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Serologic evidence of rickettsial diseases associated with tick bites in workers of urban veterinary clinics

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ABSTRACT

Background & objectives: Rickettsial and other zoonotic diseases are a latent risk for workers of veterinary clinics. The objective of this study was to evaluate the prevalence and the associated risk factors of parasitosis caused by *Rhipicephalus sanguineus,* and to estimate the seroprevalence of rickettsial diseases in workers of urban veterinary clinics of Juárez city, México.

Methods: The participants of the study were recruited from 63 private veterinary clinics and hospitals. The serological analysis of the blood samples collected was carried out using immunofluorescence assay (IFA). The statistical analysis for prevalences, risk factors, and correlation was performed with the SAS program.

Results: In total, 167 veterinary workers were included in the study. The prevalence of tick bites was 40% (67/167), and the risk factors associated with the occurrence of bites included the activities performed in the clinic and the number of labour hours spent per week. About 21% (35/167) of participants were seropositive to *R. rickettsii*, 28% (47/167) to *Ehrlichia chaffeensis,* and 24% (40/167) to *Anaplasma phagocytophilum*. A correlation was observed between: the number of workers in the clinics and the proportion of tick bites ($r^2 = 0.865$); the prevalence of bites and the seropositivity of the participants to at least one pathogen ($r^2 = 0.924$); and the number of bites per individual and infection to pathogens ($r^2 = 0.838$).

Interpretation & conclusion: Workers in urban veterinary clinics are highly exposed to tick bites and, therefore, to the diseases they transmit. Hence, it is important to implement prevention measures and perform constant monitoring of these diseases.

Key words Mexico; *Rhipicephalus sanguineus;* rickettsial diseases; tick bite; veterinarians

INTRODUCTION

Veterinary practice is considered a high-risk occupational activity. Among risk factors to which veterinary clinic workers are exposed include those of physical, chemical, or biological nature[1]. The main physical factors are accidents related to trauma caused by bites or scratches, as well as ionizing radiation; the main chemical factors include the use of anesthetics, as well as handling of drugs, detergents, and disinfectants, while the main biological factors include contact with infectious organisms such as bacteria and viruses that may cause allergies, poisoning, infections or diseases. Trauma and zoonoses are the most important events reported, at least in Latin America[2].

Regarding zoonotic diseases, they vary considerably in severity, causing a wide variety of presentations, ranging from subclinical, disabling chronic, or even lethal outcomes; nevertheless, it is important to notice that they are still undiagnosed nowadays[3]. Zoonoses that are frequently reported in veterinarians are leptospirosis[4], Q fever[5], brucellosis[6], and Lyme disease[7]. Although their prevalence is relatively low among veterinarian personnel in small-animal practice, it is important to maintain close surveillance of these and other diseases in order to maintain job health security[8]. Some zoonoses that are considered as emergent or re-emergent are rickettsiosis, anaplasmosis, ehrlichiosis, and borreliosis, which are transmitted by ticks in a life cycle that include both wildlife and domestic animals[3, 9].

Brown dog tick (*Rhipicephalus sanguineus*) is the tick of greater distribution worldwide[10], as a result of human migration, and has become one of the most important ectoparasites in neotropical animals[11]. Humans are incidental hosts that get bitten when in contact with tick-infested dogs[12].

Bites from *R. sanguineus* in humans are common and have been reported worldwide, although a higher incidence can be observed in the Mediterranean region and Central and South America, with a lower incidence in the United States[13–14]. The prevalence varies, depending on

the affected population. During the summer of 2008 in northeast Greece, a total of 537 tick bites were registered, 87% of which were identified as *R. sanguineus*[15] infection; however, in Soria, Spain, out of 63 patients reporting tick bites, only 6% were identified as *R. sanguineus* infection[16]. Tick bites from this species have also been reported in South Carolina, in animal care personnel[17].

Rhipicephalus sanguineus is important to public health because it is a potential vector of *Ehrlichia canis, Babesia canis, Babesia gibsoni, Hepatozoon canis, Rickettsia rickettsii, R. conorii, R. massiliae*, and the filaroid *Dipetalonema reconditum*, among other parasites[12]. Furthermore, its relationship with rickettsial diseases in humans has also been demonstrated. Such diseases exhibit similar clinical signs but have different etiologies and epidemiological behaviour, and they include Venezuelan ehrlichiosis caused by *E. canis*[18], Rocky Mountain spotted fever caused by *R. rickettsii*[19,] and Mediterranean fever caused by *R. conorii*[18, 20–21]. Additionally, *R. sanguineus* is implicated in the transmission of *E. chaffeensis*, which causes human monocytic ehrlichiosis[3, 11], as well as *Anaplasma platys* and *A. phagocytophilum*, which cause human granulocytic anaplasmosis[11, 22].

