ten veterinary clinics in the province of Huanuco participated on a voluntary basis. The study included 15 dogs from each clinic. Only tick-infested dogs were included, and the owner was queried to establish the factors associated with this infection.

The study variables were the following:

Lifestyle: defined as the way of life, describing generically the style, form, or manner in which living is understood (street and home).

Degree of tick infestation: the brown dog ticks (*R. sanguineus*) were counted at all stages (larvae, nymphs, adults, or blood-filled females) because they can act as a vector for *E. canis* at any stage. The criterion used for the count was cranial-to-caudal occurrence; no validated methodology for the calculation of tick infestation was found in the literature. The results were categorized in ordinal form (from 0 to 10, 11 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, and from 101 to 110).

Health condition of the dog: categorized as poor, good, or excellent. The condition was deemed good when the dog had a normal physical appearance, the diet was adequate, it had no clinical signs of disease, and the owner stated that the dog was in good physical form. The condition was deemed excellent when the dog was in good condition, and the owner followed its vaccination and antiparasitic treatment schedule properly, in addition to a proper physical appearance of the dog. The condition was deemed poor when the dog was not healthy, according to the presence of one or more clinical signs such as diarrhea, vomiting, cachexia, ecchymosis, jaundice, ataxia, dyspnea, or weight loss.

Age group: defined according to the period that the dog has lived since birth.

Type of diet: defined as the intake of any nutrient by the dog (homemade, mixed, or balanced).

Central tendency and dispersion measures for quantitative variables as well as percentages for categorical variables were used for data analysis. The bivariate analysis was carried out using the Pearson chi squared test, which assesses the existence of a relation between ehrlichiosis and relevant factors. A multivariate analysis with nonconditional logistic regression was performed with the variables that were significant in the bivariate analysis. The effect of collinearity between the evaluated variables was taken into account. We used statistical software Stata v. 11.

A rapid test for detection of anti-*E. canis* antibodies was used to diagnose *E. canis* infection; the test consists of an immunochromatographic assay on a solid phase for detection of anti-*E. canis* antibodies in serum, plasma, or blood of dogs. The duration of reading the results was 20 minutes. This test is made by the Bionote® Company, South Korea [10].

RESULTS

The frequency of *E. canis* infection in dogs infested with ticks in the province of Huanuco was 51.3% (95% CI: 43.0–59.6).

There was a statistically significant difference between the presence of *E. canis* and the following variables: the health condition of a dog (for poor condition, p = 0.016); lifestyle of the dog (for strays, p = 0.011); average number of ticks (i.e., infestation degree; p = 0.009); age (p = 0.006), and type of diet (p = 0.004; Table 1). No statistically significant correlation was detected between the prevalence of *E. canis* infection and the following variables: sex (p = 0.35), breed (p = 0.296), social status of the owner (p = 55.521), and educational level of the owner (p = 0.378).

In the multivariate analysis, independent associations were found between the prevalence of *E. canis* infection and these variables: a poor health condition of the dog (p = 0.049), average number of ticks (p = 0.018), age (p = 0.038), and the type of balanced diet (p = 0.015; Table 2).

DISCUSSION

The high prevalence of *E. canis* infection (51.3%) among tick-infested dogs in the Huanuco province could be due to its climatic characteristics and its sanitation conditions because the incubation process of *R. sanguineus* eggs depends mainly on environmental temperature and humidity. The optimal conditions for oviposition and progression through the various life cycle stages are the temperature range from 20°C to 30°C [11,12] and the environmental humidity range from 20% to 93% [13]. The average temperature in Huanuco is 24°C, similar to the annual average temperature in Belo Horizonte in Brazil, 23°C, where 7318 ticks were collected from infested dogs, and 100% of the ticks were identified as *R. sanguineus* [14]. These similar climatic conditions, in addition to the rural sanitation conditions of the dwellings, can account for greater tick proliferation.

Table 1. *E. canis* y variables relacionadas en perros de la provincia de Huánuco (n=150)

	Infected		Not infected		*p* value
	n	(%)	n	(%)	
Health condition of the dog					
Poor	39	(63.9)	22	(36.1)	0.016
Good or excellent	38	(42.7)	51	(57.3)	
Lifestyle of the dog					
Stray dogs	39	(63.9)	22	(36.1)	0.011
Dogs with an owner	38	(42.7)	51	(57.3)	
Average number of ticks (infestation degree)					
average ± standard deviation	18.47 ± 16.21		12.32 ± 8.49		0.009
Age by age group					
Adults	50	(63.3)	29	(36.7)	0.006
Pups	22	(36.1)	39	(63.9)	
Older adults	5	(50.0)	5	(50.0)	
Type of dog diet					
Homemade	53	(63.1)	31	(36.9)	0.004
Mixed	21	(38.2)	34	(61.8)	
Balanced	3	(27.3)	8	(72.7)	

