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Short communication

Detection of *Borrelia burgdorferi* outer surface protein antibodies in wild white-tailed deer (*Odocoileus virginianus*) in New York and Pennsylvania, USA

Megan S. Kirchgessner[a,1], Heather Freer[b], Christopher M. Whipps[a], Bettina Wagner[b,*]

[a] *State University of New York College of Environmental Science and Forestry, 1 Forestry Drive, 246 Illick Hall, Syracuse, NY 13210, United States*

[b] *Department of Population Medicine and Diagnostic Sciences, Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, United States*

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ABSTRACT

Borrelia burgdorferi differentially exhibits outer surface proteins (Osp) on its outer membrane, and detection of particular Osp antibodies in mammals is suggestive of the infection stage. For example, OspF is typically associated with chronic infection, whereas OspC suggests early infection. A fluorescent bead-based multiplex assay was used to test sera from New York and Pennsylvania white-tailed deer (*Odocoileus virginianus*) for the presence of antibodies to OspA, OspC, and OspF. OspF seroprevalence was significantly greater than both OspA and OspC seroprevalence for all study sites. OspA, OspC, and OspF seroprevalences were significantly greater in Pennsylvania deer than New York deer. The regional differences in seroprevalence are believed to be attributable to a heterogeneous *Ixodes scapularis* distribution. While most seropositive deer were solely OspF seropositive, deer concurrently OspC and OspF seropositive were the second most commonly encountered individuals. Simultaneous detection of OspF and OspC antibodies may occur when non-infected or chronically infected deer are bitten by an infected tick within a few months of blood collection, thereby inducing production of antibodies associated with the early stages of infection with *B. burgdorferi*.

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1. Introduction

Infection with *Borrelia burgdorferi*, the causative agent of Lyme disease, is the most common vector-mediated disease in the United States. Depending upon a variety of different factors, the bacterium differentially expresses outer surface proteins (Osp) on its outer membrane. These Osp are often highly immunogenic and induce Ab production in the mammalian host. OspA expression begins soon after the bacterium enters the tick and continues for the entire length of time that the spirochete resides within the resting tick's midgut (Pal et al., 2000). When the tick initiates feeding on a mammal, expression of OspA begins to decrease and production of OspC starts to increase (Schwan et al., 1995). OspF is typically expressed after infection of the mammalian host (McDowell et al., 2001; Wagner et al., 2012). Detectible levels of antibodies to different Osp in mammals, such as white-tailed deer (*Odocoileus virginianus*), is useful for diagnosis and may also suggest the stage of infection (Wagner et al., 2012). A fluorescent bead-based multiplex assay based on recombinant OspA, OspC, and

Abbreviations: Osp, outer surface protein; MFI, median fluorescent intensities; NC, north central; NE, northeastern.

* Corresponding author. Tel.: +607 253 3813; fax: +607 253 3440.
E-mail address: bw73@cornell.edu (B. Wagner).

[1] Virginia Department of Game and Inland Fisheries, 4010 West Broad Street, Richmond VA 23230, United States.

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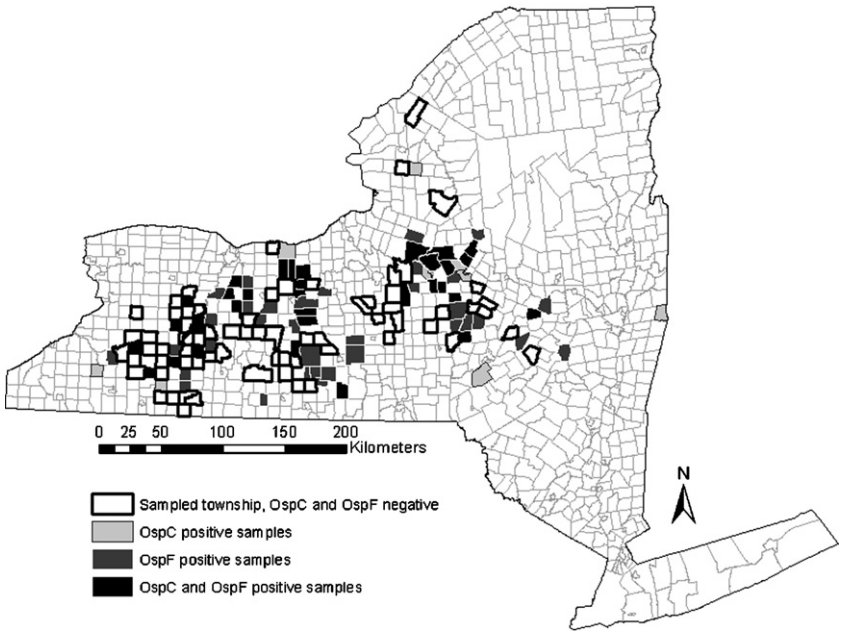

Fig. 1. Locations of sampled white-tailed deer and *Borrelia burgdorferi* outer surface protein (Osp) C and OspF seropositive deer by townships, New York, 2009.

