Serologic survey of wild cervids for potential disease agents in selected national parks in the United States

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Abstract

A total of 589 serum specimens were collected from mule deer (Odocoileus hemionus) (133) and wapiti (Cervus elaphus) (456) in eight national parks and/or adjacent lands in the western USA. Thirty two percent of the samples were collected from immobilized animals and 68% from hunter-killed animals in or near Glen Canyon National Recreation Area, Bryce Canyon National Park (NP), and Zion NP, Utah; Yosemite NP, California; Rocky Mountain NP, Colorado; Upper Yellowstone NP, Montana, and Grand Teton NP, Wyoming. Serum specimens were tested for the presence of antibodies against selected disease agents. Overall seroprevalences for mule deer were 77/133 (58%) for parainfluenza-3 virus (PI-3), 42/133 (32%) for bovine herpesvirus-1 (BHV-1), 79/133 (59%) for bovine virus diarrhea virus (BVD), 73/133 (55%) for respiratory syncytial virus (RSV), 14/133 (11%) for bluetongue virus (BT), 18/133 (14%) for epizootic hemorrhagic disease virus (EHD), 3/133 (2%) for Borrelia burgdorferi, and 1/133 (1%) for Francisella tularensis. None of the deer sera presented antibodies for Leptospira spp., Brucella abortus and Anaplasma marginale. For wapiti, overall prevalences were 262/456 (57%) for PI-3, 211/456 (46%) for BHV-1, 251/456 (55%) for BVD, 247/456 (54%) for RSV, 1/456 (<1%) for BT, 16/456 (4%) for Leptospira pomona, 13/456 (3%) for Leptospira hardjo, and 8/456 (2%) for B. abortus. No antibody titers were detected for EHD, A. marginale, and other Leptospira serotypes. This survey documents seroprevalence of selected park cervid populations to domestic livestock pathogens. Further research on the epidemiology of these potential pathogens in wild ungulates in national parks is recommended.

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1. Introduction

Wild ungulates in North America have been implicated as the source of disease agents when outbreaks occur in domestic livestock. Such is the case for the Greater Yellowstone Ecosystem wapiti (Cervus elaphus canadensis) and bison (Bison bison) being linked to brucellosis in cattle (Thorne et al., 1991a,b; Aguirre and Starkey, 1994) or, bison in Wood Buffalo National Park, Canada, being a source of brucellosis and tuberculosis (Environmental Assessment Panel, 1990; Tessaro et al., 1990). On the other hand, domestic livestock are known to transmit pathogens that are detrimental to wild ungulates. For example, commingling with domestic sheep is considered one of the factors responsible for bacterial pneumonia in bighorn sheep (Ovis canadensis) in North America (Foreyt, 1989; Callan et al., 1991). In many cases, however, disease interactions between wild ungulates and domestic livestock are not well understood.

Much of the information known about diseases in wildlife has been acquired from serologic surveys using non-probability sampling. The difficulty and expense involved in collecting probability samples in wildlife populations has prevented these deliberate and random processes from being widely utilized. Despite its disadvantages, purposive selection may be the only affordable method possible. Even though the variability of the target population may be underestimated by non-probability techniques, the information generated is valuable. Care must be taken, however, in extrapolating the results to the target population.

The objective of this study was to determine the seroprevalence of potential domestic animal pathogens in adult wild cervids in or adjacent to selected western US national parks.

2. Materials and methods

National parks were selected in the western USA, based on grazing allotments of livestock, in or adjacent to parklands sharing common range with wild wapiti and/or mule deer (Odocoileus hemionus) (Aguirre et al., 1993). Many parks qualified to participate in this survey; however, the criteria to exclude parks was based on their willingness to cooperate and the accessibility to collect blood specimens. The specimens collected were convenience samples taken by hunters or from opportunistic immobilizations. Blood specimens collected near Grand Teton National Park (NP), in Rocky Mountain NP (1976 sample), and in Zion NP were obtained by chemically immobilizing mule deer with a mixture of Ketamine (Ketaset®, Aveco Co. Inc., Fort Dodge, IA, USA) and Xylazine (TranquiVed®, Vedco Inc., St. Joseph, MO, USA) at doses of 400–500/80–100 mg per adult. Wapiti were immobilized with Xylazine at doses of 290–350 mg per adult (Clark and Jessup, 1992). Blood (5–10 ml) was taken by venipuncture from the jugular vein. After blood collection anesthesia was reversed with Yoh-
imbine HCl (Antagonil®, Wildlife Pharmaceuticals, Inc., Fort Collins, CO, USA) at intravenous doses of 0.2–0.3 mg kg⁻¹ bodyweight.

