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Geographical analysis of seroprevalence of *Ehrlichia* spp., *Anaplasma* spp., *Borrelia burgdorferi* and *Dirofilaria immitis*, in clinics and dog shelters in different Mexican states

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ABSTRACT

This study aimed to determine the seroprevalence and geographical distribution of *Ehrlichia* spp., *Anaplasma* spp., *Borrelia burgdorferi* and *Dirofilaria immitis* in dogs in Mexico, including owned dogs from veterinary clinics with regular medical care and shelter dogs. The Mexican territory was divided into eight geographical regions; 22 out of 32 states were included; 110 veterinary clinics and 53 dog shelters participated. SNAP® 4Dx Plus® (IDEXX® Laboratories) was used to detect antibodies against *Ehrlichia* spp., *Anaplasma* spp., *Borrelia burgdorferi* and *Dirofilaria immitis* antigens. A total of 3522 apparently healthy dogs were tested, 1648 from clinics and 1874 from shelters. The highest seroprevalence of infection/exposure was found for *Ehrlichia* spp. (30.9%), followed by *Anaplasma* spp. (14.6%), *D. immitis* (5.3%) and *B. burgdorferi* (0.1%). Significantly more positive dogs were older than 3 years. Regarding differences between facility types, there were only differences for *D. immitis* which was more prevalent in clinics than in shelters (OR = 1.97; 95% CI: 1.45–2.69; $P < 0.0001$). Co-infections were detected in 38.4% of the positive samples. Dogs from Mexican states located on the Atlantic and the Pacific coast were significantly more at risk for *Ehrlichia* spp. and *Anaplasma* spp. infections than dogs from interior states. Dogs in Atlantic coastal states were more at risk for *Dirofilaria immitis* infection.

1. Introduction

Vector-borne diseases of companion animals are caused by parasites, bacteria, or viruses transmitted by the bite of hematophagous arthropods (mainly ticks and mosquitoes) (Beugnet & Marié, 2009). Worldwide, the most important canine vector-borne diseases (CVBD) include anaplasmosis, ehrlichiosis, borreliosis and dirofilariosis (Baneth et al., 2012). In North America, dirofilariosis, or heartworm disease caused by *Dirofilaria immitis*, is the most critical helminthosis affecting dogs due to its clinical severity (Bowman et al., 2009). The spirochete *Borrelia burgdorferi*, the causative agent of Lyme disease, is endemic in North America, with the main clinical manifestations in dogs being arthritis and nephritis (Littman et al., 2018). Anaplasmosis and ehrlichiosis (caused by *Anaplasma* spp. and *Ehrlichia* spp., respectively) are infections that can cause fever, hemorrhages, depression, myalgia, anorexia, and thrombocytopenia in affected dogs (Rikihisa, 1991). Regarding *Anaplasma* spp. pathogens, two species commonly infect dogs, *Anaplasma phagocytophilum*, the agent of the granulocytic anaplasmosis, transmitted by *Ixodes* spp. ticks, and *Anaplasma platys*, the agent of the thrombocytic anaplasmosis, transmitted by *Rhipicephalus sanguineus* (Snellgrove et al., 2020). Regarding *Ehrlichia* spp., three species are described in North America, *Ehrlichia canis* transmitted by *Rhipicephalus sanguineus* and *Ehrlichia ewingii* and *Ehrlichia chaffeensis* transmitted by *Amblyomma* spp. ticks. The serological tests cross-react between the *Anaplasma* and *Ehrlichia* species, respectively, and without a PCR identification, it is not possible to conclude on the species based on serology (Chandrashekar et al., 2010).

Nationwide Mexican prevalences of *E. canis*, *Anaplasma* spp., *B. burgdorferi* and *D. immitis* were reported by Ochoa (2003), Nuñez (2003) and Movilla et al. (2016). The seropositivity of dogs to *Anaplasma* spp. and *E. canis* was reported in Campeche (Rojero-Vázquez et al., 2017) and in Yucatán (Rodriguez-Vivas et al., 2005; Ojeda-Chi et al., 2019) while *B. burgdorferi* was serologically reported in dogs in Mexicali (García

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et al., 2008). Regarding *D. immitis*, there are some reports on its prevalence in dogs in Yucatán Peninsula from 2007 to 2018 (Bolio-Gonzalez et al., 2007; Caro-Gonzalez et al., 2011; Torres-Chable et al., 2018).

This survey aimed to determine the seroprevalence of *Ehrlichia* spp., *Anaplasma* spp., *B. burgdorferi* and *D. immitis* in dogs in veterinary clinics and shelters in different states of Mexico during 2019–2020 including a larger number of dogs than the previous surveys.