Table 2. Multivariate analysis of the variables related to positive results of the test for E. *canis* infection (n = 150)

Variable	Odds ratio	(95% CI)	*p* value
Poor health of the dog	0.51	(0.27-0.99)	0.049
Highest average number of ticks (infestation degree)	1.05	(1.01-1.09)	0.018
Adult dogs	1.92	(1.04-3.56)	0.038
Balanced food	0.48	(0.27-0.86)	0.015

Hosmer-Lemeshow chi squared (10): 3.41

Upon evaluating the relation between the health condition of the dogs and the presence of *E. canis,* we found a significant association because the poor health condition in 63.9% of cases is related to a positive diagnostic test result. The poor health condition of a dog is a risk factor of *E. canis* infection*.* Infection is likely to depend on transmission via tick bites.

Homelessness of a dog is also a risk factor of *E. canis* infection*.* This is because when an animal is away from home, being in contact with "street dogs," which are usually infested with ticks, the risk of infection is greater. This association was not proven by the multivariate analysis.

The factor "greater number of ticks" was found to be linked to *E. canis* infection. This is because with a greater number of ticks, the risk of transmission is greater. It is difficult to control ticks due to the complexity of their life cycle and the favorable climate in the province of Huanuco; thus, reinfestation by ticks and transmission of ehrlichiosis are possible.

Upon evaluating the relation between the age of the dog, categorized by groups, and the diagnosis of *E. canis* infection, we found a significant association. To be precise, adult age of a dog is linked to *E. canis* infection. In a study conducted at the Veterinary Teaching Hospital of Londrina State University, State of Parana, southern Brazil, 87 of 381 dogs (22.8%) tested positive for ehrlichiosis; according to that study, groups at increased risk of being seropositive for ehrlichiosis compared with the general population included dogs over 1 year of age, previously exposed to ticks, and showing neurological signs [15]. Middle-aged dogs have a greater chance of being exposed to the vector than pups do. This situation is due to the habit of the owners to let the animals out on the street when they complete their vaccinations, believing that such dogs are at a lower risk of infectious diseases.

In this study, the breeds (including the cross-breeds) showed no significant relation to the presence of *E. canis* infection; i.e., when it comes to the breed, animals have the same likelihood of infection [16]. This result contradicts studies where breed analysis indicated that the German Shepherd is at a greater risk.

The type of homemade food that the dog receives is related to *E. canis* infection. This finding can be explained: an animal that consumes balanced food takes in a greater amount of nutrients and thus the immune response triggered by the host is better as compared to an animal that consumes homemade food only. Nevertheless, infection is still likely to depend on transmission via a tick bite.

Finally, upon evaluating the relation between the educational level of the dog's owner and *E. canis* infection in the dog, we found no significant association. Therefore, the educational level of dogs' owners is not related to *E. canis* infection. This is because in an area endemic for ticks, the ticks are present in any home, regardless of the educational level of the dogs' owners.

As for the risk to humans, *E. canis*, or an organism indistinguishable from it because it has been isolated from a human being in Venezuela, seems to carry a zoonotic risk, in line with another study [17]. Later, in 2006, by means of PCR analysis of the 16S rRNA gene, some researchers demonstrated that 30% of seropositive patients have symptoms consistent with ehrlichiosis; that was the first report of *E. canis* infection in humans [18]. In addition, genetic characterization of the 16S rRNA gene in two strains of *E. canis* in the region of Botucatu, São Paulo State, Brazil, revealed that a strain that naturally infects dogs is identical to the Venezuelan strain that infects humans [19]. Thus, the *E. canis* strains of Brazil may infect human beings.

According to a study by De Souza *et al*., the indirect immunofluorescence test is the gold standard for the diagnosis of human monocytic ehrlichiosis. In this test, there is cross-reactivity between *E. canis* and *E. chaffeensis*. There is only one description of *E. chaffeensis* (from a deer) confirmed by sequencing in Brazil. In contrast, *E. canis* is common and widespread among dogs. Those authors hypothesized that some, if not all of the human cases attributed to *E. chaffeensis* in Brazil are actually caused by *E. canis* [19].

The main limitation of our study is the risk of low representativeness due to the small sample size, in addition to nonprobabilistic sampling.

In conclusion, the prevalence of *E. canis* infection among dogs in the province of Huanuco is 51.3%. The variables that are related independently to *E. canis* infection are the poor health condition of a dog, the average number of ticks (infestation degree), adult age, and homemade diet of the dog.

Acknowledgements: we thank veterinarian doctors of the 10 offices for assistance with the analyses performed.

Author contributions: EHM participated in the conception and design of the study, laboratory work, and collection of data. EHM and BDM performed analysis and interpretation of data, writing of the manuscript, critical review, and approval of its final version. BDM also provided technical, statistical, and administrative advice.

