USE OF RECOMBINANT ANTIGENS OF BORRELIA BURGDORFERI AND ANAPLASMA PHAGOCYTOPHILUM IN ENZYME-LINKED IMMUNOSORBENT ASSAYS TO DETECT ANTIBODIES IN WHITE-TAILED DEER

Authors: Magnarelli, Louis A., IJdo, Jacob W., Ramakrishnan, Uma, Henderson, David W., Stafford, Kirby C., et al.

Source: Journal of Wildlife Diseases, 40(2) : 249-258

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-40.2.249

Journal of Wildlife Diseases, 40(2), 2004, pp. 249–258

USE OF RECOMBINANT ANTIGENS OF *BORRELIA BURGDORFERI* AND *ANAPLASMA PHAGOCYTOPHILUM* IN ENZYME-LINKED IMMUNOSORBENT ASSAYS TO DETECT ANTIBODIES IN WHITE-TAILED DEER

Louis A. Magnarelli,[1,5] Jacob W. IJdo,[2,4] Uma Ramakrishnan,[1] David W. Henderson,[3] Kirby C. Stafford III,[1] and Erol Fikrig[2]

[1] Departments of Entomology and Forestry and Horticulture, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, Connecticut 06504, USA
[2] Section of Rheumatology, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut 06520, USA
[3] Community Services Associates, Inc., 175 Greenwood Drive, Hilton Head Island, South Carolina 29928, USA
[4] Current address: College of Medicine, Division of Rheumatology, University of Iowa, Iowa City, Iowa 52242, USA
[5] Corresponding author (email: louis.magnarelli@po.state.ct.us)

ABSTRACT: Serum samples obtained from white-tailed deer (*Odocoileus virginianus*) in Connecticut (n=218) and South Carolina (n=20) (USA) during the period 1992–2002 were analyzed for antibodies to whole-cell or recombinant antigens (i.e., fusion proteins) of *Borrelia burgdorferi* sensu stricto and *Anaplasma phagocytophilum,* etiologic agents of Lyme borreliosis and granulocytic ehrlichiosis, respectively. In enzyme-linked immunosorbent assays (ELISAs) with whole-cell *B. burgdorferi,* the overall seropositivity rate for Connecticut (53%) exceeded that for South Carolina (30%). In separate tests of seven recombinant antigens of *B. burgdorferi* by an ELISA, seroprevalence for the VlsE antigen was highest (48%) in Connecticut followed by outer surface protein (OspF) (21%), whereas serum reactivities to the protein (p) 41-G antigen (55%) and VlsE (25%) were most frequent for South Carolina sera. In analyses for antibodies to the recombinant protein (p) 44 antigen of *A. phagocytophilum,* seroprevalences of 52% and 25% were recorded for Connecticut and South Carolina samples, respectively. These findings paralleled those determined by indirect fluorescent antibody staining methods with whole cells (43% and 30%). Moreover, there was good agreement (74%) in results of Western blot analyses and an ELISA when a subset of 39 sera was screened with whole-cell or recombinant p44 antigens of *A. phagocytophilum.* An ELISA with highly specific recombinant VlsE or p44 antigens can be used in conjunction with other antibody tests to determine whether deer living in different regions of eastern United States were exposed to *B. burgdorferi* or *A. phagocytophilum.*

Key words: Anaplasma phagocytophilum, antibodies, *Borrelia burgdorferi,* ELISA, *Odocoileus virginianus.*

INTRODUCTION

In widespread areas of eastern and upper midwestern United States, *Ixodes scapularis* ticks are abundant. White-tailed deer (*Odocoileus virginianus*) are important hosts for motile stages of this tick. The rise in populations of *I. scapularis* parallels increases in numbers of deer in or near forests. Human granulocytic ehrlichiosis (HGE) and Lyme borreliosis cases have been reported in regions where *I. scapularis* occurs (Walker and Dumler, 1996; Walker et al., 1996; Belongia et al., 1997; Magnarelli et al., 1998). Antibodies to *Anaplasma phagocytophilum,* formerly *Ehrlichia phagocytophila* (Dumler et al., 2001) and recently renamed (Anonymous, 2002), and *Borrelia burgdorferi* have been detected in deer sera (Gallivan et al., 1998; Magnarelli et al., 1999). With limited home range, deer are suitable for studies designed to help identify sites in Connecticut where these and other tick-borne infections are highly endemic.