2. Materials and methods

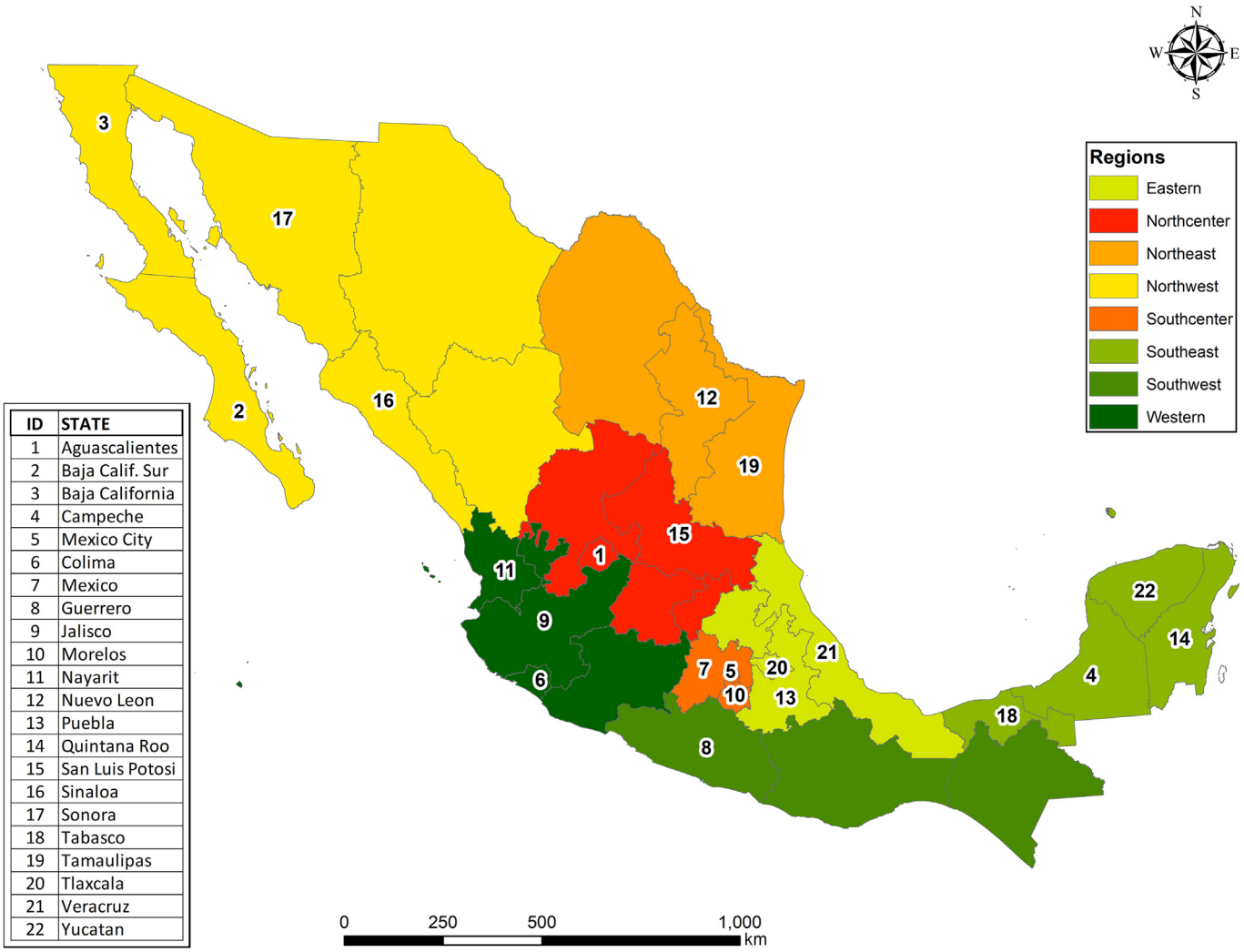
2.1. Source of data

One hundred and ten veterinary clinics and 53 dog shelters located in 22 out of the 32 Mexican states were included in this study. The sites were recruited in states from the eight national geo-economic regions, according to the methodology applied by Movilla et al. (2016): northwestern (Baja California, Baja California Sur, Sinaloa and Sonora), northeastern (Nuevo León and Tamaulipas), western (Colima, Jalisco and Nayarit), eastern (Puebla, Tlaxcala and Veracruz), northcentral (Aguascalientes and San Luis Potosi), southcentral (State of Mexico, Mexico City and Morelos), southeastern (Campeche, Quintana Roo, Tabasco and Yucatán) and southwestern (Guerrero) (Fig. 1). The 22 states included in the survey were distributed within the four quartiles of the distribution of annual rainfall by state, including the most dry (Baja California Sur, 179 mm/year) and the most wet (Tabasco, 4050 mm/year) states, based on the records on mean annual precipitation by states collected from 2000 to 2017 (SEMARNAT, 2019).