Specimens from Rocky Mountain NP (1981 and 1992 samples) and other areas were obtained from hunter-killed adult animals (over 2 years old) known to be part of the park cervid population. Hunters collected blood samples after killing the animals. This system has been employed by the Colorado Division of Wildlife since 1965 (Adrian and Keiss, 1977). Blood collection kits were mailed to hunters with special permits in specific pre-selected areas (areas surrounding the park). After collection, blood specimens were centrifuged at 2000 rpm for 10 min and serum was separated into two different labeled, screw-capped vials. Serum vials were stored in sealed, double plastic bags and frozen on dry ice in the field and stored at −22°C in the laboratory until tested.

Serum-virus neutralization (SN) tests were performed to detect antibodies to bovine herpesvirus-1 (BHV-1), bovine virus diarrhea virus (BVD), and respiratory syncytial virus (RSV) (Picton, 1993). Briefly, serum specimens were heat-inactivated at 56°C for 30 min and diluted in flat bottom 96-well microtiter plates using two-fold dilution steps with an initial serum dilution of 1: 4. An equal volume of virus (50 µl), containing 100 tissue culture median infectious doses (TCID₅₀), was added to the diluted serum. The plates were incubated for 1 h at 25°C and the appropriate cells were added at a concentration of 5 x 10⁵ cells ml⁻¹. The plates were examined for cell growth and presence of cytopathic effects after 5–7 days. Tests were performed in duplicate and the serum end-point titer was defined as the last serum dilution which inhibited the cytopathic effect (CPE) of the virus. Serum specimens were considered positive if CPE was inhibited at the 1:4 dilution.

For the determination of antibodies of parainfluenza-3 virus (PI-3) the hemagglutination-inhibition (HI) test was used. Serum specimens at a 1:4 dilution were diluted in the plates in two-fold steps. First, an equal amount of virus (25 µl) was added and after incubation at 25°C for 1 h, 50 µl of the red blood cells were added. The test was incubated overnight at 4°C. The titer was recorded as the last well to show inhibition, i.e. to form a button of red blood cells (Picton, 1993).

A competitive enzyme-linked immunosorbent assay (cELISA) was used to screen all samples for bluetongue (BT) antibodies (Afshar et al., 1987). The optical density (OD) of each well was determined at 490 nm wavelength using an ELISA microplate reader. Negative controls were defined as having OD values <20% of the OD value of the positive control serum. All samples were screened for antibodies to epizootic hemorrhagic disease virus (EHD) types 1 and 2, using a commercial agar gel immunodiffusion test (AGID) (Veterinary Diagnostic Technology, Inc., Wheat Ridge, CO, USA). Serum specimens containing antibody to EHD formed a precipitin line of identity with the neighboring control positive band.

The microscopic agglutination microtiter test (MAMT) as described by the Leptospirosis Committee of the US Animal Health Association, was used for the detection of Leptospira antibodies. Sera were screened and titrated using Leptos-
pira pomona, L. grippotyphosa, L. hardjo, and L. icterohaemorrhagiae antigens obtained from the National Veterinary Services Laboratory, Ames, Iowa. Positive results were recorded when agglutination occurred at a 1:100 dilution (Fournier, 1986).

The MAMT was used to detect antibodies against Francisella tularensis. Titers $\geq 1:128$ were considered to provide evidence of previous exposure (Stewart, 1988). Serum specimens were submitted to the US Department of Agriculture Brucellosis Laboratory, Denver, CO, for the detection of antibodies against Brucella abortus and Anaplasma marginale. The Brewer Diagnostic Card Test® (BBL Microbiology Systems, Cockeysville, MD 21030), was the macroscopic agglutination procedure used.

An ELISA test and Western immunoblotting were used to determine the presence of antibodies against Borrelia burgdorferi in mule deer (Gill et al., 1993). The OD of each well was determined at 450 nm wavelength using an ELISA microplate reader. Samples were considered positive if the OD values were $\geq 3$ standard deviations above the mean (OD=0.08) of negative controls. Western immunoblots were conducted as previously described (Gill et al., 1993).

3. Results

A total of 589 samples were tested from 133 wild mule deer and 456 wapiti in selected national parks and/or adjacent lands in the States of California, Colorado, Montana, Utah, and Wyoming. A total of 188 specimens were collected from chemically immobilized animals. Of 1570 blood sample kits mailed to hunters, 921 (59%) specimens were returned and 678 (43%) specimens were usable and not hemolyzed. Of the 678 usable samples, 401 (59%) were tested.

