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Y.W.S.O. and G.A.V.H. conceived the study; Y.W.S.O., O.C.E. and C.M.I. designed the study; Y.W.S.O. collected the samples; V.D.F.G., M.H.C.A., A.E.A.S analysed data; O.C.E. and C.M.I. performed experiments; G.A.V.H., R.M.R. and V.D.F.G wrote the manuscript; C.V.V. F.G.V.D. G.A.V.H. and M.H.C.A translated and revised the paper.

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Seroprevalence of Equine Piroplasmosis under Tropical and Subtropical Conditions in Mexico

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Abstract:
The aim of this study was to determine the prevalence of *Theileria equi* and *Babesia caballi* and the risk factors for of the diseases in horses under subtropical and tropical conditions in Mexico. During spring 2015, horses from Coahuila (n=100) and Chiapas (n=75), showing characteristic clinical symptoms were selected for sampling. Antibodies to *T. equi* and *B. caballi*, were detected throughout cELISA. Subtropical conditions with 2% of *T. equi,* 0% of *B. caballi* and tropical conditions with 80.82% of *T. equi*, and 31.50% of *B. caballi.* No differences (P>0.05) occurred between the sexes, a trend was observed as horses were younger (51% and 36%; P=0.06). The most prevalent hemoparasite occurred under tropical conditions with *T. equi*. Effective diagnostic systems and optimization of preventive and control programmes must be emphasized to reduce the risk of infection by the pathogens.

INTRODUCTION

Equine Piroplasmosis (EP) caused by *Theileria equi* and *Babesia caballi* is considered the highest significant tick-borne disease of equids (Sumbria *et al*., 2015), affecting horses, donkeys, mules, and zebras (OIE, 2008; Wise *et al.*, 2013). The disease is characterized by fever, anorexia, discomfort, jaundice, anemia, hemoglobinuria, increased pulse rates and respiration, constipation followed by diarrhea, tachycardia, and sometimes death (Scoles and Ueti, 2015). An infected horse recovered from an acute phase could become a carrier for life and serve as a source of iatrogenic-tick infection-transmission. Despite an apparent healthy look, the chronic form of EP can result in nonspecific signs as lethargy, partial anorexia, weight loss, and poor performance (Wise *et al.*, 2013). Therefore, one of the factors that influences the maintenance and spread of the infection is the movement of carrier animals to places where there are clinically healthy animals (Scoles and Ueti, 2015).

The primary tests used for qualifying horses for mobility and importation are the Indirect Fluorescent Antibody test (IFA) and the competitive Enzyme-linked Immunosorbent Assay (cELISA) (OIE, 2008), which may be used alone or in combination with clinical signs to diagnose the disease. The distribution of piroplasmosis depends on the presence of ixodid tick vectors (Diallo *et al*., 2016). However, Mexico is not considered an endemic area because the OIE routinely receives no information regarding the distribution of piroplasmosis (Wise *et al.,* 2013). Notwithstanding, Cantú-Martínez *et al.* (2012) reported EP in the northeastern part of Mexico; therefore, the aim of this study was to determine the prevalence of *T. equi* and *B. caballi* infection by cELISA and the risk factors associated with the occurrence of the disease in horses from Torreón, Coahuila (subtropical area) and Villa Corzo, Chiapas (tropical area) in Mexico.

MATERIALS AND METHODS

Study areas

Torreón, Coahuila (the subtropical area) is located between the coordinates 25°32′40″ N-103°26′30″ W, in the northern-central part of México at an altitude of 1,120 masl. The climatic conditions are hot, dry and arid. The average annual temperature ranges to 0°-40°C in the coolest month and the hottest month. The rainfall is from 39-249 mm per year, with an area of 1,947.7 km^2. Villa Corzo, Chiapas (the tropical area) is located between the coordinates 16°14′00″ N-93°16′09″ W, in southern Mexico with surrounding mountains reaching 2000 m. The climatic conditions are warm and sub-humid, with summer rains (average of 1,100 mm) and an average temperature of 25.8°C. The municipality occupies an area of 2,631 km^2 and is situated in a valley at an altitude of 600 masl (Figure 1).

Sample collection and ethical consideration

During spring 2015, horses from Coahuila (n=100) and Chiapas (n=75), showing characteristic clinical symptoms such as jaundice, lethargy, partial anorexia, weight loss, and poor performance were selected for sampling. Management of the units used in this study was in strict accordance with the Mexican Legislation of Animal Health (DOF-07-06-2012). Blood samples were collected by puncture of the jugular vein using vacuum tubes and disposable needles and centrifuged at 3,000*g* for 10 min. The serum was isolated and stored at -20º C until tested.