From December 2019 to February 2020, personnel at participating locations were instructed to test dogs meeting the following inclusion criteria: apparently healthy; older than 1 year; owned dogs with a history of regular outdoor activity and not having received anthelminthic treatments with macrocyclic lactones in 2 months nor ectoparasiticide treatments 1 month before collecting samples; shelter dogs should not have received anthelminthic treatments with macrocyclic lactones 2 months before or ectoparasiticide treatments 1 month before collecting the sample; consent from the owner to collect the sample, and, for care centers, the consent of the corresponding authority.

2.2. Diagnostic testing

Peripheral blood (0.5–1.0 ml) was collected from a vessel on dog's arm in EDTA tubes. According to the instructions by the manufacturer, whole blood samples from dogs were tested with the ELISA kit SNAP® 4Dx® Plus from IDEXX® (Westbrook, Maine, USA). The SNAP tests were performed on site immediately after blood sampling. This assay screens for the simultaneous qualitative detection of a circulating carbohydrate of *D. immitis* adult female antigen, and antibodies, both IgG and IgM, against proteins from *Ehrlichia* spp., *Anaplasma* spp. and *B. burgdorferi*. Reported sensitivity and specificity of in-clinic ELISA for detection of antibodies are 96.2 and 100% for *Ehrlichia* spp., 99.1 and 100% for *Anaplasma* spp., and



ID	STATE
1	Aguascalientes
2	Baja Calif. Sur
3	Baja California
4	Campeche
5	Mexico City
6	Colima
7	Mexico
8	Guerrero
9	Jalisco
10	Morelos
11	Nayarit
12	Nuevo Leon
13	Puebla
14	Quintana Roo
15	San Luis Potosi
16	Sinaloa
17	Sonora
18	Tabasco
19	Tamaulipas
20	Tlaxcala
21	Veracruz
22	Yucatan

Fig. 1. Mexican geo-economic regions and states included in the study. *Abbreviations*: Cd. de México, Mexico City; Edo. México, State of Mexico.

98.8 and 100% for *B. burgdorferi*. Reported sensitivity and specificity for detection of heartworm antigens are 99.2 and 100%, respectively (Chandrashekar et al., 2010). The tests were read visually.

2.3. Statistical analysis

Data from the participating veterinary clinics and dog shelters were organized by state and regional groups (northwest, northeast, west, east, northcentral, southcentral, southeast and southwest) along with information on age (> 1 to < 3, ≥ 3-year-old) (Evason et al., 2019) and sex of the patients. Chi-square test and logistic regression were applied using Minitab 18 (Minitab, Inc. State College, Pennsylvania, USA). The level of significance for differences between variables in the analysis was set at $P < 0.05$. Maps on the seroprevalence by region and state for each of the four pathogens in the study were generated using ArcMap 10.7.1 (ESRI. Redlands, California, USA).

3. Results

A total of 3522 diagnostic test results from the 110 clinics and the 53 dog shelters from 22 Mexican states were performed, which is the largest seroprevalence survey conducted in Mexico.

The presence of *Ehrlichia* spp. and *Anaplasma* spp. antibodies was detected in every region. The presence of *B. burgdorferi* antibodies was only detected in four states. *Dirofilaria immitis* antigen was identified in four regions, except the northcentral and southwestern ones (Tables 1 and 2).

3.1. Ehrlichia spp. infection

The seroprevalence to *Ehrlichia* spp. was 30.9% (1090/3522), nationwide. At the regional level it was ranked as follows: southwest (67.2%), southeast (51.3%), northwest (43.7%), west and northeast (33.2% and 29.8%, respectively), east (15.3%), and southcentral and northcentral (7.7% and 7.2%, respectively) (Table 1, Fig. 2).

The states with >30% of seropositive tests to *Ehrlichia* spp. were located in the Atlantic and Pacific coasts, while the states from the interior resulted in < 30% of positive tests (Fig. 2). The 8 states from the interior averaged 8.2% (105/1280) of positive tests, which is significantly different from the average of 46.2% (460/995) for the Atlantic states ($\chi^2 = 433$, $P < 0.0001$) and the average of 42.1% (525/1247) for the Pacific states ($\chi^2 = 387$, $P < 0.0001$).

Dogs 3-year-old or older presented a significantly higher percentage of positive results to *Ehrlichia* spp. ($\chi^2 = 13.7$, $P < 0.0001$) compared to dog under 3-year-old, 32.1% (720/2241) *vs* 25.5% (233/915), respectively (Table 2). Neither sex nor origin of the dogs (veterinary clinics *vs* shelters) appeared to be risk factors (Table 3).

