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Prevalence of Antibodies Against *Babesia bigemina* and *B. bovis* in White-Tailed Deer (*Odocoileus virginianus texanus*) in Farms of Northeastern Mexico

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Abstract: The objective of the study was to estimate the prevalence of antibodies against *Babesia bovis* and *B. bigemina* in sera from 165 white-tailed deer in northeastern Mexico. Sera were collected in spring of 2003 in 5 mixed cattle and deer farms from the states of Coahuila and Nuevo Leon, Mexico. Animals were live-trapped by the drop net technique and once immobilized blood samples were obtained by jugular venipuncture using vacuum tubes. Antibodies were detected by the indirect immunoflourecent technique using blood smears with parasited erythrocytes for *B. bovis* or *B. bigemina*. Seropositivity in the deer was relatively high, because 87% (144/165) of the sera was positive. The seroprevalences for *B. bigemina* and *B. bovis* were 53% (88/165) and 75% (124/165). The sex of the deer was a risk factor for seropositivity to *B. bovis* but no for *B. bigemina*. The risk of infection with *B. bovis* was 2.4 times greater for females than for males. In conclusion, a high prevalence of antibodies against *B. bigemina* and *B. bovis* in the white-tailed deer was found at northeastern Mexico. More studies are recommended with the aim of detecting the role of deer in the transmission of babesiosis to cattle.

Key words: Babesiosis, prevalence, white-tailed deer, Mexico

INTRODUCTION

One of the most important diseases for the cattle industry is babesiosis, where ticks of the generous *Ixodes* sp. and *Boophilus* sp. play and important role in the transmission of the disease, because they parasite diverse species of domestic and wild animals (Park *et al*., 1966; Kistner and Hayes, 1970; Soulby, 1988; Quiroz-Romero, 1990). Babesiosis is a parasitic disease caused by different species of protozoa of the generous *Babesia*, which is characterized mainly by producing high fever, progressive anemia, haemoglobinuria and jaundice; causing economical losses to cattle farmers (Soulby, 1988). This disease is gaining interest as an emergent zoonotic disease (Homer *et al*., 2000). It is a worldwide-distributed disease where ticks are present, but most important in the tropical and subtropical regions.

There are 71 species of *Babesia* that parasite different animals such as: sheep, horses, cattle, pigs, dogs, rodents and some wild ruminants such as red deer, white-tailed deer and also human beings (Levine, 1973; Soulby, 1988; Quiroz-Romero, 1990; Greene and Graig, 1993). Specific *Babesias* infect specific mammals; for example *B. bovis* infect cattle and *B. odocoili* to white-tailed deer.

In Mexico the most important species of *Babesia* affecting cattle are *B. bovis* and *B. bigemina* (Quiroz-Romero, 1990). These 2 species, however, are capable of infecting blood erythrocytes of white-tailed deer (Levine, 1973). Although clinical signs have not been observed, some papers reported deer parasited with one of these 2 species of *Babesia*. Therefore, it is possible that deer can be act as reservoirs of this important disease (Park *et al*., 1966; Kistner and Hayes, 1970).

The northeastern of Mexico include the states of Coahuila, Nuevo Leon and Tamaulipas and constitute the main area where white-tailed deer (*Odocoileus virginianus texanus*) hunting farms are located and where it is common to find them mixed with cattle and sharing the pasture. It might be possible then that the white-tailed deer could be a host of *B. bovis* or *B. bigemina* causing babesiosis in cattle. White-tailed deer could also kept tick populations and be a possible reservoir of diseases transmitted by haemoparasites (Park *et al*., 1966; Kistner and Hayes, 1970; Quiroz-Romero, 1990).

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The objective of this study was to estimate the prevalence of antibody activity in white-tailed deer sera against *Babesia bovis* and *B. bigemina* in 5 farms in northeastern Mexico.

MATERIALS AND METHODS

Localization and climate: The study was carried out in March 2003 in 5 farms in the northeastern Mexican states of Nuevo Leon and Coahuila. The farms produced cattle for meat and white-tailed deer (*Odocoileus virginianus texanus*) for hunting. The climate of the region is subhumid tropic with average temperature of 14-24°C and annual rainfall of 200-800 mm.

Animal management: Deer and cattle were fed base on natural pastures of the region with occasional supplementation in the critical months and the deer did not receive any other special management.

Study design and serum samples: Blood samples from 204 deer (males and females) 2.5-4 year old were collected. Animals were captured in order to repopulate other farms. Sample size was calculated according to the formula for proportions for finite populations considering a prevalence of 85% (for endemic regions), a 95% confidence level and a 5% precision (Segura and Honhold, 2000), plus 10% because of possible losses. Animals were captured using the drop net technique. Once the animals were immobilized, blood samples were obtained by jugular venipuncture using vacuum tubes (13×100 mm; Venoject, Terumo, Elkton, Maryland, USA) and disposable needles (31×38 mm). Serum samples were refrigerated for 72 h and sent to the laboratory of Pathology and Clinical Diagnosis of the Faculty of Veterinary Medicine and Animal Science of the Autonomous University of Nuevo Leon, where the blood samples were centrifuged at 2000×g per 10 min in order to get the sera. Sera were kept in Eppendorf tubes and stored at -20°C until tested.

