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Serosurvey for Selected Disease Agents in White-tailed Deer from Mexico

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ABSTRACT: Serum samples from 350 white-tailed deer (*Odocoileus virginianus texanus*) collected in March 1994 from northeastern Mexico were tested for the prevalence of antibody activity against five infectious diseases of ruminants. The prevalence rate was 81% for bluetongue virus (BTV) of all serotypes, 72% for epizootic hemorrhagic disease virus (EHDV), 3% for *Borrelia burgdorferi*, 69% for *Anaplasma marginale*, and 0% for *Brucella abortus, B. melitensis*, and *B. ovis*. These are diseases that affect domestic ruminants, and deer may act as a reservoir of infection. In addition, if deer are translocated, they may introduce pathogens to formerly disease-free areas. The high seroprevalence of BTV and EHDV cannot be related to the presence of hemorrhagic disease in the deer in this region. This is the first report to indicate the presence of *B. burgdorferi* infection of deer in Mexico. Despite the high prevalence of *A. marginale* titers, it is uncertain that deer play a role in the epizootiology of cattle anaplasmosis in the region. Apparently, white-tailed deer are unimportant in the epizootiology of brucellosis of both cattle and goats in northeastern Mexico.

Key words: Anaplasmosis, bluetongue, brucellosis, epizootic hemorrhagic disease, Lyme disease, serosurvey, white-tailed deer.

During the past two decades the population of white-tailed deer (WTD: *Odocoileus virginianus texanus*) in northeastern Mexico has increased considerably (Martinez et al., 1997). Because they occupy sympatric ranges, deer and domestic ruminants, also may share infectious disease agents. Infectious agents may be transmitted from wildlife to livestock, livestock to wildlife, and occasionally to humans (Chomel et al., 1994). In this context, the question is not whether deer and livestock can co-exist, but rather what management strategies can be put into effect that will ensure maintenance and expansion of wildlife populations while maintaining the livestock stocking rate without initiating disease problems. Moreover, a thorough understanding is needed of the epidemiology of deer/livestock interactions regarding disease transmission before decisions can be made about disease control. Until now, no serological study of such diseases of WTD has been attempted in northeastern Mexico. Therefore, the objective of this study, was to determine the prevalence of antibody activity in WTD sera against five common transmissible infectious diseases of deer and livestock.

Three hundred and fifty serum samples of WTD were collected in March of 1994 from six ranches in the northeastern Mexican states of Nuevo Leon, Coahuila, and Tamaulipas (See Table 1 for map coordinates). Samples were collected from animals that had been live-trapped by nets fired from helicopters. Bleeding was done by jugular venipuncture using vacuum tubes (Venoject, Terumo, Elkton, Maryland, USA) without anticoagulant. The samples were allowed to clot, were centrifuged, and the sera were stored at 4 C until arrival at the laboratory. In the laboratory the sera were stored at −20 C until tested. Age was approximated from the dentition (Dimmick and Pelton, 1994). Other parameters, such as weight and sex, also were collected from the trapped deer.

Agar gel immunodiffusion test kits were used for antibody activity against bluetongue virus (BTV; Veterinary Diagnostic Technology, Wheat Ridge, Colorado, USA), and epizootic hemorrhagic disease virus (EHDV; Veterinary Diagnostic Technology). Tests were performed according to the kit instructions.

A double sandwich enzyme linked immunosorbent assay (ELISA) was used to

TABLE 1. Number (percentage) of white-tailed deer seropositive for *Anaplasma marginale* (AM), *bluetongue virus* (BTV), *epizootic hemorrhagic disease virus* (EHDV), *Borrelia burgdorferi* (LD), *Brucella abortus* (BA) and *B. mellitensis* (BM) in six ranches in northeastern Mexico. Not all sera from each ranch were tested for each disease.