A variety of serologic tests, such as indirect fluorescent antibody (IFA) staining methods, Western blot analysis, and enzyme-linked immunosorbent assays (ELISAs), have been used to verify past or current infections of *B. burgdorferi* and *A. phagocytophilum* infections (Lane et al., 1994; Luttrell et al., 1994; Magnarelli et al., 1999; Arens et al., 2003). Although sensitivities and specificities of these assays

TABLE 1. Presence of serum antibodies to whole-cell or recombinant antigens of *Borrelia burgdorferi* or *Anaplasma phagocytophilum* in Connecticut deer, as detected by using polyvalent enzyme-linked immunosorbent assays (ELISAs) or indirect fluorescent antibody (IFA) staining methods.

Sampling		Total sera tested	*B. burgdorferi*							*A. phagocytophilum*		
			Percent positive[a]	No. of antibodies to:						Percent positive[a]	No. with antibodies to:	
County	Year			WC	C	F	p35	p41G	VlsE		WC[b]	p44[b]
Fairfield	1992	60	50	30	1	10	14	3	23	20	12	13
	1999	3	33	1	1	0	0	0	1	0	0	1
Hartford	1996	20	55	11	3	3	2	0	11	40	8	12
	1999	8	75	6	1	1	1	0	4	50	4	7
Middlesex	1996	10	40	4	1	3	0	1	5	30	3	9
	1999	6	17	1	0	1	0	0	1	50	3	3
New Haven	2000	51	41	21	2	17	5	10	24	69	35	33
	2002	9	78	7	1	3	0	2	5	67	6	4
New London	1996	2	100	2	0	1	0	0	2	50	1	2
	1999	13	62	8	1	2	3	2	8	69	9	7
Tolland	1996	7	71	5	0	1	3	0	3	0	0	6
	1999	10	50	5	2	2	0	0	7	40	4	6
Windham	1999	19	74	14	2	2	0	0	10	42	8	10
Totals		218	53	115	15	46	28	18	104	43	93	113

[a] Percent positive is based on serum reactivity to whole-cell (WC) antigens. C = OspC; F = OspF. Results for Osp E and p39 include one positive for each.
[b] Whole-cell and p44 antigens tested by IFA and ELISA methods, respectively.

were considered acceptable, there is potential for false positive reactions when whole-cell antigens are used because heat-shock, flagellin, or other proteins of these pathogens may be shared with other bacteria. Recent advances in the production and use of purified recombinant antigens (i.e., fusion proteins) in ELISAs to detect antibodies in human, dog, horse, and bovine sera (IJdo et al., 1999; Magnarelli et al., 2001a, b, c, 2002a, b) have improved laboratory analyses. The objectives of the present study were to develop and evaluate ELISAs incorporating highly specific recombinant antigens of *A. phagocytophilum* and *B. burgdorferi*, to compare assay performance, and to calculate seropositivity rates for infections in deer from the northeastern and the southeastern United States.

MATERIALS AND METHODS

Whole-blood samples were obtained from deer during the period 1992–2002 from seven of eight counties in Connecticut and Beaufort County, South Carolina (USA). Serum samples from Connecticut white-tailed deer (n=218) consisted of 99 samples collected in 1992 and 1996 and previously tested with whole-cell antigens of *B. burgdorferi* and *A. phagocytophilum* (Magnarelli et al., 1995, 1999) and 119 additional samples collected between 1999 and 2002 (Table 1). Our study group also included 59 previously untested sera, obtained from deer blood collected during November of 1999 in 33 towns located in southern and eastern Connecticut. In addition, another 60 fresh serum samples were obtained in September through December of 2000 and 2002 from deer in North Branford, Connecticut (41°20′N, 72°46′W) in New Haven County. Sera from the latter group were separated from whole blood drawn by venipuncture of tranquilized animals in accordance with research protocols approved by an institutional animal care use committee. An additional 20 deer sera, collected in 2001 from Hilton Head Island, South Carolina (32°12′N, 80°45′W), originated from blood specimens taken from the body cavities of animals killed during the fall hunting season. Details on centrifugation procedures and methods of processing blood samples for antibody or polymerase chain reaction (PCR) analyses have been reported (Magnarelli et al., 1999).

