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Prevalence of Antibodies Against *Anaplasma marginale* in White-tailed Deer (*Odocoileus virginianus texanus*) in Hunting Farms of Northeastern Mexico

[1]M.A. Cantú-Martínez, [1]M.L. Silva-Páez, [1]R. Ávalos-Ramírez,
[1]A. Wong-González, [1]J.A. Salinas-Meléndez and [2]J.C. Segura Correa
[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Nuevo León,
Monterrey, N.L., México
[2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán,
Mérida, Yucatán, México

Abstract: The objective of this study was to estimate the prevalence of antibodies against *Anaplasma marginale* in sera from white-tailed deer (*Odocoileus virginianus texanus*) in the states of Nuevo Leon and Coahuila, Mexico. One hundred and sixty five deer from 5 farms were live-trapped in 2005 using the net-canyon method. Blood was extracted by jugular venipuncture using vacuum tubes. Antibodies in the sera were detected by the indirect immunofluorecent technique. Seroprevalence was high because 69.7% of deer reacted positively to *A. marginale*. The prevalences for the municipalities of Anahuac and Acuña were 61.9 and 83.3%, respectively. The risk of a seropositive animal was 3 times greater in the municipality of Acuña. The prevalence between sexes were equal ($p>0.05$). In conclusion, a high frequency of antibodies against *A. marginale* in white-tailed deer in northeastern Mexico was found. More studies are recommended with the aim of determine the risk of deer in the transmission of *A. marginale* to cattle.

Key words: Anaplasmosis, deer, prevalence, Mexico

INTRODUCTION

The northeastern region of Mexico includes the states of Coahuila, Tamaulipas and Nuevo León, which is the main area were deer hunting farms are located. In this region, the white-tailed deer (*Odocoileus virginianus texanus*), normally graze mixed with cattle. Therefore, it might be possible then that deer could be a host to keep tick populations and a reservoir of diseases that are transmitted by haemoparasites (Luther *et al*., 1980; Chomel *et al*., 1994).

One of the most important diseases for the cattle industry is anaplasmosis, which is an infecto-contagious disease of acute or chronic course for bovines and it is widespread through the world. In Mexico, the Anaplasmosis is found in tropical and subtropical regions (Mucino and Cesar, 1982; Teclaw *et al*., 1985; Rodriguez-Vivas *et al*., 2004). Anaplasmosis is produced by the rickettsia *Anaplasma marginale* that infect the red cells causing anemia, jaundice, fever, weakness and high mortality among cattle (Osebld *et al*., 1959). This disease affects mainly to bovines, but also has been notified in the carabao, bison, African antelope, gnu, elk and the camel (Byton and Woods, 1940). In the deer, the anaplamosis commonly produce a subclinical infection, situations that allow us to assume that deer act as a healthy carrier of the infection and therefore, they have an important role in the maintenance of the rickettsia in the habitat; because in some case deer and bovines share the same grass (Anónimo, 1993).

Experimentally, some authors have clinically reproduced the disease in the black-tailed deer (*Odocoileus hemionus colombianos*), white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) when they were practiced the splectomy (Christensen *et al*., 1960; Maas and Buening, 1981). Even thought, anaplasmosis have been reported in the white-tailed deer, the degree of infection is low, therefore, it is believed that they are asymptomatic carriers (Christensen *et al*., 1960; Christensen and Mcneal, 1967; Levine, 1973).

In the northeastern region of Mexico, the specific vector of *A. marginale* is *Dermancentor andersoni*, which it is a three-host tick and fed himself indistinctly of deer-or bovine blood. Other generous of tick associated to anaplasmosis are *Boophilus*, *Rhipicephalus*, *Ixodes* y

Corresponding Author: M.A. Cantú-Martínez, Facultad de Medicina Veterinaria y Zootecnia,
Universidad Autónoma de Nuevo León, Monterrey, N.L., México

Hyalomma (Harwood and James, 1979). It is important to remember that the anaplasmosis is also transmitted via the bite of horseflies or through the use of contaminated surgical tools (Luther *et al*., 1980).