There are few official reports of rickettsial diseases in veterinarians, since it is considered that the population at risk are mainly campers, soldiers, hunter, and farmers because they are exposed to the bite of ticks when spending time on the field[23]. However, in a study conducted in Brazil, a prevalence of 69.5% was estimated for *R. sanguineus* bites in 46 employees of three veterinary clinics[24]; therefore, it is considered that personnel of such work centres are at constant risk of developing some of these diseases. The objective of the present work was to evaluate the prevalence of parasitosis caused by *R. sanguineus* and to estimate the seroprevalence of *R. rickettsii*, *E. chaffeensis*, and *A. phagocytophilum* in working personnel of urban veterinary clinics of Ciudad Juárez, Chihuahua, México.

MATERIAL & METHODS

Study design and sample size

The present study was conducted in Ciudad Juárez, Chihuahua, México located at 31° 44′ 2 2 N″ and 106° 29′ 1 3″ W coordinates, 1137 m above sea level. The climate of the region is very dry and arid, with average temperatures of 21.8 and 13.8°C during summer and winter, respectively, and average yearly precipitation of 260 mm. The city population is 1,332,131 inhabitants[25]. Participants of the study were recruited from 63 private veterinary clinics and hospitals during June 2016. The sample size was 167 people and included 105 veterinarians, 37 assistants, 19 groomers, and six administrative workers.

Epidemiologic survey

The participants were included after obtaining written informed consent. All of them agreed to answer a survey and provide a blood sample. The epidemiological questionnaire included questions related to demographic data (age, sex, education), labour data (activity performed in the clinic, total daily labour hours, number of dogs attended per week, presence of ticks in dogs treated and aggression by ticks at the last 6 months). Questions about the characteristics of the veterinary clinics (type of flooring on the patio, presence of cracks in the walls, and application of pesticides for tick control) were also included.

Blood sampling and serologic diagnosis

Samples were obtained by qualified personnel and consisted of 3 ml of whole venous blood by participants, which was deposited in BD vacutainer® red cap tubes, labeled with an identification number. Once taken, the blood samples were transported (on ice) immediately to the biotechnology laboratory at Universidad Autónoma de Ciudad Juárez (UACJ), Chihuahua, Mexico, and centrifuged at 3800 rpm for 20 min at 22°C to separate serum, which was collected and stored in 1.5 ml vials at –20°C until serologic analysis performed.

For the detection of IgG antibodies of the pathogens of interest, indirect immunofluorescence assay (IFA) commercial test kits, *viz. R. rickettsii* IFA IgG kit and *A. phagocytophilum* and *E. chaffeensis* MIF IgG kit (Fuller Laboratories, Fullerton, California, USA) were used. The initial serum dilution was 1: l64, and positive and negative controls (provided by the manufacturer) were placed in each immunofluorescence plate. The criterion for determination of a positive result was the fluorescent sero-reaction at the 1: l64 dilution. Slides were observed in a fluorescence microscope at 40× magnification

Statistical analysis

All the information collected was stored in a spreadsheet (Microsoft Excel®) as a database, ensuring confidentiality. The processing and statistical analyses were performed with the SAS program (version 9.0; SAS Institute Inc., Cary NC). The prevalence of tick bites in the participants (bitten individuals out of total participants) was

calculated and the risk factors were evaluated with the corresponding odds ratio (OR) considering confidence intervals (CI) at 95%. The seroprevalence for *R. rickettsii*, *A. phagocytophilum,* and *E. chaffeensis* was calculated, as along with the frequency distributions for the different variables using the Chi-squared test and the Fisher's exact test.

Additionally, a correlation analysis was carried out to determine the degree of association between the number of workers, the prevalence of tick bites for the last six months, and the proportion of seroprevalence to any of the pathogens evaluated. Furthermore, the association between the number of tick bites per participant and seropositivity to the pathogens was also determined.

Ethical statement

This work was approved by the Institutional Ethics Committee of Universidad Autónoma de Ciudad Juárez (CIBE-2016-05). All the participants provided a signed agreement letter before voluntarily participating in the study.