Indirect fluorescent antibody staining methods or a polyvalent ELISA were used to detect

total immunoglobulins to *A. phagocytophilum* and *B. burgdorferi.* Assays with whole-cell antigens were used as a reference to compare the performances of new tests with recombinant antigens. Details on materials and procedures for assays incorporating whole-cell antigens (strains NCH-1 or 2591) have been reported (Magnarelli et al., 1999). An ELISA containing separate recombinant protein (p) 44 antigen of *A. phagocytophilum* or the following recombinant antigens of *B. burgdorferi,* outer surface protein (Osp)C (23 kDa), OspE (19 kDa), OspF (29 kDa), p35 (47 kDa fibronectin-binding protein), p39, p41-G, and VlsE (VlsE1-HIS), were essentially the same as those used in analyses of dog, bovine, and human sera (Magnarelli et al., 2000a, 2001a, b, c, 2002a, b). Briefly, the p44 antigen, a major outer membrane component, was fused with maltose-binding protein (MBP) and was the same reagent evaluated with human sera (IJdo et al., 1999; Magnarelli et al., 2001a). This antigen was coated to flat-bottom polystyrene plates (Nunc A/S, Roskilde, Denmark) at a concentration of 2.5 μg/ml for optimal reactivity. The working dilution of commercially prepared (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA) horseradish peroxidase-labeled rabbit anti-deer immunoglobulin was 1:800 in phosphate-buffered saline solution (PBSS). Recombinant VlsE1-HIS was provided by S. J. Norris (University of Texas Medical School at Houston, Houston, Texas, USA). This His_6-tagged version of the full-length VlsE protein was expressed and purified, as described by Lawrenz et al. (1999). All other recombinant antigens of *B. burgdorferi* were cloned, expressed, and purified as glutathione S-transferase (GST) fusion proteins in *Escherichia coli,* as described before (Magnarelli et al., 2000a, 2001b, 2002b). The VlsE surface-exposed lipoprotein antigen was coated to plates at a concentration of 1 μg/ml. All other antigens were coated at 5 μg/ml. The peroxidase-conjugated anti-deer immunoglobulins were diluted to 1:800 in PBSS. Serum samples were diluted in PBSS (pH 7.2) to 1:160, 1:320, and 1:640. If positive, sera were retested at higher dilutions to determine titration endpoints. Since deer sera reacted frequently with VlsE and p44 antigens, tests were conducted with five antibody-positive and three negative sera for reactivity to these fusion proteins on different days to determine reproducibility of results. All plates contained positive control sera used earlier (Magnarelli et al., 1999) and negative controls for sera, PBSS, GST, MBP, and conjugates. In addition, human positive sera were used to verify whole-cell and recombinant antigen reactivity.

Seventeen to 35 negative control sera, tested before by IFA or ELISA methods with whole-cell antigens (Magnarelli et al., 1995, 1999), were used to calculate net absorbance values to define critical regions for positive results. Net absorbance values represent differences in optical density (OD) readings for reactions with or without antigen for each serum dilution. Statistical analyses (three standard deviations plus the mean) of net OD values were used to determine cutoff figures for a positive reaction. In an ELISA with the p44 antigen, net OD values of 0.10 and 0.06 were considered positive for serum dilutions of 1:160 and ≥1:320. In an ELISA incorporating *B. burgdorferi* recombinant OspE, critical regions of 0.13, 0.08, and 0.04 defined positive results, whereas a cutoff value of 0.04 was established for all serum dilutions for OspC, p35, and p41-G. Cutoff regions for OspF and p39 antigens were, respectively, 0.07, 0.04, and 0.04 and 0.15, 0.15, and 0.09 for serum dilutions of 1:160, 1:320, and ≥1:640. Cutoff values for tests with VlsE antigen were 0.07 and 0.03.

