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Occupational risk for personnel working in veterinary clinics through exposure to vectors of rickettsial pathogens

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ABSTRACT

Workers at veterinary clinics are exposed to zoonotic pathogens, which, though infrequently, can lead to serious consequences. The aim of the present study was to estimate the seroprevalence for rickettsial pathogens in veterinary clinic personnel in Ciudad Juárez, Chihuahua, Mexico. Voluntary participants included 106 veterinarians, 19 pet groomers, 36 veterinary assistants and 6 administrative workers who supplied blood samples taken by venipuncture. Detection of *Rickettsia rickettsii*, *Ehrlichia* spp. and *Anaplasma phagocytophilum* was conducted by indirect immunofluorescence assay (IFA) and polymerase chain reaction (PCR) techniques. To determine risk factors for the seroprevalence, the study considered social, demographic and occupational variables as well as the clinical signs and symptoms of the participants. Logistic regression procedures were performed, and the odds ratio (OR) was calculated with confidence intervals corresponding to a probability of 95%. The results indicate that 54% of the participants had a positive seroreaction to at least one of the pathogens evaluated and 2% were positive to all. The coexposure was as follows: 3% to *R. rickettsii* and *A. phagocytophilum*, 9% to *Ehrlichia* spp. and *A. phagocytophilum*, and 2% to *Ehrlichia* spp. and *R. rickettsii*. It was established that grooming posed the highest risk for *R. rickettsii* exposure. The implementation of appropriate management practices for zoonotic diseases is recommended to avoid health problems that may be life threatening to personnel in veterinary clinics.

1. Introduction

Rickettsial diseases are considered emerging zoonoses caused by obligate intracellular Gram-negative bacteria in the order Rickettsiales (Raoult and Roux, 1997). These bacteria such as *R. rickettsii* that are transmitted by blood-feeding arthropods affect the vascular endothelium, causing multi-organ failure. The symptoms can be mild; however, in some cases the disease can lead to death (Buckingham et al., 2007; Helmick et al., 1984). The most frequent clinical signs are fever, headache, myalgia, arthralgia, general discomfort, nausea, vomiting, abdominal pain and anorexia. However, the symptomatology is often unspecific and may be confused with other diseases (Biggs et al., 2016).

Rocky Mountain spotted fever, caused by *Rickettsia rickettsii*, is the most severe rickettsial disease and is the most commonly reported in Mexico; from 2009 to 2015, the Mexican Secretary of Health reported a total of 4309 confirmed cases, with the highest morbidity in the northern states (Secretaria de Salud, 2015). Other tick-borne bacteria of zoonotic importance are those of the *Ehrlichia* genus. *E. chaffeensis* causes the most commonly found, human monocytic ehrlichiosis (Ismail et al., 2010); *E. ewingii* has been reported less frequently (Biggs et al., 2016); and *E. canis*, the cause of canine monocytic ehrlichiosis, was previously exclusively considered an animal disease. However, Perez et al., 1996 detected *E. canis* in asymptomatic patients in Venezuela and later isolated the bacteria from patients who showed clinical signs suggestive of human monocytic ehrlichiosis. Furthermore, they demonstrated that the isolated strain in such patients was similar to the strain isolated from domestic dogs and *Rhipicephalus sanguineus* sensu lato ticks in Venezuela (Perez et al., 2006; Unver et al., 2001). In Mexico, reports show serologic and molecular evidence of the presence of *E. canis* in dogs from several states of the country; a seroprevalence of 22% in Baja California (Haro-Alvarez et al., 2007) and 74% in Sinaloa (Sosa-Gutierrez et al., 2013) was reported. Additionally, prevalences of 44% and 10.0% were detected by PCR in dogs from Yucatan (Rodríguez-Vivas et al., 2005) and the Durango-Coahuila region (Almazán et al., 2016). Only two cases of human ehrlichiosis have been documented. The first case reported in the state of Mexico was caused by *E. chaffeensis* and resulted in the death of the patient. The diagnosis

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was confirmed by IFA and PCR (Sosa-Gutierrez et al., 2016a, 2016b). The second patient was a dog groomer in the state of Oaxaca who did not show any symptoms and was positively diagnosed for *E.* canis by PCR (Silva et al., 2014).

