PREVALENCE AND SPATIAL DISTRIBUTION OF ANTIBODIES TO BOVINE VIRAL DIARRHEA VIRUS AND COXIELLA BURNETII IN WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN NEW YORK AND PENNSYLVANIA

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Abstract: Significant pathogens of domestic livestock and public-health related pathogens, such as bovine viral diarrhea virus (BVDV) and Coxiella burnetii, are commonly diagnosed in some wildlife species. BVDV is an economically important pathogen of domestic bovids and Coxiella burnetii is a highly infectious zoonotic bacterium. As a result of recent shifting patterns of disease, it is critical that baseline information regarding the status of both significant pathogens of domestic livestock and public-health related pathogens are established for commonly encountered wildlife such as white-tailed deer (Odocoileus virginianus). White-tailed deer are susceptible to both BVDV and C. burnetii infection, and the purpose of this study was to investigate for the presence of antibodies to these two pathogens in New York and Pennsylvania white-tailed deer. Exposure to BVDV and C. burnetii was determined using sera collected from 333 (219 males and 114 females) wild white-tailed deer in New York and 291 (130 males and 161 females) wild white-tailed deer from Pennsylvania. Samples were collected from hunter-harvested deer in central New York State in 2009 and live-captured deer in Pennsylvania in 2010. Sera were screened for anti-BVDV antibodies via a commercial blocking BVDV enzyme-linked immunosorbent assay. Coxiella burnetii phase II whole-cell antigen-coated slides were used to screen sera via an indirect microimmunofluorescence assay. Antibody prevalence was compared by sex class and location of collection. Deer in New York had higher antibody prevalence to BVDV (6.01%) than did deer in Pennsylvania (0.34%). Conversely, C. burnetii phase II antibodies were more common in Pennsylvania (20.96%) than in New York (14.41%). No statistically significant difference between locations was observed in either BVDV or C. burnetii antibody prevalence when data were analyzed by sex-class. Overall, C. burnetii seroprevalence was not significantly higher in Pennsylvania than in New York.

Key words: Bovine viral diarrhea virus, Coxiella burnetii, New York, Odocoileus virginianus, Pennsylvania, white-tailed deer.

INTRODUCTION

Economically important pathogens of domestic livestock and public-health related pathogens have been reported in some wildlife species, and some wildlife are suspected to be reservoir hosts for some pathogens. Two pathogens reported in wild white-tailed deer (Odocoileus virginianus) are bovine viral diarrhea virus (BVDV), an economically important pathogen of domestic bovids, and Coxiella burnetii, a highly infectious zoonotic bacterium. The majority of livestock pathogens are generalist species that infect multiple host species, and over 50% of human diseases are zoonotic.1,2,6 In addition, 73% of human emerging or re-emerging infectious diseases of the last 20 yr include at least one wildlife host.2 As a result of these changing patterns of disease, it is critical that baseline information regarding the status of both economically important pathogens of domestic livestock and public-health related pathogens are established for commonly encountered wildlife such as white-tailed deer.

An estimated 1 to 10% of American dairy operations have BVDV on their premises,28 and the American Association of Bovine Practitioners, the National Cattlemen’s Beef Association, and the Academy of Veterinary Consultants have formally announced resolutions designed to eradicate BVDV from the United States.8 Interspecies disease transmission between white-tailed deer and cattle has been confirmed experimentally,21
and research suggests that persistently infected (PI) white-tailed deer have the potential to be efficient sources of BVDV for both conspecifics and domestic animals.21,23 Seropositive and PI wild white-tailed deer have been diagnosed in Alabama, Indiana, South Dakota, and Mexico within the last 15 yr.4,18,22,23 *Coxiella burnetii* is a highly infectious bacterium that is considered a possible agent of bioterrorism and became nationally notifiable in 1999.3,15 Antibodies to *C. burnetii* have been documented in various wild ruminant species including white-tailed deer.17 *Coxiella burnetii*-infected white-tailed deer may pose a public health risk, most notably to individuals that directly contact the blood and internal organs of harvested white-tailed deer.

Although there have been several recent papers published that report BVDV seroprevalences in white-tailed deer sampled from a variety of locales throughout North America,18,21,27 none of them comment on the seroprevalence of deer sampled in the northeastern United States. The last report of BVDV seroprevalence from deer sampled in the Northeast was published in 1967 and assessed the seroprevalence of two discrete study sites in New York State (3 and 5.75%).13 The only report of white-tailed deer *C. burnetii* seroprevalence was published in 1993 and discussed deer sampled in Ontario, Canada.17 Considering the paucity of serologic data pertaining to BVDV and *C. burnetii* exposure of Northeastern white-tailed deer, the purpose of this study was to investigate for the presence of antibodies to these two pathogens in New York and Pennsylvania white-tailed deer.