The objective of this study was to estimate the prevalence of antibodies against *A. marginale* in white-tailed deer hunting farms in the northeastern of Mexico.

MATERIALS AND METHODS

Localization and climate: The study was carried out from March to April 2005 in 5 white-tailed deer (*Odocoileus virginianus texanus*) hunting farms located in the municipalities of Anáhuac, Nuevo León and Ciudad Acuña, Coahuila in the northeastern of Mexico. Then main objective of the farm was beef cattle production. Of the 5 farms studied, 2 were located in the municipality of Anahuac at the north of Nuevo Leon, in the coordinates 27°17' latitude north and 100°00' latitude west at an altitude of 335 m above sea level. The climate is hot and arid, being the hottest months June, July and August; the average annual temperature is 22°C with an ample range; in winter the average temperature is 8°C. The average annual rainfall ranges between the 48 and 75 mm, rains in July, August and September. The main vegetation is of the steppe type, prickly middle-size bush and cultivated areas (Cantú-Martínez *et al*., 2008).

The other 2 farms were located in the municipality of Acuña at the north of the state of Coahuila, in the coordinates 102°54'00" longitude west and 28°58′00" latitude north, at an altitude of 280 m above sea level. The climate is dry and warm with average temperature of 20-22°C. The average annual rainfall ranges from 300-400 mm, with rains in the months of May, June, July, November, December and January. The period of frost weather in the municipality is from 20-40 days (Cantú-Martínez *et al*., 2008). The vegetation is constituted of grass, bushes and pine forest.

Deer management: Deer and cattle grazed together natural grass of the region with eventual feed supplementation during the months when grass was scarce and they did not receive any other special management.

Sampling and study design: One-hundred deer (males and females) of 2.5-4 years of age, with the aim of repopulating other farms were captured. Therefore, this sample could be considered as a random sample of the population of deer estimated in 1600. Sample size was calculated using the formula to estimate prevalence in a finite population (n = 1600), considering an 85% prevalence (for endemic zones), a 95% confidence interval and a 5% precision (Thusfield, 1995).

Blood collection and serology: Animals were captured using the net-canyon method. Once the animals were immobilized a blood sample (10 mL) from each 1 was collected by jugular venipuncture using a vacuum tube (13×100 mm; Venoject, Terumo, Elkton, Maryland, USA) and disposable needles (31×38 mm). Blood samples were refrigerated for 72 h and sent to the Research Unit of the Faculty of Veterinary and Animal Science, of the Autonomous University of Nuevo León, where they were centrifuged at 2000 × g per 10 min in order to get the sera. The sera were kept in Eppendorf tubes and stores at -20°C until tested.

An indirect Immunofluorescent Antibody (IFA) test was used to determine antibody activity to *A. marginale*. Positive and negative controls in the IFA test were of bovine origin; the conjugate was of rabbit antibovine origin and the antigen was infected bovine erythrocytes prepared in a cell culture, spread on a slide, dried at room temperature and stored at -20°C. The IFA test was conducted by thawing the antigen slides at room temperature and inscribing small wells of nail polish in 2 rows of 7 wells each. Double dilutions were carried out in order to avoid errors in the IFA test. Dilutions of the test sera (10 μL) were placed in each well 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 pH 7.2 at 4°C) solution and kept in a recipient shaked continuously during 15 min. 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 with glycerin/PBS as the immersion medium. 1: 76-1: 50 dilutions of the deer sera in a buffer solution (PBS pH 7.2) were prepared to obtain the antibody titers present in the positives sera. For the use of the conjugate, the optimal dilution for the IFA test was obtained first, because when the dilutions is not optimal the fluorescence make difficult to visualize the antibodies. The IFA test for the diagnosis of bovine anaplasmosis had high sensibility (99%) and specificity (93%).

Statistical analysis: Seroprevalences and 95% confidence intervals were obtained for *A. marginale* using the formula provided by Thrusfield (1995). Association of municipality and 6 with the presence of antibodies against *A. marginale* was determined using chi-square (χ^2) test and the confidence interval of the odd ratios (Thrusfield, 1995).