Human granulocytic anaplasmosis is one of the most commonly reported rickettsial diseases in the United States (Holly et al., 2006). In Mexico, there are no reports of the disease in humans; however, it is well known that the distribution of tick-borne diseases is closely related to the distribution of the corresponding vectors (Raoult and Roux, 1997). In this regard, it has been reported that *A. phagocytophilum* is transmitted by *Ixodes* ticks (Richter et al., 1996), although recent studies acknowledge *R. sanguineus* sensu lato as a vector that transmits this pathogen (Ghafar and Amer, 2012). Additionally, in a study in which rickettsial pathogens were identified in ticks from all over Mexico, *A. phagocytophilum* showed the broadest prevalence (9%), and the tick with the highest distribution was *R. sanguineus* sensu lato (Sosa-Gutierrez et al., 2016a, 2016b).

Lethality rate related to the above diseases vary due to factors such as the virulence of the pathogen, the lack of use of preventive measures or the delay in diagnosis and treatment; however, tick-borne infections continue to cost human lives (Mutz, 2009). One example is the high annual lethality rate (of up to 63%) reported at the Children's Hospital in Sonora, Mexico from the period of 2004–2012 (Alvarez-Hernandez and Contreras-Soto, 2013).

Workers in animal health centers are exposed to several occupational risks during their daily duties from exposure to physical, chemical and biological agents. In this regard,

getting infected with a zoonotic pathogen is one of the main hazards for personnel at animal health centers.

The objective of the present study was to estimate the seroprevalence of rickettsial pathogens in exposed people associated with their professional activities in veterinary clinics in Ciudad Juárez, Chihuahua, Mexico. Additionally, the study aimed to determine the social, demographic and occupational risk factors and their association with clinical symptomatology.

2. Materials and methods

2.1. Study design

The present study was performed in Ciudad Juarez, Chihuahua, Mexico during the summer (May and June) of 2016. Participants were recruited from 63 veterinary clinics that specialized in small animal health care (mainly dogs and cats) located in different areas of the city. The sample consisted of 167 workers (106 veterinarians, 36 medical assistants, 19 pet groomers and six administrators).

2.2. Blood sampling and epidemiological survey

A total of 6 ml of blood per participant was taken by qualified personnel via venipuncture. The blood collection system included vacutainer tubes with EDTA (purple top) and without EDTA (red top) (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Each tube was labeled with the participant number and contained three ml of whole blood from the corresponding donor. The blood samples were transported on ice immediately to the laboratory of Biotechnology at the Biomedical Science Institute of the Autonomous University of Ciudad Juárez, where the red-topped tubes were centrifuged at 3000 × *g* for 30 min at 4 °C. Serum was collected and stored in 2-mL vials at −20 °C until serological analysis was performed. Blood samples in the purple-topped tubes were also stored at this temperature until the DNA extraction procedure was performed. Questions related to social and demographic characteristics, such as gender, age and level of education, were included in the epidemiological survey. In addition, the participants provided information related to the activities they perform in their job, previous tick bites or blood transfusions received and if they had any symptoms or clinical signs related to rickettsial diseases up to three months prior to the study.

2.3. Indirect immunofluorescence assay

To detect the antibodies against *R. rickettsii*, *Ehrlichia* spp. and *A. phagocytophilum*, the *R. rickettsii* IFA IgG and *A. phagocytophilum* and *E. chaffeensis* MIF IgG commercial kits (Fuller Laboratories, Fullerton, California, USA) were used according to the manufacturer's instructions. Samples were considered positive when they had a seroreaction at a 1:64 dilution. Afterwards, positive samples were diluted to 1:256 to detect any possible recent infection. Slides were observed using a fluorescence microscope (Leica DM2000) under 40x magnification.