Serology

The cELISA test was carried out using commercially available kits (VMRD, Inc; USDA - 501B.20 and 501A.20) to detect antibodies to *T. equi* and *B. caballi*. Briefly, 50 µl of diluted control and 175 of equine serum samples were pipetted into the wells of the microplates coated with *T. equi* and *B.caballi* antigens, respectively, and incubated for 30 min at room temperature. Plates were washed and the primary antibody was added to each well in the plate. The plates were incubated for an additional 30 min and washed, after which the secondary antibody conjugated with Horseradish peroxidase was added. After incubating the plates for 30 min, 50 μl of substrate solution was added to each well. The plates were again incubated for 15 min, and their reaction was stopped by adding stop solution and immediately read on a plate reader (RT-6000 Microplate Reader, RAYTO, Nanshan, Shenzhen, China) at a wavelength of 630 nm. Interpretation of the results was done as described by the protocols of Jaffer *et al.* (2010) and OIE (2008). Serological procedures were performed at the Biomedical Research Center of the Autonomous University of Coahuila.

Statistical analysis

The total prevalence between localities, sexes, and age groups is expressed in percentages. To determine the statistical difference in the prevalence of infection, the chi-square test was used. The analyses were performed using the statistical package MYSTAT 12, Version 12.02.00-2007, assessment of significant differences was considered a $P \leq 0.05$ (I_{95}).

Fig. 1. Location of the sample collection areas (left: subtropical, right: tropical).

RESULTS

The level of seropositivity was Coahuila, 2% for *Theileria equi,* and 0% for *Babesia caballi* and Chiapas; 80.82% for *Theileria equi*, and 31.50% for *Babesia caballi* (Table 1). In Chiapas, 12.32% of horses tested positive for both hemoparasites. We found an overall prevalence of 42% with a low prevalence (P<0.01) under subtropical conditions (Torreón, Coahuila; 2%), while a high prevalence under tropical conditions (Villa Corzo, Chiapas; 95%).

Table 1. Prevalence of horses testing positive for antibodies for *T. equi* and *B. caballi* using c-ELISA according to location, sex, and age in Mexico (n=175).

	Number of horses (n)	Seropositives (n)	Clean infection (n)	*T. equi* (n)	*B. caballi* (n)	Mixed (n)
Location						
Torreón, Coahuila	100	2[a]	98[a]	2[a]	0[a]	0[a]
Villa Corzo, Chiapas	75	71[b]	4[b]	57[b]	23[b]	9[a]
Age						
≤5	65	33[c]	32[a]	25[a]	11[a]	3[a]
>5	110	40[d]	70[a]	34[a]	12[a]	6[a]
Sex						
Male	49	21[a]	28[a]	16[a]	6[a]	1[a]
Female	73	29[a]	44[a]	23[a]	11[a]	5[a]
Geldings	53	23[a]	30[a]	20[a]	6[a]	3[a]
Total	175	73	102	59	23	9

Hemoparasites (+8) = 10.66%.
Different superscripts indicate a statistical difference within column and variable (a,b P<0.05; c,d P=0.06).

DISCUSSION

Such a very low prevalence under subtropical conditions could be due to the absence of a vector for this microorganism. Horses from Torreón, Coahuila are commonly moved throughout the country for sporting events. We suppose that this situation could have aided the spread of the disease in a mechanical way or by direct contact with the vector. Furthermore, seroconversion indicates that animals have been exposed to the causal agents in any possible way. Torreón, Coahuila is a region similar to those of the study area of Motloang et al. (2008) where the prevalence was up to 48% for *B. caballi* and 98% for *T. equi*, a geographical area inhabited by the *Rhipicephalus evertsi* tick, while in areas near to Torreón, *Rhipicephalus sanguineus* s.l. is the most prevalent tick (Castillo-Martínez *et al.*, 2015), although its role in the transmittance of the equine piroplasmosis is still unclear (Scoles and Ueti 2015). Medrano-Bugarini et al. (2019), reported a prevalence of 5.9% for *T. equi* and *B. caballi*, respectively, in soft ticks *Otobius megnini* from horses living in Ciudad Juárez, Chihuahua. In the study area, the presence of *O. megnini* parasitizing horses was reported by González-Álvarez et al. (2018);

however, the role of this tick as potential vector of the aforementioned pathogens needs to be investigated.

The high prevalence in Chiapas (95%), could be explained because of the tropical climate of this region, which benefits the survival of ticks from the *Boophilus*, *Dermacentor*, *Haemaphysalis, Hyalomma*, *Ixodes* and *Rhipicephalus*, genera which could be vectors of the disease (Scoles and Ueti 2015). Our results agree with those reviewed by Scoles and Ueti (2015), they state that in endemic regions the infection percentages range from 60-90% of animals infected with one or both hemoparasites. These results could be explained by both climatic conditions and husbandry practices. First, temperature, humidity, and rainfall are determinant factors that influence tick distribution (Alsaad *et al.*, 2012). Secondly, horses housed outdoors, working in rangeland or coexisting with livestock are more likely to get infected by ticks carrying the disease (Hawkins *et al.*, 2015). The presence of wildlife, including some bird species, could be playing another important role in the tick vectors and piroplasm distribution. Grazing or spending all day in the field, is a risk factor for acquiring Equine Piroplasmosis (Moretti *et al.*, 2010), and this is a common practice in horses of Chiapas; furthermore, sanitary care, stalling and working conditions are inadequate to prevent infection.