Ranch (Map Coordinates)	AM	BTV	EHDV	LD	BA	BM
Las Margaritas (29°13′N 101°14′W)	38 (73)	54 (73)	55 (77)	2 (3.6)	0 (0)	0 (0)
La Grulla (24°13′N 101°14′W)	8 (80)	9 (90)	9 (90)	0 (0)	0 (0)	0 (0)
San Emeterio (27°44′N 100°05′W)	9 (53)	17 (100)	15 (83)	0 (0)	0 (0)	0 (0)
La Azufrosa (27°15′N 99°50′W)	10 (91)	12 (80)	11 (73)	3 (19)	0 (0)	0 (0)
Santa Niño (27°35′N 99°51′W)	11 (65)	16 (89)	14 (87)	0 (0)	0 (0)	0 (0)
Palo Blanco	6 (56)	14 (87)	11 (69)	2 (14)	0 (0)	0 (0)

determine the antibody activity against *Borrelia burgdorferi* (Synbiotics Corporation, San Diego, California, USA). Positive samples were confirmed with western blot (Tizard, 1995). An indirect immunofluorescent antibody test (IFA; Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas, USA) was used for *Anaplasma marginale*. Positive and negative control sera for the ELISA were deer sera and the positive and negative controls in the IFA were of bovine origin. In the IFA, the conjugate was of rabbit antibovine 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 IFA test was conducted by thawing the antigen slides at room temperature and inscribing small wells of nail polish in three rows of seven wells each. 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) solution from a wash bottle. Conjugate was then applied (10 μl) and the slides were incubated as before. After another 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.

Antibody activity against *Brucella abortus, B. melitensis* and *B. ovis* was determined using the Rose Bengal and the Rivanol tests (Alton et al., 1988). The antigens used were specific for the species of *Brucella* being tested (Productora Nacional de Biologicos Veterinarios, Mexico, D.F., Mexico). Positive and negative controls were of bovine origin.

The results of the serological testing are listed in Table 1. All 350 sera from six ranches were tested for antibody activity against BT and EHDV. Of these, 81% (283) and 72% (52), respectively, were positive. Of 125 sera tested for antibody activity against *B. burgdorferi*, 6% (7) were positive. Sera from 118 deer representing all of the six ranches were tested for activity against *A. marginale* of which 69% (82) were positive. For anaplasmosis, the prevalences on the ranches ranged from 53 to 91%. After a one way analysis of variance, there was no significant difference (0.18) among the six ranches tested due to the small number of animals tested at some of the ranches (Statgraphics, Software Systems, Inc., 1987). Sera from 165 deer were tested for antibody activity against *B. abortus, B. melitensis* and *B. ovis* of which all were negative.

The ages of the WTD sampled ranged

from 1.5 to 3.5 yr. Almost all were females. Because of the lack of diversity in gender data and inconsistency in collecting age data, these parameters were not analyzed across prevalence rates.

This survey is the initial part of a larger effort to determine the importance of WTD in the epidemiology of disease for domestic ruminants. The study determined the prevalence of antibody activity in deer against five infectious diseases of ruminants that have some importance for the cattle ranching industry in northeastern Mexico.

One of the major sources of concern in the otherwise successful brucellosis eradication program in the USA is the reservoir of infected wild ruminants, particularly bison and elk (McCorquodale and DiGiacomo, 1985; Davis et al., 1990). In Mexico, deer are the major wild ruminant that would be a potential brucellosis reservoir creating a quandry for ranchers who derive income from both cattle and hunting leases. Fortunately, this study shows that if *B. abortus* exists at all in the deer population, it is at a very low level. This agrees with the <6% that was found in the local cattle by Teclaw et al. (1985a).

Of more importance is *B. melitensis* infection which is common in goats in this part of Mexico (Siller, 1994). Fortunately no positive deer were found since this would have been a serious setback for the proposed Mexican eradication program.