Specificity studies were continued as an extension of previous work (Magnarelli et al., 1986, 1999) to assess potential for false positive reactions in an ELISA incorporating new lots of recombinant antigens. Six broadly reactive positive control deer sera, two containing homologous antibodies to *A. phagocytophilum* and four homologous antibody-positive samples from animals inoculated with *B. burgdorferi;* plus six cattle sera with homologous antibodies to *Anaplasma marginale;* and one cow serum positive for *Brucella* antibodies were tested with the full panel of antigens. Positive cattle sera were included in these tests because these animals are closely related to deer. Companion blood samples for the two deer sera with antibodies to *A. phagocytophilum* were shown earlier (Magnarelli et al., 1999) to have DNA of this agent. Also, cattle with *A. marginale* antibodies were known to have red blood cells infected with this pathogen. In all analyses of cattle sera, peroxidase-labeled goat anti-bovine antibodies (Kirkegaard and Perry Laboratories) were diluted in PBSS to 1:8,000.

Western blot and PCR analyses were conducted to assess ELISA results for *A. phagocytophilum* antibodies. Matching whole blood and serum samples, obtained from 39 deer during 1996 in Connecticut, were used to determine concordance of results. Details on the preparation and use of lysates of infected and uninfected HL-60 cells and on other materials and procedures for immunoblotting have been described (Magnarelli et al., 1999). Similarly, methods used to prepare genomic DNA for PCR analyses and procedures applied to detect the DNA of *A. phagocytophilum* in whole-

blood samples have been reported (Magnarelli et al., 1999).

A *z*-test was used to determine significant differences in percentages of positive results. Analysis included the Yates' correction as a part of the statistical software program (SigmaStat, SPSS Inc., Chicago, Illinois, USA).

RESULTS

Sera tested from seven counties in Connecticut contained antibodies to *B. burgdorferi* (53% positive) or *A. phagocytophilum* (43% positive) when whole-cell antigens were incorporated in assays (Table 1). Differences in these percentages were statistically significant ($z=1.994$, $P=0.046$). When 10 or more sera were tested, seropositivity for *B. burgdorferi* antibodies ranged from 40% to 74%, while those for *A. phagocytophilum* ranged from 20% to 69%. In general, overall serum reactivities to recombinant *B. burgdorferi* antigens were much less frequent than that recorded for whole-cell antigens. An exception was noted for VlsE results, where the number of seropositives ($n=104$) nearly equaled the 115 positives for whole-cell antibodies. Positive findings for OspE ($n=1$), p39 ($n=1$), OspF ($n=46$), and the remaining recombinant *B. burgdorferi* antigens were much lower (seropositivity=21% or less). Moreover, 17 sera were positive to one or more recombinant antigens and negative by an ELISA with whole-cell antigen. In analyses for *A. phagocytophilum* antibodies, seroprevalence for the recombinant p44 antigen (52% positive) exceeded that calculated by IFA staining methods with whole-cell antigen (43%). Of the total 218 sera analyzed, 88 (40%) contained antibodies to whole-cell or recombinant antigens of both pathogens.

Serologic test results for 20 deer sera from South Carolina revealed comparatively fewer seropositives. Reactivity to recombinant p41G was most frequent ($n=11$ positives); antibody concentrations varied (1:160 to 1:1,280). The highest antibody titer (1:1,280) was recorded for two sera reactive to VlsE antigen. Six sera (30%) contained antibodies to whole-cell *B. burgdorferi*. Of these, five sera also had antibodies to recombinant p41G ($n=5$), VlsE ($n=1$), OspC ($n=1$), or OspF ($n=1$). Similarly, there were six seropositives for *A. phagocytophilum* whole-cell antibodies by IFA staining methods (titers=1:160 to 1:640), five of which also reacted to the p44 antigen by ELISA (titers=1:640 to 1:1:2,560). Three deer sera contained antibodies to *B. burgdorferi* and *A. phagocytophilum*.