3.2. Anaplasma infection

The seroprevalence for *Anaplasma* spp. in Mexico was 14.6% (515/3522), being highest in the southeast and southwest (26.4% and 32.1%, respectively) (Table 1). In contrast, the lowest seroprevalence was detected in the southcentral region (2.0%) (Fig. 3).

The states with > 11% of positive tests to *Anaplasma* spp. were

Table 1
Percent positive test results in dogs by regions and states for *Ehrlichia* spp., *Anaplasma* spp., *Borrelia burgdorferi* and *Dirofilaria immitis*.

State	*Ehrlichia* spp.	*Anaplasma.* spp.	*Borrelia burgdorferi*	*Dirofilaria immitis*
	(*n*/*N*)	(*n*/*N*)	(*n*/*N*)	(*n*/*N*)
Northwest				
Baja California	51.3 (81/158)	32.9 (52/158)	0 (0/158)	0 (0/158)
Baja Califonia Sur	40.2 (68/169)	18.9 (32/169)	0 (0/169)	3.0 (5/169)
Sinaloa	52.3 (68/130)	16.9 (22/130)	0 (0/130)	9.2 (12/130)
Sonora	32.7 (52/159)	8.8 (14/159)	0 (0/159)	3.8 (6/159)
Regional mean	43.7 (269/616)	19.5 (120/616)	0 (0/616)	3.7 (23/616)
Northeast				
Nuevo León	25.8 (40/155)	7.1 (11/155)	0 (0/155)	1.3 (2/155)
Tamaulipas	33.5 (57/170)	11.8 (20/170)	1.2 (2/170)	37.1 (63/170)
Regional mean	29.8 (97/325)	9.5 (31/325)	0.6 (2/325)	20.0 (65/325)
West				
Colima	47.3 (79/167)	27.5 (46/167)	0.6 (1/167)	0 (0/167)
Jalisco	19.4 (32/165)	6.1 (10/165)	0 (0/165)	0 (0/165)
Nayarit	32.7 (53/162)	17.9 (29/162)	0 (0/162)	0.6 (1/162)
Regional mean	33.2 (164/494)	17.2 (85/494)	0.2 (1/494)	0.2 (1/494)
East				
Puebla	0 (0/145)	0 (0/145)	0 (0/145)	0 (0/145)
Tlaxcala	0 (0/114)	0 (0/114)	0 (0/114)	0 (0/114)
Veracruz	39.2 (65/166)	19.3 (32/166)	0 (0/166)	12.0 (20/166)
Regional mean	15.3 (65/425)	7.5 (32/425)	0 (0/425)	4.7 (20/425)
Northcentral				
Aguascalientes	4.0 (6/149)	0.7 (1/149)	0 (0/149)	0 (0/149)
San Luis Potosí	10.1 (16/158)	10.8 (17/158)	0 (0/158)	0 (0/158)
Regional mean	7.2 (22/307)	5.9 (18/307)	0 (0/307)	0 (0/307)
Southcentral				
Ciudad de México	3.1 (5/163)	0 (0/163)	0 (0/163)	0 (0/163)
México	0.9 (2/231)	0 (0/231)	0 (0/231)	0 (0/231)
Morelos	21.8 (36/165)	6.7 (11/165)	0 (0/165)	0.6 (1/165)
Regional mean	7.7 (43/559)	2.0 (11/559)	0 (0/559)	0.2 (1/559)
Southeast				
Campeche	66.0 (103/156)	39.7 (62/156)	0 (0/156)	10.9 (17/156)
Quintana Roo	37.7 (63/167)	24.6 (41/167)	0 (0/167)	12.0 (20/167)
Tabasco	55.3 (94/170)	19.4 (33/170)	0.7 (1/170)	16.5 (28/170)
Yucatán	47.0 (78/166)	22.9 (38/166)	0 (0/166)	4.2 (7/166)
Regional mean	51.3 (338/659)	26.4 (174/659)	0.2 (1/659)	10.9 (72/659)
Southwest				
Guerrero	67.2 (92/137)	32.1 (44/137)	0.7 (1/137)	0 (0/137)
Country mean	30.9 (1090/3522)	14.6 (515/3522)	0.1 (5/3522)	5.2 (182/3522)

Abbreviations: *n*, number positive; *N*, number tested.

Table 2
Percent positive test results in dogs by age, sex, and dog origin for *Ehrlichia* spp., *Anaplasma* spp., *Borrelia burgdorferi* and *Dirofilaria immitis*.