Bluetongue and EHDV are related viruses (Reoviridae: Orbivirus) of ruminants that use *Culicoides variipennis* as the biological vector in the USA. Epizootic hemorrhagic disease is primarily a problem in deer populations, but BT can cause serious problems in sheep as well as deer (Thomas, 1981). Enzootic stability is the epidemiological pattern that caracterizes other vector-borne diseases such as malaria and bovine babesiosis (MacDonald, 1950; Mahoney and Ross, 1972; Smith and Kakoma, 1989) when infection rates are sufficient to ensure that a high percentage of young animals is infected. The immunity resulting from this infection then provides lifelong protection from disease symptoms as long as either a chronic infection or multiple reinfections sustain the level of the immune response. When transmission rates are low enzootic instability occurs in which previously uninfected adults react to the eventual challenge with severe disease.

The results of this survey indicate a high prevalence of infection with the viruses of BTV and EHDV in deer, although there are no reports of clinical disease in the deer. Stallknecht et al. (1996) found a similar situation where there was a 90% prevalence of antibody activity in areas of Texas adjacent to our collection sites. This indicates stability of these viruses in the deer population. However we have no data for fawns and yearlings so we can not graph the age-class seroprevalence. Although an epizootic could have occurred one to two years ago, this seems unlikely both because of the large deer populations and none of the ranchers reported major deer mortality. The graph of age-class seroprevalence in the report of Stallknecht et al. (1996) was characteristic of an enzootically stable situation (Mahoney, 1969).

Ticks of the genera *Boophilus* and *Dermacentor* are biological vectors for anaplasmosis although biting flies, particularly tabanids, are important for mechanical transmission (Stich et al., 1989). The area in which these deer were trapped is endemic for *Boophilus* spp. in populations high enough to maintain babesiosis in enzootic stability. Indeed, many captured were infested with adult *Boophilus* spp. Of the 289 tick specimens collected on WTD in this study, 60% were *B. annulatus* (Martinez, 1995). White-tailed deer can be infected with *A. marginale* and very low parasitemias do occur (Keel et. al., 1996) which may potentially infect a biological vector, especially considering that female ticks ingest approximately three to four times their engorged weight in blood during the feeding process (Balashov, 1972). Since anaplasmosis in cattle in the area is in an enzootically stable condition (Teclaw

et. al., 1985b), an additional reservoir of infection is immaterial.

Deer are dead end hosts for *B. burgdorferi* since the primary vector, the deer tick, *Ixodes scapularis*, uses deer as a host only in the adult stage and rarely transmits the infection transovarially (Burgdorfer et. al., 1988). That means the primary function of deer in the epidemiology of Lyme disease is vector maintenance (Duffy et. al., 1994). Chomel et al. (1994) mention cross reactions with other *Borrelia* spp., especially *B. coriaceus* which has not been reported from Texas or northeastern Mexico. Considering that Lyme disease has been found in humans and dogs in Monterrey, Nuevo Leon, Mexico (Salinas et al., 1995), we know that *B. burgdorferi* exists in northeastern Mexico and that deer may be involved in vector maintenance. In Connecticut (USA) serological prevalence in dogs mimics the local disease incidence in humans (Magnerelli et. al., 1985) so the low percentage of serologically positive deer would also reflect the low level of *B. burgdorferi* infection in the region. Although cattle can be clinically infected with *B. burgdorferi* (Burgess et. al., 1987), it is not an important bovine disease.

The results of this survey indicate that in northeastern Mexico, because of the presence of tick vectors, specifically *Boophilus* spp. (Martinez, 1995), deer may be peripherally involved in the epidemiology of anaplasmosis of cattle. Because the deer are also in an enzootically stable situation regarding BTV and EHDV, there does not seem to be much danger of disease outbreaks of these, either. If for some reason such as climate changes associated with a prolonged El Niño, the vector responsible for transmission was in low numbers for several years, the resulting susceptible deer population could be in danger of a major outbreak. Deer in northeasten Mexico do not seem to be involved in brucellosis transmission and play only an indirect role in transmission of borreliosis which does not appear to be very prevalent. For the diseases studied here, either the prevalence in the deer was very low or the disease is already endemic in the areas to which the deer are being translocated. Further studies of tick infestations, which are underway, as well as surveys for other economically important rumiant diseases, will further illuminate the role of deer in the epidemiology of other diseases of domestic ruminants.

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