OspF proteins was developed recently and was validated for detection of antibodies to *B. burgdorferi* in dogs and horses (Wagner et al., 2011a,b).

Although white-tailed deer are deadend hosts and not considered competent *B. burgdorferi* reservoirs, they are hypothesized to be potential sentinels for *B. burgdorferi* range expansion (Gill et al., 1993; 1994). The successful vector-mediated transmission of the spirochete to deer (Magnarelli et al., 2010) and the vigorous immune response of deer against the spirochete suggest their utility as sentinels (Gill et al., 1994). Spirochetemia has been reported in deer (Bosler et al., 1984), but research has shown that ticks feeding on spirochetemic deer do not become infected with *B. burgdorferi* (Telford et al., 1988). Some methods of *B. burgdorferi* surveillance, including small-mammal trapping or active tick surveillance, are very labor-intensive and expensive (Gill et al., 1994). Thus, white-tailed deer surveillance is a valuable method of *B. burgdorferi* distribution monitoring because of the ease of collecting samples from hunter-harvested deer (Gill et al., 1994).

The purpose of the current study was to use a fluorescent bead-based multiplex assay to detect antibodies in white-tailed deer to *B. burgdorferi* Osp proteins. These antibodies were then used as markers to determine the distribution of infected ticks feeding on wild white-tailed deer in New York and Pennsylvania.

2. Materials and methods

We utilized sera collected from wild deer from four separate study areas in Pennsylvania (approximately 39–42° N, 74–80° W) and from across central New York (approximately 40–45° N, 71–79° W) (Figs. 1 and 2). Blood was collected via jugular veinipuncture from 232 live-captured deer in Pennsylvania from January until March 2010. The blood was stored at 4 °C, centrifuged (target speed 1300 × *g* for 10 min), and serum was separated and frozen at −80 °C (Martinez et al., 1999). All work was performed under a Penn State University Institutional Animal Care and Use Committee permit (#26886). In New York, 267 samples from hunter-harvested deer were collected in November 2009. Blood samples were obtained from the thoracic cavity (Passler et al., 2008) or as it drained from the nasal and oral cavities. Blood was processed as above.

Multiplex assays were performed as described by Wagner et al. (2011a) with the following exceptions: deer sera were diluted 1:400 in PBS containing 1.0% BSA and 0.05% sodium azide (PBN), detection of deer serum antibodies was performed using 50 μl of unconjugated rabbit anti-deer IgG (rabbit anti-deer IgG (H+L), Bethyl Laboratories, Inc., Montgomery, Texas, USA) diluted 1:800 in PBN followed by 50 μl of phycoerythin conjugated goat anti-rabbit (R-Phycoerythrin AffiniPure F(ab')$_2$ Fragment Goat Anti-Rabbit IgG (H+L), Jackson ImmunoResearch Laboratories, Inc., West Grove, Pennsylvania, USA) diluted 1:100 in PBN. Data were reported as median fluorescent intensities (MFI). Assay interpretation ranges were previously established for a canine and for an equine Lyme multiplex assay. Both assays resulted in similar interpretation ranges (Wagner et al., 2011a,b). The equine cut-off values were used here to classify the white-tailed deer samples because it is believed that horse responses better represent white-tailed deer serological responses.

Pearson's Chi-squared test with Yates' continuity correction was used to assess the significance of the number of positive samples by geographic location and by Osp Ag within each individual site. R version 2.13.0 (R

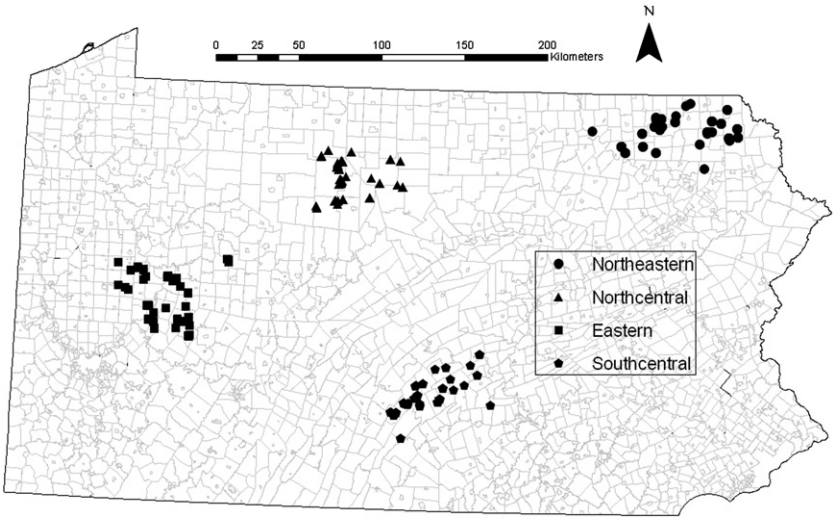

Fig. 2. Northeastern, north central, south central, and eastern Pennsylvania sample sites, 2010.