RESULTS

All the 167 individuals who participated in the study declared that they had treated dogs parasitized by ticks in their work areas, and they agreed that the month of June is when the greatest infestation occurs at the location of the study. Tick control by application of some pesticides in a clinic is a common practice at least once a year, as stated by 98% of the respondents. The prevalence of bites in the participants of the study was 40% (67/167). Of these 67 people, 11 (16%) were bitten more than once in the last three months prior to the study. The lower limbs were the anatomical region with the highest frequency of bites (43%), followed by the upper extremities (31), trunk (12), head (9), and only 5% were bitten in more than one anatomical region. A total of 26% (18/67) of the bitten individuals experienced a sensation of pain or discomfort in the area of the bite.

As shown in Table 1, risk factors associated with tick bites that showed a statistically significant difference were only the activity performed and the number of daily working hours. The prevalence for the three evaluated diseases showed a homogenous distribution in the study population: 21% (35/167) were positive for *R. rickettsii*, 28% (47/167) for *E. chaffeensis*, and 24% (40/167) for *A. phagocytophilum*. The frequency distribution of seropositive individuals to different diseases is shown in Table 2. A strong correlation was observed between the number of workers in the clinics with the proportion of bites per tick ($r^2 = 0.865$) as well as with the proportion of seropositive participants to at least one of the pathogens ($r^2 = 0.924$) and between the number of bites and infections ($r^2 = 0.838$).

Table 1. Risk factors associated with tick bites

Variable	Percent tick bite prevalence	OR [CI 95%]
Demographic characteristics of participants		
Gender		
Female	43 (45/104)	1.42 [0.74–2.71]
Male	35 (22/63)	1
Scholarship		
Pregrade	32 (13/41)	0.92 [0.29–1.31]
Graduated	43 (54/126)	1
Age (yr)		
18–25	47 (21/44)	2.15 [0.9–5.0]
26–40	42 (32/76)	1.71 [0.8–3.72]
>40	30 (14/47)	1
Working conditions of participants		
Activity		
Grooming	68 (13/19)	10.83 [1.0–114.1]*
Assistant	25 (9/36)	1.66 [0.1–16.2]
Veterinarian	46 (49/106)	4.29 [0.48–38.5]
Administrative	16 (1/6)	1
Weekly working hours		
>40	50 (49/92)	3.86 [1.53–9.47]*
21–40	34 (14/41)	2.0 [0.7–5.73]
–20	20 (7/34)	1
Dogs attended weekly		
>50	44 (20/45)	1.13 [0.51–2.52)
21–50	41.67 (20/48)	1.01 [0.4–2.5]
1–20	41.51 (22/53)	1
Characteristics of veterinary clinics		
Pest control in the clinic		
No	43 (3/7)	1.25 [0.24–5.12]
Yes	40 (64/160)	1
Yearly pesticide application		
–4	45 (25/56)	1.49 [0.69–3.2]
5–9	40 (20/50)	1.23 [0.5–2.72]
>10	35 (19/54)	1
Type of flooring on the yard		
Dirt	42 (14/33)	1.17 [0.52–2.64]
Concrete	41 (19/46)	1.05 [0.42–2.59]
Mixed	38 (34/88)	1
Wall crackings		
Yes	46 (52/114)	2.12 [1.0–2.48]*
+No	28 (15/53)	1

*Indicate significant differences ($p < 0.05$).

Table 2. Percent variables associated with seropositivity to rickettsial tick-borne diseases