RESULTS AND DISCUSSION

The IFA test with a sera dilution of 1: 76 and a conjugated dilution of 1: 50 was used, because they gave the best visibility of the specific forms of *Anaplasma*. Of the 175 blood samples collected only 165 were used, because 10 were discarded by haemolysis. The results of the study are given in Table 1. Sixty of the 165 deer sampled became from the municipality of Acuña and 105 from Anahuac. Twenty-eight (17.0%) of the total samples analyzed, came from male and 137 (83.0%) from female deer.

The overall prevalence of seropositive animals against *A. marginale* was 69.7% (115/165). This seroprevalence is similar to the notified (69.0%) in a previous study in white-tailed deer (Martinez *et al*., 1999) and for beef cattle in southern Mexico (Rodriguez-Vivas *et al*., 2004). However, it is higher than that obtained (56%) by Chomel *et al*. (1994), when they examined 276 samples from black-tailed deer (*Odocoileus hemionus colombianus*) and mule deer (*Odocoileus hemionus*) in California, USA. The prevalence differences obtained between Mexico and USA could be due to the most rigorous tick control in the USA. Robinson *et al*. (1968) in Texas (near to the area here studied) found a seropositivity of 47% (out of 30 sera), using the agglutination in capillary tube test. In Missouri, Maas and Buening (1981) in white-tailed deer obtained a seroprevalence of 1% (7/616) using the capillary tube test. In addition, Smith *et al*. (1982) in Illinois, in a study with 175 white-tailed deer and 54 bovines, found prevalences of 6.9 and 13.0%, respectively. The prevalences obtained by Chomel *et al*. (1994), Robinson *et al*. (1968), Maas and Buening (1981) and Smith *et al*. (1982) using the capillary tube test, could be greater with the IFA test. Teclaw *et al*. (1985) found with the IFA test a 99% confidence interval and with the capillary tube test 80%.

Fifty of the 60 animals sampled in Acuña were positive (83.3%) and 65 of those sampled in Anahuac were positives (61.9%). Differences between municipalities could be due to differences in management and biosecurity measures. The relatively high seroprevalences in both municipalities point out the importance of the anaplasmosis in the region. The results here obtained reinforce the theory that the white-tailed deer (*Odocoileus virginianus texanus*) can act as a reservoir of *A. marginale* and be a serious problem in those places where the mechanisms of infection involve common vectors between wild and domestic ruminants, therefore, it is necessary to carry out future research in order to know the role of deer in the transmission of the *A. marginale* to cattle. As mentioned by Martinez *et al*. (1999) in the endemic regions, with enzootic stability, the presence of deer may be it is not an important risk factor for cattle. However, management factors such as the frequency of baths against ticks could modify the enzootic status and deer could be then a significant risk factor in the preservation and spread of *Anaplasma* sp. Teclaw *et al*. (1985) in 2156 bovines from 40 farms of the states of Nuevo León, Tamaulipas y Coahuila, Mexico found a range of within herd prevalences from 0-86%. The risk of finding a seropositive animal was 3.1 times higher in the municipality of Acuña as compared with the municipality of Anahuac.

Twenty-three of the 115 deer seropositive to *A. marginale* were males (13.9%) and 92 females (55.8%). Similar to other study in cattle in Mozambique (Alfredo *et al*., 2005), there was not association of 6 of deer ($p>0.05$) on the seroprevalence A. marginale. However, a previous study on another tick transmitted disease, *Babesia bovis,* showed sex differences (Cantú-Martínez *et al*., 2008).

Table 1: Result serological for municipality and sex in deer white line (*Odocoileus virginianus* texanus) in the northeast of Mexico

	Acuña		Anáhuac	
	Males	Females	Males	Females
Positive	10	40	13	52
Negative	0	10	5	35
Total	10	50	18	87

CONCLUSION

Based on the results of this study, it is probable that the white-tailed deer could be a reservoir of the disease therefore, further research is needed to know the role that deer play in the transmission of the anaplasmosis in cattle.

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