Seropositivity rates for an ELISA with VlsE antigen were highly concordant with those determined by an ELISA with whole-cell *B. burgdorferi* antigens. Of the 218 Connecticut sera screened by both assays, 92 and 90 sera were positive and negative, respectively, in both assays (84% agreement). Results for the remaining 36 sera differed; 23 samples were positive to whole-cell antigen and negative to VlsE, while the reverse was noted for 13 sera.

Antibody test results for *A. phagocytophilum* revealed a concordance of 71% when findings for an ELISA incorporating the p44 antigen were compared with those of IFA staining methods with whole-cell antigens. Seventy-six sera were positive in both tests, while 88 samples were negative. Thirty-seven other sera reacted to the p44 antigen by an ELISA but were negative by IFA methods. The remaining 17 sera were positive by IFA and negative by an ELISA.

Concentrations of antibodies varied. In analyses for *B. burgdorferi* immunoglobulins, antibody titers generally ranged between 1:160 and 1:5,120 (Table 2). Maximal titration endpoints and an elevated geometric mean (698) were recorded when VlsE antigen was incorporated into an ELISA. Two sera had antibody titers of 1:10,240 to this antigen. Similar results were obtained when the p44 recombinant antigen was used in an ELISA. Five sera had antibody titers of 1:10,240, while one serum sample had an endpoint of 1:40,980.

Matching whole blood and serum samples were analyzed to assess concordance

TABLE 2. Frequency distributions and geometric means of reciprocal antibody titers for reactivity of Connecticut deer sera to whole-cell or recombinant antigens of *Borrelia burgdorferi* or *Anaplasma phagocytophilum*.

Antigens	No. of positive sera	Antibody titers[a]					Geometric means
		80	160–320	640–1,280	2,560–5,120	10,240–40,980	
B. burgdorferi whole cell	115	NA	61	51	3	0	427
OspC	15	NA	14	1	0	0	266
OspE	1	NA	1	0	0	0	320
OspF	46	NA	20	22	4	0	453
p35	28	NA	22	6	0	0	312
p39	1	NA	0	1	0	0	1,280
p41-G	18	NA	16	2	0	0	235
VlsE	104	NA	41	45	16	2	698
A. phagocytophilum whole cell	93	57	24	9	3	0	145
p44	113	NA	36	52	19	6	823

[a] Reciprocal antibody titers as determined by ELISA (≥1:160) with recombinant antigens or whole-cell *B. burgdorferi*. Serum dilutions of ≥1:80 were positive by IFA staining methods with whole-cell *A. phagocytophilum*. Geometric means computed for positive results only. NA = not applicable.

of PCR, p44, and immunoblot results for *A. phagocytophilum*. There was good agreement (74%) in antibody test results by ELISA and immunoblotting methods.

TABLE 3.. Comparison of laboratory results for detection of antibodies to or DNA of *Anaplasma phagocytophilum* in 39 matching serum and blood samples obtained from Connecticut deer in 1996.

Laboratory analyses[a]	No. positive (%)[b]
p44 ELISA and IB	
Positive in both tests	19 (49)
Negative in both tests	10 (26)
Positive by ELISA, negative by IB	10 (26)
Negative by ELISA, positive by IB	0 (0)
p44 ELISA and PCR[c]	
Positive in both tests	6 (15)
Negative in both tests	6 (15)
Positive by ELISA, negative by PCR	23 (59)
Negative by ELISA, positive by PCR	4 (10)
IB and PCR[c]	
Positive in both tests	0 (0)
Negative in both tests	10 (26)
Positive by IB, negative by PCR	19 (49)
Negative by IB, positive by PCR	10 (26)

[a] ELISA contained p44 recombinant antigen, while immunoblotting (IB) was performed with whole-cell lysates of *A. phagocytophilum*.