In our study, most of the infection cases were 80% for *T. equi* while for *B. caballi* was 31% (P<0.05). Previous reports about the prevalence also for *B. caballi* 82.8% (Asgarali *et al*., 2007) and 50.2% (Rosales *et al.*, 2013) and *T. equi* 20.3% (Gallusová *et al*., 2014); 88.5% (Posada-Guzmán *et al.*, 2015) and 78.3% (Vieira *et al*., 2013), greatly differ. There are some conditions that can help to explain our differences; for example, *T. equi* and *B. caballi* share the same vector ticks (Mahmoud *et al.*, 2016). However, several factors can affect the epidemiology of the disease, such as the vectorial competence, behavior, abundance, and distribution (Scoles and Ueti, 2015). Other explanations could be related to management practices or due to the vector prevalence for both microorganisms, and finally, Malekifard et al. (2014) stated that *T. equi* is more common and pathogenic than *B. caballi*, and highly involved in the development of clinical signs, besides, the infection is not eliminated when the animal has recovered in a natural way or after treatment (Hussain *et al.*, 2014). In this study, samples were obtained from animals with clinical signs. Therefore, if *B. caballi* is less pathogenic, we could have left out animals without clinical signs but still infected during sampling, affecting the prevalence result. Furthermore, false negative or false positive results could be obtained due to cross-reactions. This is due to a weakening of the specific immune response, as well as the lack of antibody determination in the carriers due to the disease´s long-term (Bahrami *et al.*, 2014).

The percentage of mixed infections in our study was higher (12.3%) with respect to those obtained by Malekifard et al. (2014), and Wang *et al.* (2014) of 0.8% and 7.6%, respectively, although quite similar to the 12.3% and 13% reported by Hussain et al. (2014) and Rosales et al. (2013) respectively. However, there are other studies reporting prevalence as high as 50% (Vieira *et al.,* 2013) and 62.3% (Posada-Guzmán *et al.*, 2015). This prevalence of mixed infections could be explained by the presence of vectors for both pathogens, associated with poor sanitary conditions and husbandry practices.

No statistical differences were found between the sexes (P>0.05; Table 1). However, the prevalence in horses <5 years old was 51% and 36% in horses >5 years old (P=0.06). These results are in contrast with those obtained by Asgarali et al. (2007), who observed a high prevalence in horses >5 years (79%), and they mention that, in terms of age, it is not clear why there was a higher seropositivity in the older animals compared to the younger age group. It is important to consider that *T. equi* persists as a life-long infection, whereas the expected persistence of *B. caballi* in its host is 1.5 years on average. Consequently, a positive result for *T. equi* may reflect an actually infected animal or may be a chronic and long-persisting status, without symptoms. In our study, sampling was directed to animals with symptoms of the disease. We could have omitted positive horses >5 years old, and this may be the reason for our high seropositivity reported in younger horses. In fact, infection caused by *B. caballi* is usually controlled by the animal´s immune system, with a spontaneous tendency to clean or self-limit the infection in a natural manner or after a treatment, the period in which animals can be carriers of the microorganism for up to four years (Alsaad *et al.*, 2012). As well, it has been suggested that the infection can be lifelong and, although the pathogen might be present, it can be undetectable (Rosales *et al.*, 2013), stress and other factors can cause severe immunosuppression, resulting in the manifestations of the disease (Asgarali *et al.*, 2007).

The prevalence of infected females, males, and geldings was similar (39.7%, 43.0%, and 43.4%, respectively; P>0.05). Our results are in line with that reported by Kamyingkird et al. (2014) where the results were not different in relation to gender but differ from the report of Moretti *et al.* (2010) who found the highest prevalence for females, since males do not graze in order to breed control, and as a result, males are less exposed to tick vectors, while females, males and geldings in this study were raised under similar conditions.

CONCLUSION

Our results showed a very low *T. equi* seroprevalence in the subtropical area, while in the tropical area, there was observed a very high prevalence, with *T. equi* as the most prevalent hemoparasite. Diagnosing infected animals is crucial in order to provide the accurate treatment to prevent further hemoparasite transmission to susceptible animals. Additional research is required to know the prevalence in apparently healthy animals, as well as collect and identify ticks to characterize potential vectors by molecular techniques.

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CONFLICT OF INTEREST

Authors declare have no conflicts of interest.

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