Variable	No. of seropositive dogs (%)				
	Total	*Ehrlichia* spp.	*Anaplasma* spp.	*B. burgdorferi*	*D. immitis*
Age					
< 3 years ($n = 915$)	276 (30.2)[a]	233 (25.5)[a]	131 (14.3)[a]	3 (0.3)[a]	49 (3.9)[a]
≥ 3 years ($n = 2241$)	829 (37.0)[b]	720 (32.1)[b]	320 (14.3)[a]	2 (0.1)[a]	133 (5.9)[b]
Sex					
Female ($n = 1939$)	682 (35.2)[a]	508 (29.9)[a]	286 (14.7)[a]	1 (0.1)[a]	91 (4.7)[a]
Male ($n = 1489$)	552 (37.1)[a]	480 (32.2)[a]	217 (14.6)[a]	4 (0.3)[a]	86 (5.8)[a]
Facility type					
Clinic ($n = 1648$)	596 (36.2)[a]	488 (29.6)[a]	221 (13.4)[a]	4 (0.2)[a]	114 (6.9)[a]
Shelter ($n = 1874$)	676 (36.1)[a]	602 (32.1)[a]	294 (15.7)[a]	1 (0.1)[a]	68 (3.6)[b]

Note: Different superscript letters indicate significant differences between variable states, $P < 0.05$.

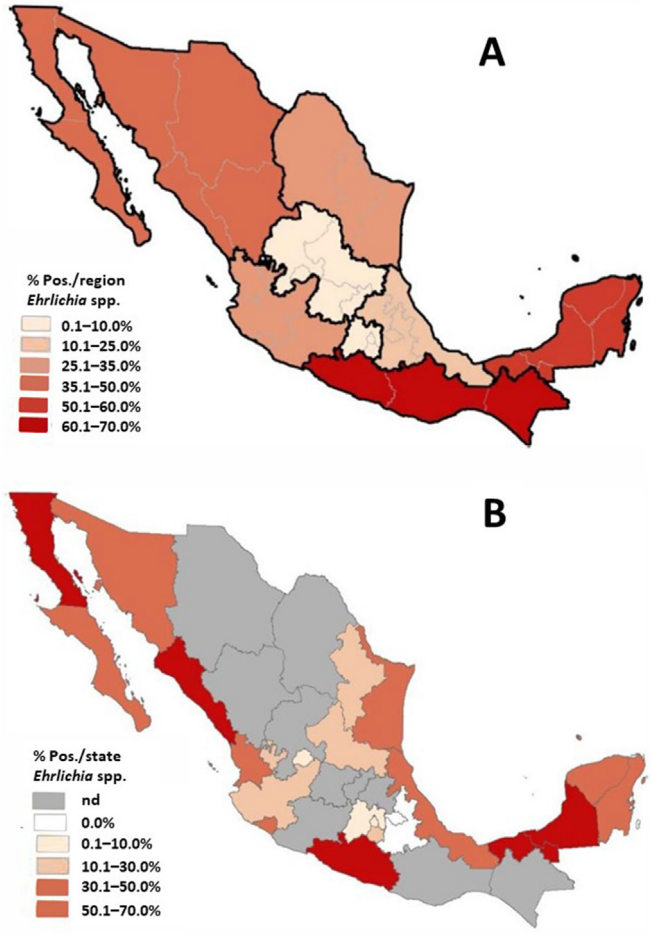


Fig. 2. Seroprevalence of *Ehrlichia* spp. in dogs grouped according to the percentage of positive tests by region (**A**) and state (**B**).

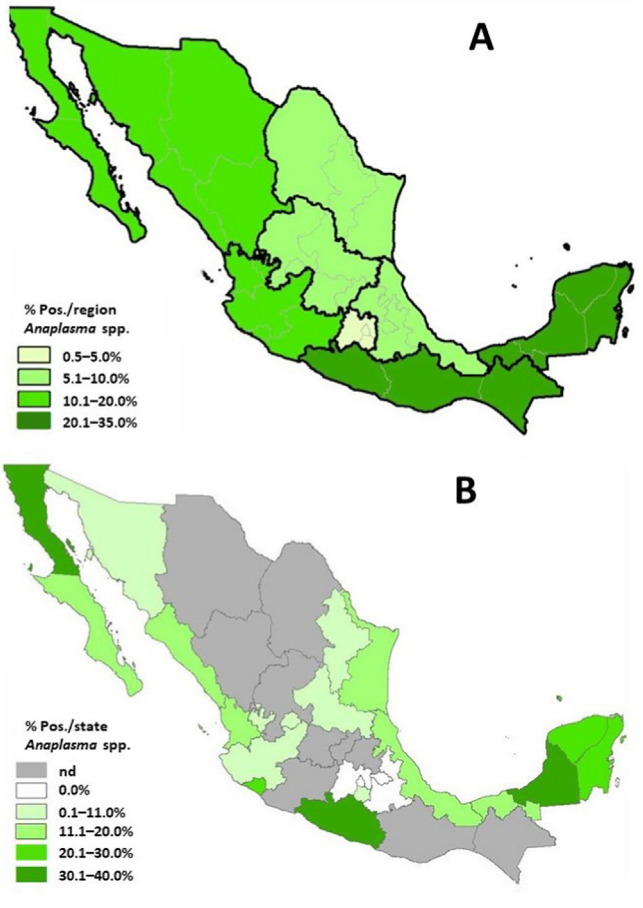


Fig. 3. Seroprevalence of *Anaplasma* spp. in dogs grouped according to the percentage of positive tests by region (**A**) and state (**B**).