From the mule deer samples, 77/133 (58%) were positive for PI-3 virus, 42/133 (32%) were positive for BHV-1 virus, 79/133 (59%) were positive for BVD virus, 73/133 (55%) were positive for RSV virus, 14/133 (11%) were positive for BT, and 18/133 (14%) were positive for EHD virus. Seroprevalences from the wapiti samples were 262/456 (57%) for PI-3 virus, 211/456 (46%) for BHV-1 virus, 251/456 (55%) for BVD virus, 247/456 (54%) for RSV virus, 1/456 (0.2%) for BT, and none were positive for EHD. Seroprevalences for the viral agents plus a 95% confidence interval for the proportion are summarized for both species in Table 1.

Seroprevalences for the bacterial antigens in mule deer were 3/7 (43%) for B. burgdorferi in Yosemite NP, and 1/25 (4%) for F. tularensis in Zion NP. Antibodies for Leptospira spp., B. abortus, or A. marginale were not detected in the deer sera. For wapiti, seroprevalences for L. pomona were 3/50 (6%) in Rocky Mountain NP in 1981 and 4/100 (4%) in 1992, 5/143 (3%) in Upper Yellowstone NP, and 4/146 (3%) in Grand Teton NP; for L. hardjo they were 1/17 (6%) in Rocky Mountain NP, 1976, 3/50 (6%) in 1982, and 2/100 (2%) in 1992, and for B. abortus they were 5/146 (3%) in Grand Teton NP, and 3/143
Table 1
Seroprevalence and 95% Confidence Interval upper bound (in parenthesis) to selected viral antigens in sera collected from wild cervids in western US national parks

<table>
<thead>
<tr>
<th>National park</th>
<th>Species</th>
<th>Number tested</th>
<th>PI-3*</th>
<th>BHV-1b</th>
<th>BVDc</th>
<th>RSVd</th>
<th>BT*</th>
<th>EHDc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glen Canyon NRA</td>
<td>mule deer</td>
<td>53</td>
<td>25(61)b</td>
<td>12(34)b</td>
<td>37(82)b</td>
<td>41(89)b</td>
<td>4(15)b</td>
<td>5(17)b</td>
</tr>
<tr>
<td>Bryce Canyon NP</td>
<td>mule deer</td>
<td>48</td>
<td>30(76)</td>
<td>16(47)</td>
<td>13(40)</td>
<td>15(44)</td>
<td>5(19)</td>
<td>6(22)</td>
</tr>
<tr>
<td>Zion NP</td>
<td>mule deer</td>
<td>25</td>
<td>20(96)</td>
<td>12(68)</td>
<td>25(100)</td>
<td>15(79)</td>
<td>4(30)</td>
<td>7(46)</td>
</tr>
<tr>
<td>Yosemite NP</td>
<td>mule deer</td>
<td>7</td>
<td>2(65)</td>
<td>2(65)</td>
<td>4(94)</td>
<td>2(65)</td>
<td>1(40)</td>
<td>0(&lt;10)</td>
</tr>
<tr>
<td>Deer total</td>
<td></td>
<td>133</td>
<td>77(66)</td>
<td>42(39)</td>
<td>79(68)</td>
<td>73(63)</td>
<td>14(16)</td>
<td>18(19)</td>
</tr>
<tr>
<td>Rocky Mountain NP (1976)</td>
<td>wapiti</td>
<td>17</td>
<td>9(77)</td>
<td>5(51)</td>
<td>11(87)</td>
<td>0(0)</td>
<td>1(14)</td>
<td>0(&lt;2)</td>
</tr>
<tr>
<td>Rocky Mountain NP (1981)</td>
<td>wapiti</td>
<td>50</td>
<td>27(68)</td>
<td>21(56)</td>
<td>29(72)</td>
<td>10(31)</td>
<td>0(&lt;2)</td>
<td>0(&lt;2)</td>
</tr>
<tr>
<td>Rocky Mountain NP (1992)</td>
<td>wapiti</td>
<td>100</td>
<td>19(27)</td>
<td>40(50)</td>
<td>22(30)</td>
<td>24(32)</td>
<td>0(&lt;2)</td>
<td>0(&lt;2)</td>
</tr>
<tr>
<td>Upper Yellowstone NP</td>
<td>wapiti</td>
<td>143</td>
<td>121(92)</td>
<td>71(58)</td>
<td>114(86)</td>
<td>110(84)</td>
<td>0(&lt;2)</td>
<td>0(&lt;2)</td>
</tr>
<tr>
<td>Grant Teton NP</td>
<td>wapiti</td>
<td>146</td>
<td>86(67)</td>
<td>74(59)</td>
<td>75(59)</td>
<td>103(78)</td>
<td>0(&lt;2)</td>
<td>0(&lt;2)</td>
</tr>
<tr>
<td>Wapiti subtotal</td>
<td></td>
<td>456</td>
<td>262(62)</td>
<td>211(31)</td>
<td>251(60)</td>
<td>247(39)</td>
<td>1(&lt;3)</td>
<td>0(&lt;2)</td>
</tr>
</tbody>
</table>