**MATERIALS AND METHODS**

**Study area and field procedures**

Wild white-tailed deer were surveyed in the central portion of New York State (approximately 40–45°N, 71–79°W) (Fig. 1) and in four separate study areas of Pennsylvania (approximately 39–42°N, 74–80°W) for evidence of exposure to BVDV, *C. burnetii*, or both. Three-hundred and thirty-three hunter-harvested white-tailed deer (219 males and 114 females) were sampled at private deer processing facilities, and at New York State Department of Environmental Conservation deer check stations, on the first weekend of the New York State regular firearm season. Blood samples were obtained either from the thoracic cavity or as it drained from the nasal and oral cavities.25 The blood was transferred to a 10-ml glass serum tube (Vacutainer™, Becton Dickinson and Company, Franklin Lakes, New Jersey 07417, USA) and stored at 4°C prior to centrifugation (different centrifuges were used depending on the location with the target of 1,300 g for 10 min). After separation, serum was collected and frozen at −80°C.8,18 Blood was collected from 291 live-captured white-tailed deer in Pennsylvania from January until March 2010 (130 males and 161 females). Deer were captured via rocket nets and drop nets and blood samples were collected by jugular venipuncture using 10-ml glass serum tubes. All work was performed under an Institutional Animal Care and Use Committee (IACUC) permit (IACUC #26886) granted by Penn State University. Blood was processed in both the field and laboratory in the same manner as described for New York.

**Laboratory procedures**

**Bovine viral diarrhea virus:** A commercially available blocking BVDV enzyme-linked immunosorbent assay (ELISA) (SVANOVIIR BVDV p80-AB ELISA kit, Svanova Biotech AV, Uppsala, SE-751 45 Sweden), currently validated for use in bovids, was modified for use in white-tailed deer. The laboratory protocol was performed according to the manufacturer’s instructions, but the optical density value (%) cutoff for positive samples was lowered to 19% based upon serum neutralization (SN) results of dual-tested samples. The SVANOVIIR BVDV p80-AB ELISA was used to screen the serum samples for the presence of anti-BVDV antibodies. All seropositive, and a subset of seronegative, samples tested by the ELISA were confirmed by SN for BVDV1 (BVD1 Singer strain) and BVDV2 (BVD2 strain 125). Serum neutralization tests were carried out following standard methods.7 In brief, serum samples were inactivated at 56°C for 30 min, then serially diluted twofold in minimum essential medium with Earle’s salts (MEME) supplemented with 10% fetal bovine sera (FBS), streptomycin, and penicillin in a 96-well cell culture microtiter plate. An equal volume of MEME containing 100 tissue culture infective dose (TCID50) of BVDV1-Singer or BVDV2-strain 125 isolate/ml was added to each well. The plates were incubated at 37°C for 4 days before examination for cytopathic changes.7

**Coxiella burnetii:** *Coxiella burnetii* phase II whole-cell antigen-coated slides were used to screen sera via indirect microimmunofluorescence assays. *Coxiella burnetii* phase II, stored at −80°C until just prior to inoculation, was used to infect 1-day-old cells of the VERO line in MEME plus 10% FBS. No antibiotics were added to the
media. Flasks were incubated at 37°C for 72 hr. Trypsin was added and cells were suspended in 16 ml of MEME plus 10% FBS. A glass pipette was used to dispense approximately 0.4 ml of the suspension to each well of a Teflon®-coated slide (Teflon-coated immunological slides with multiple wells, Cel-Line Associates, Newfield, New Jersey 08344, USA). Slides were incubated at 37°C for 3 hr, then fixed for 5 min in 10% formalin and then for an additional 5 min in 100% methanol. Slides were stored at −20°C until stained. Indirect microimmunofluorescence assays were carried out as follows. Approximately 0.4 ml of sample sera diluted 1:20 in blocking