located in the Atlantic and Pacific coasts of the country (Fig. 3). The eight interior states averaged 3.13% (40/1280) of positive tests to *Anaplasma* spp., significantly different from the 22.7% (226/995) of Atlantic states ($\chi^2 = 208$, $P < 0.0001$) and from the 19.9% (249/1247) of Pacific states ($\chi^2 = 177$, $P < 0.0001$).

No age effect and no sex effect were observed. The origin of dogs, veterinary clinics *vs* shelters, did not appear to be a risk factor (Table 3).

3.3. *Borrelia burgdorferi* infection

Percent positive test results to *B. burgdorferi* were reported for only five cases out of 3522 samples (0.1%) (Table 1). There were two cases in the northeastern (2/325; 0.6%), one in the western (1/494; 0.2%), one in the southeastern (1/659; 0.1%), and one in the southwestern (1/137; 0.7%) regions (Fig. 4). Due to the low seroprevalence, no risk factor was found (Table 3).

3.4. *Dirofilaria immitis* infection

For *D. immitis*, the nationwide seroprevalence was 5.2% (182/3522) (Table 1). The percent of positive test results were highest in the northeast (20.0%) compared to the other regions. The lowest percentage was detected in the southcentral (0.2%) and western (0.2%) regions. No positive results were reported for the northcentral (0.0%) and southwestern (0.0%) regions (Fig. 5).

The states with > 10% of positive tests to *D. immitis* were located in

Table 3
Risk factors for positivity to CVBD agents.

Dependent variable risk factor	OR	95% CI	*P*-value
Seropositivity to one of the tested CVBD agents			
Age	1.36	1.15–1.60	<0.0001
Sex	1.09	0.94–1.25	0.251
Veterinary clinic *vs* dog shelter	1.00	0.87–1.15	0.955
Positivity to *Ehrlichia* spp.			
Age	1.39	1.17–1.65	<0.0001
Sex	1.11	0.96–1.29	0.145
Veterinary clinic *vs* dog shelter	0.89	0.77–1.03	0.108
Positivity to *Anaplasma* spp.			
Age	1.00	0.80–1.24	0.980
Sex	0.99	0.81–1.19	0.885
Veterinary clinic *vs* dog shelter	0.83	0.69–1.01	0.056
Positivity to *B. burgdorferi*			
Age	0.27	0.05–1.63	0.154
Sex	5.22	0.58–6.75	0.140
Veterinary clinic *vs* dog shelter	4.56	0.51–0.81	0.175
Positivity to *D. immitis*			
Age	1.80	1.21–2.68	0.004
Sex	1.24	0.92–1.69	0.156
Veterinary clinic *vs* dog shelter	1.97	1.45–2.69	0.0001

Notes: Levels by factor: Age (≥ 3-year-old *vs* < 3-year-old); Sex (male *vs* female); Facility type (clinic *vs* shelter).
Abbreviations: OR, odds ratio; CI, confidence interval.

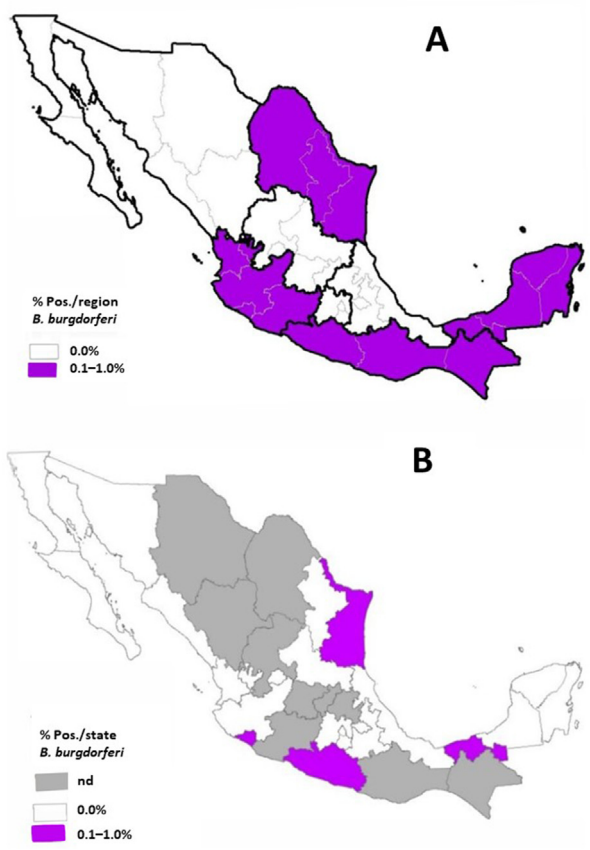


Fig. 4. Seroprevalence of *Borrelia burgdorferi* in dogs grouped according to the percentage of positive tests by region (**A**) and state (**B**).