Development Core Team, 2008) was used to analyze the data. A *P*-value < 0.05 was considered significant.

3. Results and discussion

The median and range of MFI values and the OspA, OspC, and OspF seroprevalences for New York and Pennsylvania deer are listed in Table 1. At all sites, the number of OspF seropositive deer were significantly greater than the number of OspA and OspC seropositive deer ($P \leq 0.03$). This was expected because OspF antibodies represent robust markers that can be detected throughout infection with *B. burgdorferi* (Wagner et al., 2012). The number of OspA, OspC, and OspF seropositive deer were significantly greater in Pennsylvania than New York (P < 0.003 for OspA, OspC, and OspF). Significant regional differences in

Table 1
Median and range values for fluorescent intensity and seroprevalence (classified according to validated equine ranges) for white-tailed deer *Borrelia burgdorferi* outer surface protein (Osp) antibodies using a fluorescent bead-based multiplex assay.

Osp	Range[a]	Median[a]	Seropositive samples[b]
New York			
OspA	20–9402	681	38/267[c] (14.23%; 10.55%, 18.93%)
OspC	11–10,209	402	53/267[d] (19.85%; 15.51%, 25.05%)
OspF	19–19,975	721	89/267[e] (33.33%; 27.95%, 39.19%)
Pennsylvania (total)			
OspA	195–9883	1540	78/232[c] (33.62%; 27.85%, 33.62%)
OspC	51–21,241	607	74/232[d] (31.90%; 26.24%, 38.15%)
OspF	83–18,901	2387	172/232[e] (74.14%; 68.14%, 79.35%)
Northeastern Pennsylvania			
OspA	274–8564	1759	30/75[c] (40.00%; 29.66%, 51.31%)
OspC	51–21,241	1747	30/75[d] (40.00%; 29.66%, 51.31%)
OspF	83–18,901	1808	49/75[e] (65.33%; 54.05%, 75.11%)
North Central Pennsylvania			
OspA	297–8051	928	6/28[c] (21.43%; 10.21%, 39.54%)
OspC	65–1650	1182	4/28[d] (14.29%; 5.70%, 31.49%)
OspF	173–12,221	2448	25/28[e] (89.29%; 72.81%, 96.29%)
Eastern Pennsylvania			
OspA	195–9883	1540	28/87[c] (32.18%; 23.30%, 42.57%)
OspC	163–12,115	2282	25/87[d] (28.74%; 20.29%, 38.99%)
OspF	190–13,502	3211	66/87[e] (75.86%; 65.90%, 83.63%)
South Central Pennsylvania			
OspA	263–8351	1353	14/42[c] (33.33%; 21.01%, 48.44%)
OspC	123–4608	2102	15/42[d] (35.71%; 22.99%, 50.83%)
OspF	405–13,037	2807	32/42[e] (76.19%; 61.47%, 86.52%)

[a] Median fluorescent intensity.
[b] Total of seropositive animals/total number of animals tested (percent positive; 95% lower confidence limit, 95% upper confidence limit).
[c] Equine seropositive OspA fluorescent intensity: >2000 median fluorescent intensity.
[d] Equine seropositive OspC fluorescent intensity: >1000 median fluorescent intensity.
[e] Equine seropositive OspF fluorescent intensity: >1250 median fluorescent intensity.

Table 2
Classification of *Borrelia burgdorferi* Osp seropositive white-tailed deer into single, double, and triple expression categories.

Seropositive status	Pennsylvania[a]	New York[a]
OspA only	12/232 (5.17%; 2.98%, 8.82%)	9/267 (3.37%; 1.78%, 6.28%)
OspC only	8/232 (3.45%; 1.76%, 6.66%)	16/267 (5.99%; 3.72%, 9.51%)
OspF only	83/232 (35.78%; 29.89%, 42.13%)	46/267 (17.23%; 13.17%, 22.22%)
OspA and OspC	6/232 (2.59%; 1.19%, 5.53%)	2/267 (0.75%; 0.21%, 2.69%)
OspA and OspF	29/232 (12.5%; 8.85%, 17.38%)	8/267 (3.0%; 1.53%, 5.8%)
OspC and OspF	29/232 (12.5%; 8.85%, 17.38%)	16/267 (6.0%; 3.72%, 9.51%)
OspA, OspC, and OspF	31/232 (13.36%; 9.57%, 18.34%)	19/267 (7.12%; 4.61%, 10.85%)

[a] Total of seropositive animals/total number of animals tested (percent positive; 95% lower confidence limit, 95% upper confidence limit).