Figure 1. Number of sampled white-tailed deer and bovine viral diarrhea virus and *Coxiella burnetii*-seropositive deer by township, New York, 2009. ■ = 1–3 sampled per township; □ = 4–6 deer sampled per township; ▼ = 7–10 deer sampled per township; yellow = one seropositive deer per township; red = two seropositive deer per township. A. Total number of sampled and bovine viral diarrhea virus seropositive deer. B. Total number of sampled and *Coxiella burnetii*-seropositive deer.
buffer (0.01 M phosphate buffered saline [PBS], 1% bovine serum albumin, 0.1% Tween20) was added to each slide well and incubated at 37°C for 30 min in a humidified chamber. Wells were rinsed with PBS and washed in a PBS bath for 10 min. After air drying, fluorescein isothiocyanate-conjugated rabbit anti-deer Ig G (Anti-deer IgG [H + L] antibody, FITC labeled, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Maryland 20878, USA) diluted 1:80 in blocking buffer was applied to each well and the slide was incubated at 37°C for 30 min in a humidified chamber. The slides were rinsed and washed again with PBS using the same method and then dipped in Evan’s blue stain for 5 min. The slides were rinsed in distilled water, allowed to air dry, and then mounted in 50% glycerol-PBS mounting medium. Slides were examined using fluorescence microscopy at ×400 magnification. A Pearson’s chi-square analysis (x² = 0.10) was used to measure the strength of association between antibody prevalence and sex or location of harvest or capture.

**RESULTS AND DISCUSSION**

Of 291 white-tailed deer serum samples tested in Pennsylvania, only one animal (0.34%) was seropositive for BVDV and 61 (20.96%) were seropositive for *C. burnetii* phase II (Table 1). No significant difference was observed between antibody prevalence to *C. burnetii* phase II in males (24.62%; 32/130) versus females (18.01%; 29/161) in Pennsylvania (χ² = 1.23, df = 1, P = 0.27; Table 1). Of 333 white-tailed deer serum samples tested in New York, 20 (6.01%) had antibodies against BVDV and 48 (14.42%) had antibodies against *C. burnetii* phase II (Table 1). No significant difference was observed between antibody prevalence to BVDV in males (5.48%; 12/219) versus females (7.02%; 32/114) in New York (χ² = 0.28, df = 1, P = 0.60; Table 1). Similarly, no significant difference was observed between *C. burnetii* phase II antibody prevalence in males (16.44%; 36/219) versus females (10.53%; 12/114) in New York (χ² = 1.62, df = 1, P = 0.20; Table 1). *Coxiella burnetii* phase II antibody prevalence was not significantly higher in Pennsylvania than in New York (χ² = 3.24, df = 1, P = 0.07), whereas BVDV antibody prevalence was significantly higher in New York than in Pennsylvania (χ² = 14.38, df = 1, P < 0.05).

These data indicate that *C. burnetii* phase II antibodies are present in the wild populations of white-tailed deer in New York and Pennsylvania. The seroprevalence results (Table 1) were some-

**Table 1.** Prevalence of antibodies to bovine viral diarrhea virus and *Coxiella burnetii* in wild white-tailed deer from New York and Pennsylvania, USA.

<table>
<thead>
<tr>
<th>State</th>
<th>Total number seropositive animals/total number of animals tested</th>
<th>Percent positive</th>
<th>95% lower confidence limit</th>
<th>95% upper confidence limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>New York</td>
<td>20/233</td>
<td>8.55</td>
<td>4.97</td>
<td>13.97</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>8/114</td>
<td>7.02</td>
<td>3.60</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>12/129</td>
<td>9.45</td>
<td>5.48</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>1/291</td>
<td>0.34</td>
<td>0.06</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0/161</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>1/130</td>
<td>0.77</td>
<td>0.08</td>
</tr>
</tbody>
</table>

- Bovine viral diarrhea virus (BVDV).
What intermediate when compared to data obtained from serosurveys of wild cervids in other locations, which ranged from 4.4% (white-tailed deer in Nova Scotia) to 69% (Hokkaido deer [Cervus nippon yesoensis] in Japan). Based upon the results of C. burnetii serosurveys conducted in various parts of the world, it appears that the bacterium exhibits a regional heterogeneity in prevalence that is particular to the study species. For example, C. burnetii phase II seroprevalence of red deer (Cervus elaphus) is greater in the northern deer populations of Spain, whereas southern roe deer (Capreolus capreolus) populations exhibit a higher seroprevalence than do northern populations. Wild animals feeding on C. burnetii-contaminated domestic livestock pastures have previously been associated with a high prevalence of C. burnetii antibodies in wild animals, and 6 to 44% of environmental samples collected from six geographically diverse areas of the United States tested positive for C. burnetii DNA via polymerase chain reaction. The variable prevalence exhibited in relatively close geographic range, such as reported in Spain and the northeastern U.S., suggests that a combination of factors may affect local C. burnetii exposure. These differential factors may include the degree of pasture or environmental contamination, differences in landscape characteristics, species-specific social behavior or movement patterns, and host population density.