* Parainfluenza-3-virus.
** Bovine herpesvirus-1.
* Bovine virus diarrhea virus.
* Respiratory syncytial virus.
* Bluetongue virus.
* Epizootic hemorrhagic disease virus.
* Number positive
* Percent positive upper 95% confidence interval.

(2%) in Upper Yellowstone NP. No antibody titers were detected for *A. marginale* and other *Leptospira* spp. serotypes in the wapiti specimens tested.

4. Discussion

Much of the information known about diseases in wildlife has been acquired from serologic surveys using non-random techniques. The size and distribution of the samples, the lack of randomization, the titer and duration of infection, and the host longevity may bias the information obtained from these surveys. Samples from a single herd may be insufficient and the restraining technique may bias the composition of the collection, resulting in a non-representative sample of animals tested. Nevertheless, given the constraints imposed by limited finance, population estimators, and capture technology, convenience sampling of wild populations may be one of the few methods possible to execute in the field. This study may be criticized on points of statistical inference to target populations. It is, however, offered for its points of agreement to other studies and to provoke thought for possible questions to be solved in future studies.

Prevalences of antibodies to PI-3 (19–80%), BHV-1 (23–51%), and BVD (22–100%) in mule deer and wapiti from the national parks surveyed in this study,
indicate that these populations have been exposed to viral agents similar to, or identical to, the bovine pathogens. Exposure to these viruses has been demonstrated in other wild cervid populations in North America (Couvillion et al., 1980; Ingebriksen, 1986; Johnston et al., 1986b). Several studies demonstrate the presence of bovine respiratory viruses in areas where wild ungulates have been exposed to domestic livestock (Stauber et al., 1977; Kingscote et al., 1987; Lamontagne et al., 1989). Parainfluenza-3 virus prevalences, from 0% prior to 1977 to 100% by 1984, in an Alaskan bison population were believed to be the result of cattle introduction (Zarnke and Erickson, 1990). On the other hand, infection with these respiratory viruses has been described in cervids which had no contact with domestic ruminants for at least 50 years (Sadi et al., 1991). To date, no evidence of severe clinical effects or latent infections with PI-3 has been demonstrated in free-ranging mule deer or wapiti; however, the virus has been isolated from mule deer and pronghorn antelope (Antilocapra americana) (Thorsen et al., 1977).

Bovine herpesvirus-1 and two other antigenically related viruses have been isolated from wild ungulates (Sadi et al., 1991). Under laboratory conditions, BHV-1 infection in mule deer induced mild respiratory clinical signs, and the virus was shed by immunosuppressed cervids (Thorsen et al., 1977; Lamontagne et al., 1989). In our study, titers to PI-3 and BVD were higher in 1980 than in 1992 (53% and 58% versus 19% and 22%, respectively) in Rocky Mountain NP wapiti. By comparison, the BHV-1 seroprevalence rose between 1975 and 1980, from 29% to 42%, and then remained at 40% in 1992. These fluctuations in prevalences may reflect the rising and falling exposure and infection levels of these agents (Thrusfield, 1986). These viruses may have been circulating among these cervid populations for several years following their primary contact with domestic livestock or infected wildlife. Because these viruses are contagious and shed intermittently, their transmission could continue within the population. The maintenance of these respiratory viruses in mule deer and wapiti may be due to their persistence after initial infection and shedding during stressful situations. Seroprevalences to BRSV in this study were high in all the parks sampled and over 70% in Glen Canyon National Recreation Area, Grand Teton NP, and Upper Yellowstone NP. These figures may indicate that this or a similar virus is enzootic in these wild cervid populations. High seroprevalence of infection to BRSV or a related virus has been reported in mule deer (20–60%), pronghorn antelope, and mountain goats (Oreamnos americanus) (42%) (Dunbar and Foreyt, 1986; Johnson et al., 1986a,b). No documentation exists to suggest that BRSV causes clinical disease in wild cervids; however, bighorn sheep develop a respiratory syndrome (Foreyt and Evermann, 1988).