2.4. Pcr

To detect *Ehrlichia* spp., *E. canis* and *A. phagocytophilum* DNA, PCR tests were performed on samples from individuals who expressed possible clinical signs suggesting rickettsial diseases during the sampling procedure. Their serum samples were positive for *Ehrlichia* spp. and/or *A. phagocytophilum* at a 1:256 dilution.

The extraction of DNA from blood samples was made using Nucleospin Blood commercial kits (Macherey-Nagel; Hoerdt, France) according to the manufacturer's instructions. To detect *Ehrlichia* spp. and *A. phagocytophilum* DNA, the region encoding gene 16S rRNA was amplified following the methodology described by Inokuma et al. (2000), and Ghafar and Amer (2012), respectively. Each reaction contained 200 ng of DNA, 1 µl of sense primer (20 mM), 1 µl of antisense primer (20 mM), 12.5 µl of a master mix 2X containing reaction buffer (pH 8.5), 400 mM of dNTPs, and 3 mM of $MgCl_2$ combined with the enzyme Go Taq DNA polymerase (50 U/ml). Finally, molecular-grade water was added to obtain a total volume of 25 µl. The amplification products of 345 bp for *Ehrlichia* spp. and 262 bp for *A. phagocytophilum* were analyzed by electrophoresis on a 2% agarose gel and visualized using ultraviolet light.

2.5. Statistical analysis

Data obtained from the epidemiological surveys and the results of the serological tests were analyzed using the procedures PROC FREQ TABLES and PROC LOGISTIC in SAS (Statistical Analysis System Institute Inc., Cary, NC, USA. Version 9.0).

Seroprevalence in the blood samples of the participants was estimated for each pathogen individually and the exposure to two or three pathogens simultaneously.

Multiple logistic regression was performed by adding the following categorical variables: gender, age (18–25 years, 26–40 years and > 40 years), education (undergraduate and graduate), activities at the clinic (grooming, assistant, administrative and veterinarian), working hours per week (1–20 hours, 21–40 hours and > 40 h per week), occurrence of a tick bite (yes/no), number of times bitten by a tick (1–3 times and ≥4), blood transfusions received (yes/no) and number of dogs served weekly (1–20, 21–49 and ≥50). For comparison, the reference value of each variable was selected according to those previously reported as risk factors associated with the disease. The symptoms and clinical signs evaluated in the study were edema, fever, arthralgia, headache, myalgia, general weakness and uncoordinated movements.

Differences were considered statistically significant at $p \leq 0.05$. The odds ratio (OR) was calculated with confidence intervals corresponding to a probability of 95% for all variables in the present study.

The present research procedures comply with the guidelines for medical research in humans included in the code of ethics of the World Medical Association's Declaration of Helsinki and with the guidelines of the Bioethics Committee of the Biomedical Science Institute of the Autonomous University of Ciudad Juárez. The individuals agreed to participate in the present study on a voluntary basis by signing a

Table 1
Overall seroprevalence of rickettsial pathogens evaluated at different dilutions by indirect immunofluorescence (1:64 positives/total. 1:256 positives /1:64 positives).

Pathogen	Sample dilution	
	1:64	1:256
R. rickettsii	21 % (35/167)	11 % (4/35)
Ehrlichia spp.	28 % (47/167)	30 % (14/47)
phagocytophilum	24 % (40/167)	13 % (5/40)

document in which their participation was fully detailed.

3. Results

In the present study of 167 participants, 77 (46%) did not show evidence of any of the evaluated pathogens. The proportions of samples that were positive for *R. rickettsii*, *Ehrlichia* spp. and *A. phagocytophilum* by indirect immunofluorescence were similar ($p > 0.05$), ranging from 20.9 to 28% at the 1:64 dilution; however, that proportion was considerably lower ($p < 0.05$) for *R. rickettsii* and *A. phagocytophilum* at the 1:256 dilution, as shown in Table 1.

From all 167 participants, 90 (54%) exhibited a positive seroreaction to at least one of the pathogens evaluated in the study. A total of 5 participants (3%) exhibited coexposure to *R. rickettsii* and *A. phagocytophilum*, 15 (9%) to *Ehrlichia* spp. and *A. phagocytophilum*, and 3 (2%) to *Ehrlichia* spp. and *R. rickettsii*. Four participants (2%) were positive for all three pathogens evaluated in the study (Fig. 1).