Pennsylvania were noted when sera were analyzed between sample groups. The number of OspC seropositive deer was significantly greater in the northeastern (NE) site when compared to the north central (NC) site ($P = 0.026$). The number of OspF seropositive deer was significantly greater in the NC site when compared to either the NE ($P = 0.031$) or the south central ($P < 0.000$) sites.

These results indicate that antibodies to *B. burgdorferi* OspA, OspC, and OspF are more prevalent in Pennsylvania deer than New York deer. Regional differences in seroprevalence may be attributable to a heterogeneous *Ixodes scapularis* distribution. Alternatively, because the Pennsylvania deer were sampled in January through March, whereas the New York deer were sampled in November, the regional differences in prevalence and Ab production intensity levels may be reflective of seasonal variation. However, the latter may only influence antibodies to OspC which are markers of early infection (Akin et al., 1999, Pal et al., 2004) and disappear in the chronic disease stage if no re-infection occurred, while antibodies to OspF are robust and long-lasting indicators during all stages of infection (Magnarelli et al., 1997; McDowell et al., 2001; Wagner et al., 2012). The time between exposure to *B. burgdorferi* and a detectable Osp Ab response was found to be 3–5 weeks in experimentally infected dogs and OspC antibodies lasted until 4–5 months post infection (Wagner et al., 2012). In white-tailed deer, Magnarelli et al. (2010) reported antibodies to *B. burgdorferi* VslE antigen in samples collected from deer throughout the year and documented sustained Ab titers showing minimal changes in deer that were sampled at intervals ranging from 17 days to over 5 years. Responses to *B. burgdorferi* VslE antigen highly correlated to those to OspF antigen in dogs and horses (Wagner et al., 2012, 2013). These data suggest that the regional differences noted in this study are likely not attributable to seasonal variation but rather are due to variable *I. scapularis* and/or *B. burgdorferi* geographic distribution.

The significantly greater percentage of OspF seropositive deer suggests that the majority of sampled deer were chronically infected with *B. burgdorferi*. The significantly lower percentage of deer OspC seropositive suggests that fewer sampled deer were acutely infected. Considering the reported OspA kinetics in other species, the small percentage of OspA seropositive deer is not surprising. In humans, OspA is believed to be rarely expressed (Pal et al., 2000), but low and transient humoral OspA responses have been detected in the early stages of some *B. burgdorferi* infections in humans and dogs (Akin et al., 1999; Wagner et al., 2012) and in some patients diagnosed with chronic Lyme arthritis (Akin et al., 1999). However, because deer, most notably yearlings and adults, may be bitten thousands of times each year by *I. scapularis* (Gill et al., 1994), the primary vector in the NE United States (Oliver et al., 1993). It is believed that repeated exposure to low levels of OspA may lead to a detectable Ab response in deer and explain the presence of OspA seropositive deer in this study.

While the proportion of OspF seropositive deer was significantly greater than either the percentage of OspA or OspC seropositive deer in the majority of sampling sites, many deer exhibited concurrent seropositivity to two or more Osp (Table 2). In both New York and Pennsylvania, most seropositive deer were solely OspF seropositive, but deer concurrently OspC and OspF seropositive exhibited the second highest prevalence. Simultaneous detection of OspF and OspC antibodies may be observed when the animal was infected within the past few months or may occur when chronically infected deer are bitten by an infected tick within a few months of blood collection, thereby inducing production of antibodies associated with the early stage of infection (Wagner et al., 2012). The most infrequently encountered class of seropositives was concomitant OspA and OspC seropositive deer, which was not surprising; OspA production is suggestive of repeated low-level exposure to *B. burgdorferi*, whereas OspC production is more indicative of early infection. The regional differences observed between the sampled populations of deer in New York and Pennsylvania and the differential Osp Ab production in individual deer suggest that the *B. burgdorferi* fluorescent bead-based multiplex assay is able to adequately measure relative Osp Ab production in white-tailed deer. Furthermore, the preponderance of deer with detectable levels of OspF suggests that the majority of sampled deer were chronically infected with *B. burgdorferi*. Although white-tailed deer are not considered competent *B. burgdorferi* reservoirs, differential Osp Ab production in individual deer and regional differences in Osp Ab production supports the hypothesis that white-tailed deer are appropriate sentinels for *B. burgdorferi*.

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