Bluetongue and EHD are closely related but antigenically different viral diseases of North American domestic and wild ruminants (Callis, 1985). The diseases in deer may be inapparent or produce a clinical syndrome referred to as hemorrhagic disease, since concurrent infections are common. Bluetongue/epizootic hemorrhagic disease complex is enzootic in white-tailed deer (Odocoileus virginianus) populations in southeastern USA (Walton and Osburn, 1992). This
complex has shown changes in geographic distribution and local variation within physiographic regions (Stallknecht et al., 1991). The spread of hemorrhagic disease from deer to cattle, or vice versa, has not been proven and a long-term reservoir status has not been reported in deer (Hoff and Trainer, 1978). Serologic evidence and clinical disease have been reported in mule deer in several western States (Thorne et al., 1982; Work et al., 1992). We report overall seroprevalences of 11% for BT and 14% for EHD viruses in mule deer in Utah. Lower seroprevalences (3% for BT, and 5% for EHD) have been reported in Idaho (Stauber et al., 1977); and similar or higher seroprevalences (22–55% for BT and 7–36% for EHD) were described for this species in New Mexico and Texas (Hampy et al., 1979; Couvillion et al., 1980). According to this study, hemorrhagic disease antibodies were not present in any wapiti samples collected after 1976. Wapiti populations remain at elevations above the common vector habitat during the transmission season and possibly are not exposed to these viruses (Jessup et al., 1984). Antibody titers in wapiti have been documented in lower habitats (Trainer and Jo&m, 1969). In addition, BT and EHD viruses have been isolated from experimentally inoculated wapiti with mild clinical signs (Stott et al., 1982).

The negative results for B. abortus antibodies in all parks, except upper Yellowstone NP and Grand Teton NP areas, were expected given the intense national effort to eradicate the disease in domestic cattle. One of the few remaining nidi of bovine brucellosis in the country is found in the Greater Yellowstone Ecosystem bison and wapiti herds (Thorne et al. 1991a,b). Seroprevalences in this study of 2–3%, are lower than those reported recently for those populations (Thorne et al., 1978; Boyce, 1989).

This study reports similar seroprevalences to L. pomona and L. hardjo in mule deer (0%) and wapiti (2–6%) when compared with earlier serologic studies (Adrian and Keiss, 1977). On the other hand, seroprevalences were lower when compared with L. pomona in white-tailed deer (6–100%) and L. hardjo in pronghorn antelope (2–14%) (Shotts and Hayes, 1970; Thorne et al., 1982; Fournier et al., 1986; New et al., 1993). L. pomona and L. hardjo are primarily pathogens of livestock. Occasional isolation and evidence of infection to these serovars have been identified in wild ungulates in North America, but the source in most cases can be traced to domestic animals (Kistner et al., 1982). Most serologic surveys in California, Colorado, and Idaho have failed to demonstrate the presence of leptospiral antibodies in wild cervids. Confirmed clinical disease has not been reported in mule deer or wapiti (McGowan, 1963; Kingscote et al., 1987).

During this study, three of seven deer presented specific antibodies to Lyme disease in Yosemite NP. Wapiti samples were not tested because a reliable anti-wapiti conjugate was not available at the time of this survey. Most serologic surveys of B. burgdorferi in North American ungulates have focused on white-tailed deer. This species may serve as an indicator of disease and vector activity in endemic areas of eastern USA (Magnarelli et al., 1986; Mahnke et al., 1993). Borrelia spp. (27%) and antibodies (38%) were identified in Columbian black-tailed deer (Odocoileus hemionus columbianus) in northern California using direct immunofluorescence (Lane and Burgdorfer, 1988).
Tularemia is a vector-borne bacterial zoonosis that causes hemorrhagic septicemia in small mammals. The disease is enzootic in western USA and, although maintenance hosts are lagomorphs, *F. tularensis* has been isolated from mule deer and can be transmitted to humans by dressing deer carcasses (Emmons et al., 1976). We found antibodies in one of 133 mule deer, which correlates with previously published reports for that region (Merrell and Wright, 1978). Wapiti and mule deer have been reported as susceptible hosts of anaplasmosis, presenting subclinical infection among other North American wild ruminants (Kuttler, 1984). This survey supports work by other investigators suggesting that these species are not important sources of the disease to domestic livestock (Kingscote et al., 1987; Zaugg, 1988).

5. Conclusion

The prevalences to the respiratory viruses selected for this study document the exposure of mule deer and wapiti to bovine pathogens or closely related agents. To date, the epidemiology of these viruses in wild ungulate populations is poorly understood. Further research on these potential pathogens in wild ungulates in national parks is recommended.

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