Regarding samples that were found positive in the PCR test (3 participants), Fig. 2 shows a representation of the bands in the agarose gel that match the amplicons for *Ehrlichia* spp. and *A. phagocytophilum*.

The results of the logistic regression analysis of data used to detect the risk factors and clinical signs associated with rickettsial infection are shown in Tables 2 and 3, respectively. In Table 4, the symptoms of several study participants with a clinical diagnosis of both *Ehrlichia* spp. and *A. phagocytophilum* are shown.

4. Discussion

The values for seroprevalence of *R. rickettsii* (21%), *Ehrlichia* spp. (28%) and *A. phagocytophilum* (24%) obtained in the present study are higher than those reported in other studies in which the prevalence of rickettsial diseases in at-risk populations was evaluated. In such studies, individuals considered at risk were those who worked in parks, forests, or fields in the presence of vectors, such as ticks of the *Ixodes*, *Amblyomma* and *Dermacentor* genera, among others (Piesman and Eisen, 2008; Eisen and Eisen, 2007; Brown et al., 2005). In Germany, an ehrlichiosis seroprevalence of 19.5% in forest workers was reported (Batzing-Feigenbaum et al., 2000). In North Carolina, USA, an *E. chaffeensis* seroprevalence of 4% and *R. rickettsii* seroprevalence of 19% in a cohort of field workers was reported (Wallace et al., 2016). In the present study, the population at risk consisted of personnel of the veterinary clinics in which dogs infested by *R. sanguineus* sensu lato, the most widely distributed tick in Mexico, are treated (Sosa-Gutierrez et al., 2014).

Studies on detection of antibodies against rickettsial microorganisms in humans show varied seroprevalence results. This is probably associated with the heterogeneity of the target population involved. For example, in a sample of 300 individuals between one and 17 years old in the southern United States (Marshall et al., 2003), a seroprevalence of 12% was registered by the IFA method, and in Baja California, Mexico, the observed seroprevalence was 4% in 384 samples of volunteers older than one year of age (Field-Cortazares et al., 2015). The results of the present study are consistent with those obtained by Teoh et al. (2017), who evaluated antibodies to *R. felis* and *R. typhi* from 131 veterinarians in Australia, with a seroprevalence of 35%. Given that both studies were performed with vulnerable human populations, such as personnel working in veterinary clinics, a higher seroprevalence