Funding source: self-funded by the main author (EHM).

Conflicts of interest: the authors have no conflicts of interest related to the publication of this article.

References

1. Frisby H [internet]. Ehrlichiosis [citado el 18 de noviembre del 2015]. Disponible en: http://www.peteducation.com/dogs/ehrlichia.htm.
2. Waner T, Harrus S. Ehrlichiosis monocítica canina [Internet]. In: Carmichael LE ed. Recent Advances in Canine Infectious Diseases. Ithaca, New York: International Veterinary Information Service; 2000 [citado el 25 de enero de 2015]. Disponible en: http://www.ivis.org/advances/Infect_Dis_Carmichael/waner_es/ivis.pdf
3. Tesouro M, Sainz A. Ehrlichiosis canina. Veterinaria Información. Revisión del Consejo General de Colegios Veterinarios de España 1993;132:114–9.
4. Estares L. Prevalencia de ectoparásitos de *Canis familiaris* en los distritos de San Juan de Lurigancho, San Martín de Porres, Comas e Independencia de Lima Metropolitana [Tesis Bachiller]. Lima: Facultad de Medicina Veterinaria, UNMSM; 1999.
5. Leiva M, Naranjo C, Peña MT. Ocular signs of canine monocytic ehrlichiosis: a retrospective study in dogs from Barcelona, Spain. Vet Ophthalmol. 2005;8(6):387–93.
6. Perez M, Bodor M, Zhang C; Xiong Q, Rikihisa Y. Human Infection with *Ehrlichia canis* accompanied by clinical signs in Venezuela. Ann N Y Acad Sci. 2006;1078:110-7.
7. Chavera A, Viera F, Samamé H. Ehrlichiosis Canina en el Peru. Anales del VII Congreso Nacional de Ciencias Veterinarias; 1982; Ica, Perú.
8. Adrianzen J, Chávez A, Casas EC. Seroprevalencia de la Dirofilariosis y Ehrlichiosis canina en tres distritos de Lima. Rev Investig Vet Peru. 2003;14(1):43–8.
9. San Miguel SY. Prevalencia de *Ehrlichia canis* en caninos de la provincia de Sullana [Tesis Bachiller]. Lima: Facultad de Medicina Veterinaria, UAP; 2006.
10. Catálogo de productos para pequeñas especies [Internet]. Hwaseong-si, Gyeonggi-dp: BioNote; 2011 [citado el 25 de enero de 2015]. Disponible en: http://www.bionote.co.kr/ANIMAL/eng/
11. Dantas-Torres F. Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Parasit Vectors. 2010;3:26. doi: 10.1186/1756-3305-3-26.
12. Dantas-Torres F. Brown Dog Tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Arachnida: Acari: Ixodidae). Vet Parasitol. 2008;152(3-4):173–85. doi: 10.1016/j.vetpar.2007.12.030.
13. Alcaíno H, Gorman T, Jiménez F. Ecología del *Rhipicephalus sanguineus* (Ixodidae) en la Región Metropolitana de Chile. Arch Med Vet. 1990;22(2):159-68.
14. Silveira JA, Passos LM, Ribeiro MF. Population dynamics of *Rhipicephalus sanguineus* (Latrielle, 1806) in Belo Horizonte, Minas Gerais state, Brazil. Vet Parasitol. 2009;161(3-4):270-5. doi: 10.1016/j.vetpar.2009.01.028.
15. Trapp SM, Dagnone AS, Vidotto O, Freire RL, Amude AM, de Morais HS. Seroepidemiology of canine babesiosis and ehrlichiosis in a hospital population. Vet Parasitol 2006;140(3-4):223-30.
16. Hoyos L, Li O, Alvarado A, Suárez F, Díaz D. Evaluación del examen hematológico en el diagnóstico de ehrlichiosis canina. Rev Inv Vet Peru. 2007;18(2):133-39.
17. Pérez M, Rikihisa Y, Wen B. Ehrlichia canis-like agent isolated from a man in Venezuela: antigenic and genetic characterization. J Clin Microbiol. 1996;34(9):2133-39.
18. Perez M, Bodor M, Zhang C, Xiong Q, Rikihisa Y. Human Infection with *Ehrlichia Canis* Accompanied by Clinical Signs in Venezuela. Ann N Y Acad Sci. 2006;1078:110-7.
19. de Paiva Diniz PP, Schwartz DS, de Morais HS, Breitschwerdt EB. Surveillance for zoonotic vector-borne infections using sick dogs from Southeastern Brazil. Vector Borne Zoonotic Dis. 2007;7(4):689-97.

Correspondence: *Edward Huerto-Medina*
Address: Jr. Jorge Chávez 332, Paucarbamba, Amarilis, Huánuco, Peru
Phone Number: 062-962700265
E-mail: edwardhm_10@hotmail.com