An indirect immunofluorescent antibody test (IFA) was used to determine antibody activity to *Babesia bovis* and *B. bigemina* using prepared slides (donated by Dr Gale Wagner, Department of Pathobiology, Texas A and M University, College Station, Texas, USA). Positive and negative control sera for the IFA were of bovine origin. In the IFA, the conjugate was of rabbit antiruminant origin and the antigen was infected bovine erythrocytes prepared in cell culture, spread on a slide, dried at room temperature and stored at -20° C. The slides were thawed at room temperature and inscribing small wells of nail polish in two rows of seven wells each. Dilutions of the sera of 1:50 (10 μL) were placed in each web and incubated at 37°C in a humidity chamber for 30 min. Negative and positive control sera were applied to each antigen slide. After incubation the slides were washed with phosphate buffered saline (PBS) solution from a wash bottle. Conjugate was then applied (10 μL) and the slides were incubated as before. After a second wash to remove unattached conjugate, the slides were read using a Zeiss UV microscope (Carl Zeiss, Oberkochen, Germany) with glycerin/PBS as the immersion medium.

Statistical analysis: Seroprevalences and 95% confidence intervals were obtained for *B. bigemina* and *B. bovis* and for the combination of the 2 *Babesias* (Thrusfield, 1990). Association of sex and the presence of antibodies in deer sera were determined using univariable logistic regression (EGRET, 1999).

RESULTS AND DISCUSSION

Of the 204 samples only 165 (60%) were used because 39 (40%) got hemolysed. Of the 165 samples, 33 blood samples (20%) belong to male and 132 (80%) to female deer.

The overall seropositivity for *B. bovis* and *B. bigemina* was 87% (144/165). Seropositivity for *B. bovis* 75% (124/165) was greater in comparison with *B. bigemina* 53% (88/165). Of the *B. bovis* positive deer 20 (16.1%) were males and 104 (83.9%) were females, whereas for *B. bigemina* 15 (17%) were males and 73 (83%) were females.

The results of this study hold the hypothesis that Babesia from cattle might parasite the white-tailed deer, which produce antibodies against those protozoa, although they do not show clinical signs of the disease. Some authors mention than that could be due to some type of premonition or due to the presence of a factor in the deer serum that avoid the development of a high parasitemia and the presence of disease. Holman *et al.* (1993) tried to develop *B. bovis in vitro* using erythrocytes of white-tailed deer, but did not achieve it; they only got it when they substituted the deer sera for cattle sera. Therefore, the white-tailed deer is a reservoir of *B. bigemina* and *B. bovis*.

The estimate seroprevalences of *B. bovis* (75%) and *B. bigemina* (53%) found in this study, the fact that in the northeastern region of Mexico the cattle prevalences go from 50-56% (Teclaw *et al.*, 1985) and the absence of clinical cases in the studied farms confirm the endemic

Table 1: Seroprevalence and odds ratio by sex for *Babesia bigemina* and *B. bovis* in white-tailed deer in 5 farms in northeastern Mexico

Risk factor	N	No. of positives	Prevalence	OR	IC95
Sex		*B. bigemina*			
Females	132	73	55.30	1.49	0.69,3.19
Males	33	15	45.45	1.00	
Sex		*B. bovis*			
Females	132	104	78.79	2.41	1.08,5.38
Males	33	20	60.60	1.00	

IC95 = OR 95% confidence interval

instability of bovine babesiosis. Therefore, the risk of the occurrence of economically important outbreaks is high in the region (FAO, 1984). Endemic stability with respect to babesiosis occurs when the disease, the vector and the host are all present but immunity is acquired sufficiently early in the life of the majority of animals that clinical disease occurs only rarely, if at all. However, the management and climatic factors that affect the serological status and susceptibility to the disease should be taken in consideration. Some of the risk factors that affect the seroprevalence in cattle and also could affect the seroprevalence in the white-tailed deer are: stock density, tick control (Solorio-Rivera *et al*., 1999; Alfredo *et al*., 2005) and contact of animals from different species. The high seropositivity of babesiosis in deer shows the importance of the diseases in the region and suggests that in the control of cattle babesiosis, the presence of white-tailed deer should be taken in consideration. More research is required to find the role of the white-tailed deer in the transmission of *Babesia* sp. to cattle.

Sex of the deer was associated with the seroprevalence of *B. bovis*, but not for *B. bigemina* (Table 1). The risk of infection with *B. bovis* was 2.4 times greater for females than for males. The authors of this study did not find literature on the association of sex and the presence of antibodies against Babesia. However, it could be associated to differences in behavior of male and female deer or to management differences. Much more studies are required in order to corroborate this finding.

CONCLUSION

The white-tailed deer presented a high seroprevalence of antibodies against *B. bovis* and *B. bigemina*. Association was found between sex of the deer and the presence of antibodies against *B. bovis*.

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