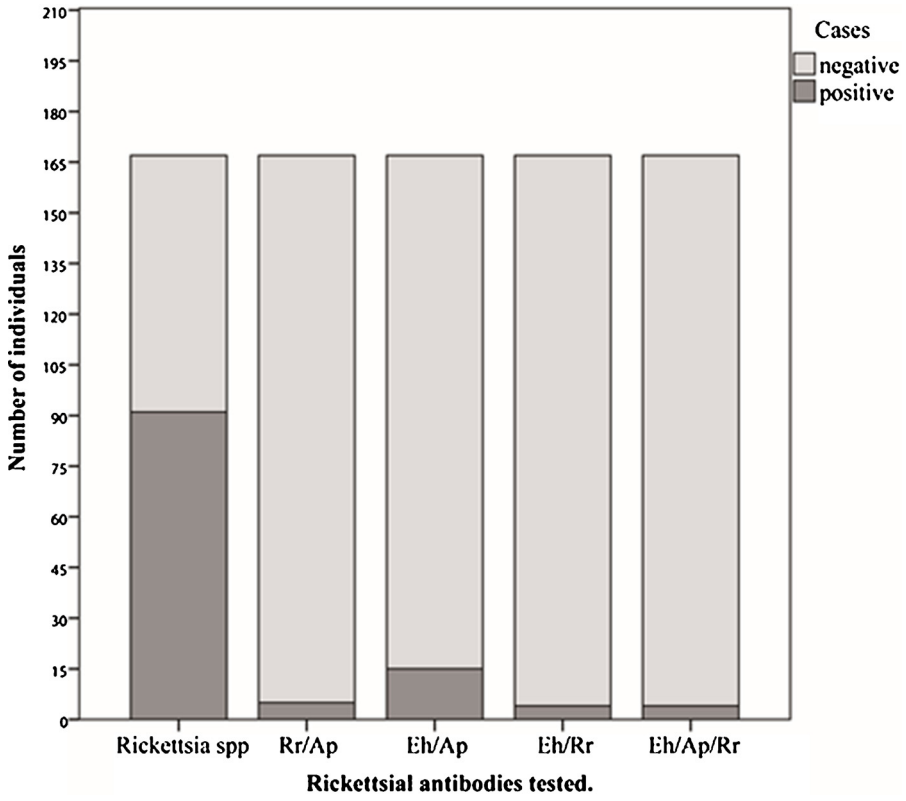

Fig. 1. Proportion of participants exposed to *Rickettsia* spp. and coexposed to *Rickettsia rickettsii* (Rr), *Anaplasma phagocytophilum* (Ap) and *Ehrlichia* spp. (Eh).

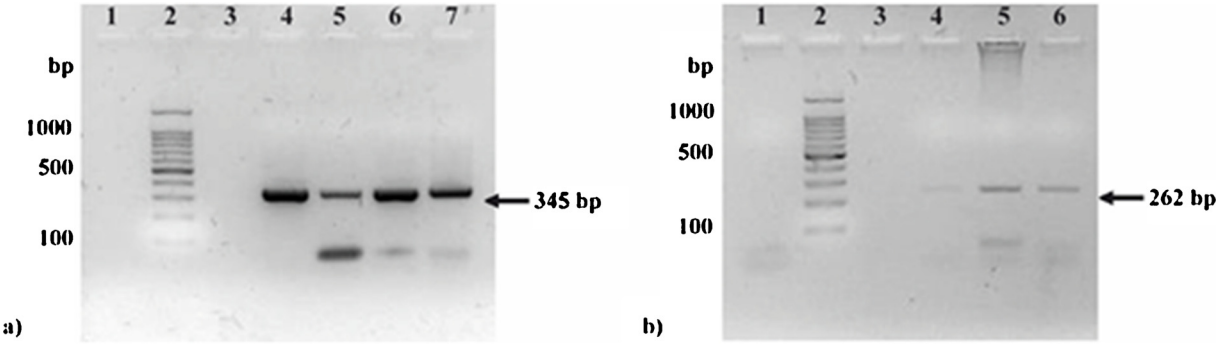

Fig. 2. PCR amplification products for the 16S rRNA gene.
Panel a) Amplification for *Ehrlichia* spp., size of amplicon: 345 bp. Lane 2: molecular weight marker of 100 bp; Lane 3: negative control; Lane 4–6 samples of participants; Lane 7: positive control.
Panel b) Amplification for *A. phagocytophilum*, size of amplicon: 262 bp. Lane 2: molecular weight marker of 100 bp; Lane 3: negative control. Lane 4 and 5 samples of participants; Lane 6: positive control.

Table 2
Characteristics evaluated in the study and their association with seroprevalence.