the Atlantic coast (Fig. 5). The six Atlantic states averaged 15.5% (155/995) of positive tests to *D. immitis*, significantly different from the average of 1.0% (27/2527) from the other 16 states in the study (χ^2 = 306.7, $P < 0.0001$).

Animals 3 years-old or older presented a significantly higher percentage of positive results to *D. immitis* when compared to dogs under 3 years-old, 5.9% (133/2251) *vs* 3.9% (49/1271), respectively ($\chi^2 = 6.9$, $P = 0.008$). Dogs tested at clinics presented a significantly higher percentage of positive results when compared to those tested at shelters, 6.9% (114/1648) *vs* 3.6% (68/1874) (Table 2) ($\chi^2 = 19.35$, $P < 0.0001$) (see also Table 3).

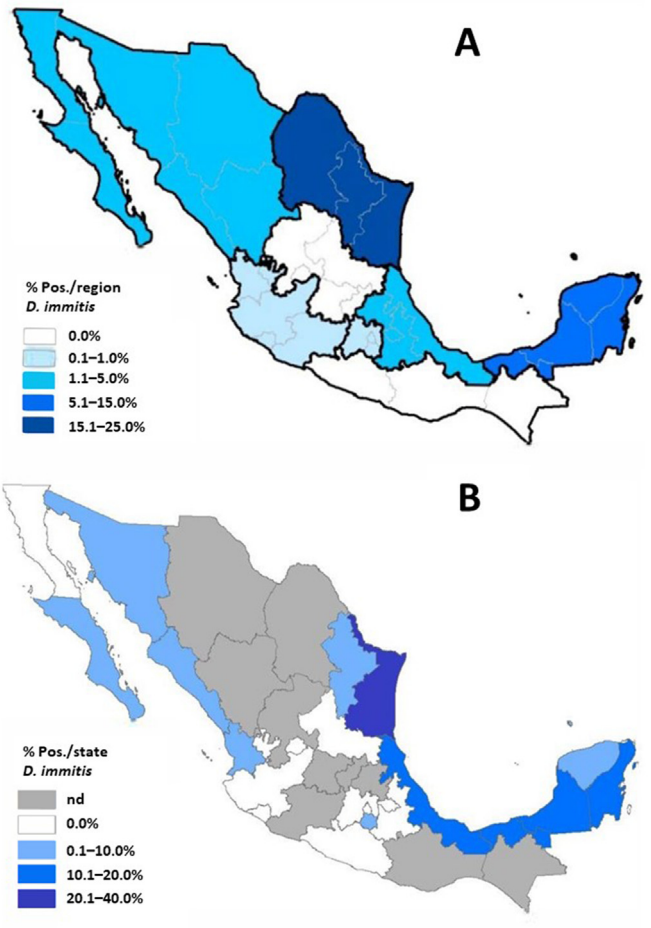


Fig. 5. Seroprevalence of *Dirofilaria immitis* in dogs grouped according to the percentage of positive tests by region (**A**) and state (**B**).

3.5. Co-infections

Co-infections, defined as a positive result to two or more agents in the same sample (Evason et al., 2019), were detected in 38.4% (488) of the 1272 positive samples. The following co-infections were detected: *Anaplasma* spp. and *B. burgdorferi* ($n = 3$); *Anaplasma* spp. and *D. immitis* ($n = 36$); *Anaplasma* spp. and *E. canis* ($n = 420$); *B. burgdorferi* and *D. immitis* ($n = 1$); *B. burgdorferi* and *E. canis* ($n = 3$); *E. canis* and *D. immitis* ($n = 89$). There were a few cases including 3 different pathogens: *Anaplasma* spp., *B. burgdorferi* and *D. immitis* ($n = 1$); *Anaplasma* spp., *B. burgdorferi* and *E. canis* ($n = 2$); *Anaplasma* spp., *E. canis* and *D. immitis* ($n = 29$).