Variable	*R. rickettsii*		*E. chaffeensis*		*A. phagocytophilum*	
	Bitten	Not bitten	Bitten	Not bitten	Bitten	Not bitten
Gender						
Female (104)	22.2 (10/45)	20.3 (12/59)	33 (16/45)	22 (13/59)	26.6 (12/45)	32 (19/59)
Male (63)	18.1 (4/22)	19.5 (8/41)	18.2 (4/22)	34.1 (14/41)	13.6 (3/22)	14.6 (6/41)
Scholarship						
Pregrade (41)	53.8 (7/13)[A]	21.4 (6/28)	100 (13/13)[A]	3.5 (1/28)	76.9 (10/13)[A]	7.1 (2/28)
Graduated (126)	9.2 (5/54)[a]	22.2 (16/72)	12.9 (7/54)[a]	36.1 (26/72)	9.2 (5/54)[a]	31.9 (23/72)
Age (yr)						
18–24 (44)	14.2 (3/21)	30.4 (7/23)	9.5 (2/21)[Aa]	52.2 (12/23)	14.3 (3/21)	30.4 (7/23)
25–40 (76)	12.5 (4/32)	20.5 (9/44)	34.4 (11/32)	29.5 (13/44)	18.8 (6/32)	31.8 (14/44)
> 40 (47)	21.4 (3/14)	24.2 (8/33)	57.1 (8/14)[A]	3 (1/33)	35.7 (5/14)	15.2 (5/33)
Activity						
Grooming (19)	30.7 (4/13)	50 (3/6)	30.7 (4/13)	28.5 (2/7)	23 (3/13)	50 (3/6)
Assistant (36)	33.3 (3/9)	25.9 (7/27)	11.1 (1/9)	29.6 (8/27)	11.1 (1/9)	25.9 (7/27)
Veterinarian (106)	10.2 (5/49)	21.1 (12/57)	10 (5/49)[A]	88.8 (24/27)	10.2 (5/49)[A]	59.2 (16/27)
Administrative (6)	0 (0/1)	0 (0/5)	100 (1/1)	40 (2/5)	100 (1/1)	20 (1/5)
Weekly working hours						
>40 (92)	12.2 (6/49)[A]	32.5 (14/43)	26.5 (13/49)	27.9 (12/43)	16.3 (8/49)[A]	37.2 (16/43)
21–40 (41)	7.1 (1/14)	25.9 (7/27)	28.5 (4/14)	22.2 (6/27)	28.5 (4/14)	14.8 (4/27)
1–20 (34)	28.5 (2/7)	14.8 (4/27)	28.5 (2/7)	37 (10/27)	28.5 (2/7)	22.2 (6/27)
Dogs attended weekly						
>50 (45)	20 (4/20)	16 (4/25)	35 (7/20)	28 (7/25)	20 (4/20)	24 (6/25)
21–49 (48)	25 (5/20)	32.1 (9/28)	55 (11/20)[A]	14.2 (4/28)	25 (5/20)	25 (7/28)
1–20 (53)	13.6 (3/22)	19.3 (6/31)	22.7 (5/22)[a]	22.5 (7/31)	31.8 (7/22)	25.8 (8/31)
Pest control						
No (7)	100 (3/3)	25 (1/4)	33 (1/3)	25 (1/4)	0 (0/3)	0 (0/4)
Yes (160)	18.7 (12/64)[a]	18.7 (18/96)	31.2 (20/64)	26 (25/96)	21.8 (14/64)	27 (26/96)
Yearly pesticide application						
1–4 (56)	8 (2/25)	25.8 (8/31)	16 (4/25)[Aa]	48.3 (15/31)	16 (4/25)[A]	51.6 (16/31)
5–9 (50)	20 (4/20)	26.6 (8/30)	60 (12/20)[A]	6.6 (2/30)	35 (7/20)[A]	6.7 (2/30)
>10 (54)	21 (4/19)	11.4 (4/35)	31.5 (6/19)	17.1 (6/35)	26.31 (5/19)	17.1 (6/35)
Type of flooring on the patio						
Dirt (33)	0 (0/14)	26.3 (5/19)	21.4 (3/14)	31.5 (6/19)	7 (1/14)	26.3 (5/19)
Concrete (46)	15.7 (3/19)	22.2 (6/27)	36.8 (7/19)	22.2 (6/27)	10.5 (2/19)[A]	40.7 (11/27)
Mixed (88)	20.5 (7/34)	24 (13/54)	29.4 (10/34)	27.7 (15/54)	23.5 (8/34)	24 (13/54)
Wall crackings						
Yes (114)	11.5 (6/52)	19.3 (12/62)	25 (13/52)[a]	27.4 (17/62)	7.6 (4/52)[A]	40.3 (25/62)
No (53)	26.6 (4/15)	31.5 (12/38)	53.3 (8/15)[A]	23.6 (9/38)	26.6 (4/15)	18.4 (7/38)

Upper case letters between columns of bitten and not bitten participants in the same seropositivity of a pathogen agent indicate significant differences (p <0.05). Lower case letters among the variables inside the same row and classification indicate significant differences (p <0.05). Figures in parentheses indicate percentages.

DISCUSSION

Practically, all the interviewed participants expressed having attended dogs with tick infestation, which shows the wide distribution of this parasite in the city. In the case of urban veterinary clinics, the main practice is keeping/taming companion animals such as dogs and cats, unlike rural veterinary clinics, where the practice includes taming farm animals. This increases the risk of tick bite, as observed in the present study, wherein the prevalence reached 40%. A similar study conducted by Epp and Waldner[26] found that the risk to zoonoses is 8.6 times higher in veterinarians with small animal practice than those with exclusive equine practice.