Variable	*R. rickettsii*		*Ehrlichia* spp.		*A. phagocytophilum*	
	Prevalence	OR (CI 95%)	Prevalence	OR (CI 95%)	Prevalence	OR (CI 95%)
Gender						
Female	22/104	1.14 (0.51-2.5)	29/104	0.96 (0.48-1.93)	31/104	2.54 (1.12-5.79)*
Male	12/63		18/63		9/63	
Age (years)						
> 40	13/76	0.96 (0.36-2.56)	24/76	1.95 (0.81-4.66)	20/76	1.09 (0.4-2.94)
25 to 40	10/44	0.68 (0.27-1.66)	14/44	1.97 (0.75-5.17)	10/44	1.32 (0.56-3.14)
18 to 25	11/47	Ref	9/47	Ref	10/47	Ref
Education						
Undergraduate	13/41	1.9 (1.05-3.45)*	14/41	0.74 (0.33-7.52)	12/41	0.69 (0.31-1.52)
Graduate	21/126		33/126		28/126	
Activity						
Grooming	7/19	3.05 (1.0-8.87)*	6/19	1.23 (0.43-3.53)	6/19	1.58 (0.54-4.59)
Assistant	10/36	2.01 (0.82-4.93)	9/36	0.9 (0.37-2.11)	8/36	0.98 (0.39-2.42)
Administrative	0/6	0	3/6	2.6 (0.51-13.91)	2/6	1.71(0.29-9.9)
Veterinarian	17/106	Ref	29/106	Ref	24/106	Ref
Labor hours (weekly)						
> 40	20/92	1.3 (0.47-3.56)	25/92	0.68 (0.3-1.58)	24/92	1.15 (0.46-2.88)
21 to 40	8/41	1.13 (0.35-3.65)	10/41	0.59 (0.22-1.61)	8/41	0.79 (0.26-2.38)
1 to 20	6/34	Ref	12/34	Ref	8/34	Ref
Tick bite						
Yes	12/67	0.77 (0.35-1.69)	20/67	1.15 (0.58-2.28)	14/67	0.75 (0.35-1.57)
No	22/100		27/100		26/100	
Times bitten by a tick						
≥4	3/11	1.95 (0.43-8.83)	4/11	1.42 (0.36-5.55)	5/11	4.35 (1.08-17.37)*
1 to 3	9/56		16/56		9/56	
Blood transfusions						
Yes	0/6	0	2/6	1.28 (0.22-7.28)	4/6	6.94 (1.22-39.46)*
No	34/161		45/161		36/161	
Dogs served (weekly)						
> 50	8/45	2.01 (0.8-5.2)	14/45	1.55 (0.64-3.77)	10/45	1.15 (0.46-2.88)
21 to 49	14/48	1.06 (0.37-3.01)	15/48	1.54 (0.63-3.8)	12/48	0.8 (0.26-2.38)
1 to 20	9/53	Ref	12/53	Ref	15/53	Ref

Prevalence, positive cases/total.
CI, confidence interval.
OR, odds ratio.
* Variable associated with each pathogen ($p < 0.05$).

value would be expected than in other studies performed with a non-specific population. However, in a study performed at blood donation centers with donors between 18 and 60 years old in Costa Rica, antibodies to *E. canis* were detected in 35% of the samples analyzed by IFA; thirty of the positive samples (86%) showed low titres (1:64 to 1:256), and the rest of the positive samples showed high titres (1:1024 to 1:1892) (Bouza-Mora et al., 2017).

Nevertheless, it is important to mention that a potential limitation to the interpretation of these results is that the diagnostic kits used in our research may reflect a cross reaction between *E. canis*, *E. chaffeensis* and *E. ewingii*. Although *E. chaffeensis* and *E. ewingii* have not been reported in northern Mexico, dogs are frequently reported as positive for *E. canis* by veterinarians (Sosa-Gutierrez et al., 2013; Haro-Alvarez et al., 2007). Additionally, it is important to consider that previous documentation questions the reliability of the serologic diagnosis of *R. rickettsii* at its initial stages by indirect immunofluorescence; thus, PCR-based tests are considerably easier and more reliable for diagnosis (Mutz, 2009).

Table 3
Clinical signs and symptoms reported by the participants up to three months prior to the study and their association with the rickettsial pathogens.

Clinical signs	*R. rickettsii*		*Ehrlichia* spp.		*A. phagocytophilum*	
	Prevalence	OR (IC 95%)	Prevalence	OR (IC 95%)	Prevalence	OR (IC 95%)
Edema in limbs	8/30	1.55 (0.62-3.87)	8/30	0.91 (0.37-2.22)	8/30	1.19 (0.48-2.93)
Fever	5/26	0.91 (0.31-2.64)	5/26	0.56 (0.19-1.58)	5/26	0.72 (0.25-2.05)
Arthralgia	16/75	1.11(0.52-2.37)	27/75	2.02 (1.02-4.0)*	19/75	1.14(0.56-2.33)
Headache	15/79	0.85 (0.39-1.81)	28/79	1.99 (1.0-3.95)*	23/79	1.71 (0.83-3.51)
Myalgia	9/51	0.78 (0.33-1.81)	20/51	2.12 (1.04-4.33)*	18/33	2.33 (1.11-4.87)*
Weakness	10/52	0.90 (0.39-2.05)	14/52	0.99 (0.43-1.90)	20/52	2.97 (1.41-6.20)*
Uncoordinated movements	1/7	0.64 (0.07-5.51)	2/7	1.02(0.19-5.46)	3/7	2.49 (0.53-11.64)