The level of C. burnetii exposure in wildlife may also be dependent upon the prevalence of C. burnetii infection in nearby domestic livestock. Coxiella burnetii can be isolated from the blood, lungs, spleen, and liver of infected domestic livestock during the acute phase of the disease and from the uterus and mammary gland in the chronic phase. The pathogenesis of C. burnetii in white-tailed deer has not been reported but it is assumed to be somewhat similar to the pathogenesis reported in goats, sheep, or cows. Recreational sportsmen and wildlife professionals in New York and Pennsylvania should be aware of potential exposure to C. burnetii because of their direct contact with blood and various organs, particularly the liver, spleen, and mammary gland. Findings such as this add credence to the recommendation to take precautions when field-dressing deer, such as wearing latex gloves.

The results of this study indicate that BVDV is present in the wild population of white-tailed deer in Pennsylvania and New York, and there are differences in prevalence between the two regions. The disparate seroprevalence results suggest that New York State deer may pose a greater risk of BVDV transmission to susceptible domestic ruminants than do Pennsylvania deer. Wild ruminants frequently share grazing space, feed, and water with pastured domestic animals, and interspecies BVDV transmission between white-tailed deer and cattle has been confirmed experimentally. In addition, phylogenetic relatedness of common BVDV isolates from deer and cattle suggest the potential for cross-species transmission. This implies that in areas where both domestic cows and wild white-tailed deer co-exist, and where BVDV has been reported in cattle or deer (or both), interspecific transmission of the pathogen may occur. Differences in the average cattle density for the 30 counties where deer were sampled in New York versus the 14 counties sampled in Pennsylvania may account for the significantly higher BVDV antibody prevalence in New York. According to the United States Department of Agriculture 2007 Census of Agriculture, beef cow density was similar in the sampled areas of New York and Pennsylvania (1.92 beef cows/km² and 1.74 beef cows/km², respectively). However, the 2007 average milk cow density of the sampled counties in New York (13.74 milk cows/km²) was considerably greater than the mean density in the sampled counties of Pennsylvania (5.01 milk cows/km²). The disparity between BVDV seroprevalence in New York and Pennsylvania may be due to the fact that the sampled portion of New York included more than double the density of both dairy cows and total calves and cows than did the sampled areas of Pennsylvania.

The BVDV seroprevalence disproportion between New York and Pennsylvania suggests that the New York wild deer population may be a more-likely reservoir species for BVDV when compared to the Pennsylvania deer population. In other words, as a result of regional variations, including different deer population densities and differential land use by deer due to varying landscape configuration and composition, the New York white-tailed deer population may be able to maintain the virus within its population without periodic interspecific transmission events. In order for deer to be considered reservoir hosts for BVDV, the virus must be maintained within the deer population without continual input from maintenance hosts such as.
domestic cows. For this to occur, two conditions must be met: the virus needs to be transmitted from PI deer to pregnant does; and infection of the fetus must occur prior to 67 days of gestation (in order to produce PI fawns which can then infect other conspecifics).\textsuperscript{20} In New York, most female white-tailed deer breed in November and December,\textsuperscript{2} making the critical gestational period approximately mid-January to mid-February. Quinn\textsuperscript{21} found evidence of sexual segregation between males and females in the relative probability of use of particular land cover types in all seasons but winter, when both sexes selected coniferous and mixed cover. This suggests that males and females are more likely to cohabit during the critical gestational period in nonagricultural areas, thereby increasing the likelihood that pregnant females may encounter a PI deer rather than a PI domestic cow. Persistently infected white-tailed deer are reported to be efficient shedders of the virus,\textsuperscript{21} and transmission of BVDV among white-tailed deer resulting in the birth of a PI fawn has been reported.\textsuperscript{20}

The social interactions of white-tailed deer may facilitate the persistence of a variety of infectious pathogens including BVDV and \textit{C. burnetii}. Female white-tailed deer offspring typically exhibit less than 5\% rates of dispersal,\textsuperscript{10} and female offspring tend to establish overlapping home ranges in close proximity to that of their mother.\textsuperscript{24} These social groupings may be particularly susceptible to BVDV infection and perpetuation.\textsuperscript{20} Male deer, on the other hand, typically disperse 5–10 km at between 12 and 18 mo of age. Dispersal distances up to 40 km have been reported.\textsuperscript{16,19} Male distribution behavior suggests that dispersing males that are PI may transmit BVDV to unrelated social groups across a larger geographic scale than do females.\textsuperscript{20} This social structure of white-tailed deer creates an environment that is suitable for the maintenance of both BVDV and \textit{C. burnetii}, but local variations in landscape ecology, population densities, and deer management strategies will alter population structure and the potential for pathogen continuance. This situation emphasizes the need for regional analyses concerning BVDV and \textit{C. burnetii} seroprevalence and distribution in wildlife species.

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LITERATURE CITED


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