The high rates of parasitism (tick bites) observed in the present study can be related to the season of greatest activity of the vector, as observed in the study carried out by Louly *et al*[24] in veterinary clinics of Brazil (69.5%),

and by Ghane *et al*[9] in farm workers (60.3%). According to Parola *et al*[27], the brown dog tick increases its period of activity, aggressiveness, and predilection for feeding on humans when exposed to high temperatures and/or long periods of summer-like weather. In the month of June in Ciudad Juárez, temperatures can be as high as 44°C[25].

The likelihood of being attacked by ticks for more than one occasion occurred in 16% of the individuals surveyed (with the highest frequency of four occasions in the last three months prior to the study) and contrasts with a cohort work carried out in workers of the forest in North Carolina where some individuals were bitten up to 8 times in a year[28]. Nevertheless, the occurrence of multiple bites in the participants of the present study cannot be considered as a representative to make conclusive assertions.

The anatomical region in which tick bites occurred most frequently were the lower extremities (43%) while the lowest occurrence was for the head (9%). This agrees with data reported by Robertson *et al*[29], where 46% of the tick bites were observed on the lower extremities and 5% in the head. However, these results may suggest that the bites are the product of an active search for the vector towards the host and not necessarily the direct manipulation of dogs by animal health personnel.

According to Abarca and Otero[3], there is still a significant subdiagnosis of rickettsial diseases. This can lead to the wrong prognosis of the patients, given the fact that these conditions are considered potentially lethal infections, and that by not distinguishing or suspecting of them, the process may lead to death. These aspects must be considered since, in the present study, only 26% of the bitten individuals experienced a sensation of pain or discomfort in the area of the bite, which is often ignored and not taken as an actual clinical suspicion of the infection. Ghane *et al*[9] also found a similar percentage of workers (24.5%) who felt sick after the bite of a tick.

Considering gender as a risk factor, although female participants showed a numerically higher prevalence than males (43 and 35%, respectively), no significant difference was observed (p >0.05), which differs with other studies, wherein biting rates were higher in males[15, 24].

Pet groomers showed a 10.8 times more risk of being bitten when compared to administrative workers. This could be attributed to the fact that groomers spend more time in contact with dogs infested with ticks than the rest of the employees. In 2014, a case of ehrlichiosis was registered in a dog groomer from the City of Oaxaca, México, who stated that he had been bitten by ticks on two occasions[30]. Another observed associated risk factor was for employees who worked >40 h per week (OR = l 3.86), in comparison to those working only 20 h. A similar relationship has been reported by Epp and Waldner[26] in veterinary clinics in Canada, associated with the development of zoonoses. They also observed that the amount of years of experience in employment is a risk factor, assuming that more experienced veterinarians are less exposed to zoonotic diseases.

In the present study, a significant association was observed between the prevalence of tick bite and the presence of cracks in the facilities (OR = 2.12). The *R. sanguineus* is considered an endophilic, anthropophilic tick that predominates in intradomestic urban environments, sheltering in curtains and carpets[31], and is frequently located in cool and humid places, such as wall cracks, to complement its life cycle[19, 32].

Immunofluorescence tests designed to detect rickettsial diseases are useful in determining pathogens in blood tests of personnel working in veterinary clinics. Teoh *et al*[33] obtained evidence that 16% of participants in their study were seropositive to *R. felis*, 4.6% to *R. typhi*, and 35.1% to both. Paulino *et al*[34] evaluated 90 serum samples of working personnel of veterinary clinics using IFA, and they observed that 21 (23.33%) and 18 (20%) were positive for *E. canis* and *E. chaffeensis*, respectively. These results appear very similar to the present study, with 21% positive cases to *R. rickettsii*, 28% to *E. chaffeensis*, and 24% to *A. phagocytophilum*. Results from this study also suggest that there is a correlation between the number of infections and the number of bites.

Since it has been documented[35] that ticks can transmit a wide variety of bacteria, and even co-infection is common, results of the present study may have a high impact on public health in aspects of diagnosis and treatment.

CONCLUSION

Working personnel in veterinary clinics are exposed to various situations of contracting the pathogen/infection. It is important to raise awareness in animal health professionals and students about the risks that exist in their professional practice, as well as to emphasize the importance of preventing and reducing such risks, by applying good practices that include the use of protective equipment, timely diagnosis, and continuous training, among others. It is recommended to implement a program capable of monitoring the populations of ticks as the main vectors of rickettsial diseases in the city in such a way that the health authorities can take the appropriate measures regarding control or eradication campaigns.

Finally, it is suggested to increase the communication between human and animal health professionals, allowing a proper approach to the problem of zoonotic diseases being a latent public health risk.

Conflict of interest: None.

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