Prevalence, positive cases/total.
CI, confidence interval.
OR, odds ratio.
* Clinical signs and symptoms associated with each pathogen ($p < 0.05$).

Table 4
Symptomatology and PCR results of several participants with clinical picture compatible with *Ehrlichia* spp. and *A. phagocytophilum* at the time of the study.

Patient ID	Symptoms and signs						PCR test results	
	Fever	Headache	Weakness	Arthralgia	Myalgia	Petechiae	*Ehrlichia* spp.	*A. phagocytophilum*
200		X	X	X			+	–
16	X	X	X	X	X	X	+	+
105			X	X			+	+
290	X	X	X	X	X	X	–	–
293	X	X				X	–	–

X, symptom and sign present.
+, positive to PCR test.
-, negative to PCR test.

The range of prevalence for zoonotic infections in veterinarians in the United States has been reported to be very wide (13%–65%). This may be due to the wide variety of species involved in professional practice (Cediel and Villamil, 2004). Practically, no evidence of a relationship of *Ehrlichia* spp. with any of the variables studied was observed in the present study, which highlights the fact that *R. rickettsii* is observed at an odds ratio of 3.05 associated with grooming activities (which include cleaning, showering, combing and cutting dogs´ hair coat) and that *A. phagocytophilum* is found to be associated with female personnel who were repeatedly bitten by ticks and had received blood transfusions. However, it has been reported that males are more susceptible at a 1.4:1 ratio (Dumler, 2013). There are not many studies that seek to determine the risk factors for ehrlichiosis, and reports indicate that living in areas with large tick infestations, performing activities in pastures with tall grass and owning pets that may carry ticks into the home may increase the risk (Maguiña et al., 2004).

While patients that resulted seropositive to *R. rickettsii* were asymptomatic in the present study, the observed signs for *Ehrlichia* spp. were myalgia, arthralgia and headache, and those for *A. phagocytophilum* were rash, weakness and myalgia, Furthermore, five participants showed symptoms and clinical signs that suggested the presence of these diseases at the time of the study and two of them were positive by PCR (Table 4), however, due to the small amount of samples, these results could not be confirmed by sequencing procedures. These results are consistent with Maguiña et al. (2004) and Schotthoefer et al. (2013), who observed that in most people the infection passes asymptomatically, so it is not common practice to request any professional health assistance, which may pose a subsequent health risk. When symptoms do occur, patients typically experience fever, shaking chills, headache, nausea, myalgia, general discomfort, and maculopapular and petechial eruptions. Considering that many of these symptoms can be present in different health conditions, it is difficult to reach an accurate diagnosis on time, causing a subsequent delay in treatment, which may be detrimental to the outcome of the patient involved (Mutz, 2009).

5. Conclusion

Evidence based on the results of serological investigations indicates the presence of several pathogens associated with tick-borne rickettsial diseases among the personnel of clinics in Ciudad Juarez, Chihuahua, Mexico. Furthermore, the association between clinical signs and the molecular diagnosis of *Ehrlichia* spp. and *A. phagocytophilum* was confirmed (Table 4).

It was possible to find an association between occupational variables and the three pathogens evaluated, establishing that grooming posed the highest risk of exposure to *R. rickettsii*.

Finally, it is necessary to promote the implementation of protective measures for personnel working in veterinary clinics. These measures must include good management practices that allow the prevention of zoonotic diseases and constant surveillance for signs that may indicate health alterations.

Conflict of interest

The authors declare that they have no conflict of interest.

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