4. Discussion

This survey provides an assessment of the geographical distribution of four CVBD in Mexico. To the best of our knowledge, it is the largest survey including apparently healthy dogs conducted in Mexico. Seroprevalence of *Anaplasma* spp. was 14.6% (515/3522) compared to 9.9% (169/1706) observed by Movilla et al. (2016). It was similar for *Ehrlichia* spp. (most likely *E. canis*) in the present study (i.e. 30.9% (1090/3522)) compared to Movilla et al. (2016) (30.8% (526/1706)), as well as for

B. burgdorferi, 0.1% (5/3522) compared to 0.2% (4/1702) and *D. immitis*, 5.2% (182/3522) compared to 5.3% (91/1706). The close similarity in the findings from these two studies, conducted several years apart shows the stability of the prevalences of dog infections since at least 5 years. Based on molecular demonstrations of the presence of *E. canis* in Mexico, the authors considered that the seroconversions observed in dogs were due to *E. canis* infection.

The seroprevalences of *Ehrlichia* spp. and *Anaplasma* spp. observed in this study show significant differences between coastal and inner states, which is consistent with conditions that favor the survival and reproduction of the vector *R. sanguineus* (Pujalte et al., 2018).

The percent of positive test results to *B. burgdorferi* was extremely low, with only five positive cases out of 3522 samples tested nationwide (0.1%). Lyme borreliosis is endemic in temperate and cold climates where the *Ixodes* vector ticks are prevalent, whereas hot climates like in Mexico are less suitable for *Ixodes* spp.

Regarding *D. immitis*, northwestern, northeastern, eastern, and southeastern regions presented higher seroprevalence than the other regions. Another study performed in the Yucatán Peninsula, in the southeastern region, determined a prevalence of 8.3% for *D. immitis* (Bolio-Gonzalez et al., 2007), like the 7.9% reported by Movilla et al. (2016) for the southeastern region and the 10.9% from the present study. When analyzing the data at a state level, this study found that the six Atlantic states averaged 15.6% of positive tests to *D. immitis*, much higher than the average of 1.0% from the other 16 states. Previous studies have shown a higher prevalence in dogs sampled in the Gulf of Mexico's area than in those tested in other Mexican regions, a potential explanation being the abundance of certain mosquito vectors in this specific region (Movilla et al., 2016).

Regarding age, 3-year or older dogs presented a significantly different percentage of positive results for *E. canis* and D. immitis than younger animals. In previous surveys conducted in Mexico, older age was also reported as a risk factor for a higher *E. canis* and *D. immitis* prevalence, indicating that older dogs have been exposed for more extended periods to mosquito or tick bites (Bolio-Gonzalez et al., 2007; Caro-Gonzalez et al., 2011; Movilla et al., 2016; Torres-Chable et al., 2018).

The co-infection between *Anaplasma* spp. and *Ehrlichia* spp. was significantly more common than the others, which is in favor of a common vector, *R. sanguineus*, and is an indirect proof that the species is *Anaplasma platys* (Snellgrove et al., 2020). *Dirofilaria immitis* was the only pathogen showing significantly higher seroprevalence in dogs sampled in veterinary clinics (6.9%) when compared to shelter dogs (3.6%), contrary to the general assumption that dogs under clinical supervision would present a lower prevalence (Self et al., 2019). It may be related to higher mosquito control measures in shelters, but further research is needed to investigate this unexpected result.

5. Conclusions

The overall trends in this survey were consistent with previous studies conducted in Mexico. The regional distribution indicated a significantly higher risk of being infected on the Atlantic and Pacific coastal states than in the center of the country. It is most probably related to climatic conditions most favorable to tick vectors and mosquitoes on the coasts when the interior part seems too hot and dry.

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Ethical approval

Blood samples were collected according to protocols approved by the Boehringer Ingelheim Animal Health Institutional Animal Care and Use Committee. Those responsible for the animals were informed about these protocols. All owners and care centers read and approved the study protocol and the inclusion of their animals.

CRediT author statement

Felipe Bedoya: Conceptualization, Writing - original draft, Writing - review & editing. Frederic Beugnet: Conceptualization, Writing - original draft, Writing - review & editing. Emilia Tobias: Conceptualization, Writing - original draft, Formal analysis, Writing - review & editing. Erick Garcia-Mendizabal: Conceptualization, Writing - original draft, Investigation, Writing - review & editing. Samantha Hay-Parker: Conceptualization, Writing - original draft, Investigation, Writing - review & editing. Nancy Montes: Conceptualization, Writing - original draft, Investigation, Writing - review & editing. Jose Uribe: Conceptualization, Investigation, Writing - review & editing. Enrique Mondaca: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: this research was funded by Boehringer Ingelheim Animal Health, Mexico, of which all the authors were employees or under contract.

Data availability

All data generated or analyzed during this study are included in this published article. Raw data are available from the corresponding author upon reasonable request with no time embargo.

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