[b] Percent positive equals number of positive samples per category divided by 39.

[c] Whole deer blood samples tested by polymerase chain reaction (PCR) methods with hge-44 primers.

Of the 39 sera tested by both procedures, 19 and 10 were positive or negative, respectively, by both assays (Table 3). A comparison of PCR and ELISA results, however, showed a lower concordance value (31%). When findings were positive in both tests, antibody titers ranged between 1:320 and 1:1,280. A similar range of titers (1:320 to 1:5,120) was noted when sera were positive by an ELISA and negative by immunoblotting methods. Titration endpoints were also elevated (1:320 to 1:40,980) when samples were positive by ELISA and negative for DNA. Concordance was lowest (26%) when results of immunoblotting were compared to PCR findings.

Antibody-positive and -negative sera were retested to assess reproducibility of results. Titers for five sera with *B. burgdorferi* antibodies to VlsE were the same ($n=1$) or varied by twofold ($n=2$) or fourfold ($n=2$) in the second trial. In tests of five positive sera for *A. phagocytophilum* antibodies, results were the same ($n=1$) or differed by twofold ($n=3$) or fourfold ($n=1$). Results for the three negative sera remained unchanged when reanalyzed by ELISA with the VlsE or p44 antigens.

Minor cross-reactivity occurred when

cattle sera containing antibodies to *A. marginale* or *Brucella* were tested with whole-cell or recombinant antigens of *B. burgdorferi* or *A. phagocytophilum.* One serum with *A. marginale* antibodies was positive to p41G and VlsE antigens at titers of 1:160 and 1:320, respectively, while another sample in this group reacted with p44 (titer=1:640) antigen. The serum sample with *Brucella* antibodies reacted to whole-cell *B. burgdorferi* in an ELISA at a titer of 1:320. The remaining four cattle sera and the positive control deer sera were negative to all heterologous antigens included in this study.

DISCUSSION

Serologic test results for *B. burgdorferi* and *A. phagocytophilum* show widespread occurrence of these pathogens in Connecticut and presence in South Carolina. As suggested before (Little et al., 1998; Magnarelli et al., 1999; Arens et al., 2003), some deer may be simultaneously infected by multiple pathogens. Although deer do not appear to be reservoirs for *B. burgdorferi,* coinfections may be significant for *A. phagocytophilum* infections. For example, concurrent infections in mice resulted in increased concentrations of both pathogens and more severe Lyme arthritis, compared to mice that had only *B. burgdorferi* infection (Thomas et al., 2001).

Although antibodies were produced to whole cells and to one or more recombinant antigens of *B. burgdorferi,* seropositivity to VlsE greatly exceeded serum reactivities to all other fusion proteins of this pathogen and was comparable to overall results for an ELISA with whole cells. There were several positives for OspF antibodies but at much lower frequency. A similar pattern of reactivity was noted when human sera from patients who had erythema migrans were tested for class-specific antibodies (Magnarelli et al., 2002b). The VlsE antigen was judged to be the most suitable test antigen for diagnosis of early infections because of its high sensitivity and specificity. Other investigators have demonstrated utility of this antigen, regardless of whether a full-length recombinant VlsE (Lawrenz et al., 1999; Bacon et al., 2003; Schulte-Spechtel et al., 2003) or a peptide corresponding to the invariant IR6 region of the VlsE antigen (Liang et al., 2001; Bacon et al., 2003) was used in an ELISA. The VlsE antigen evaluated in the present study is a desirable test antigen for confirming past or current *B. burgdorferi* infections in deer. Information on serum reactivity to OspF is also useful for confirmatory purposes.

The variable findings for antibody-positive deer sera at different sites in Connecticut parallel those reported for horses there (Magnarelli et al., 2000b) and for deer elsewhere (Gallivan et al., 1998). It is clear that there are numerous foci for both pathogens in Connecticut, where *I. scapularis* ticks are abundant. There also is evidence of these infectious agents in deer and horses in the southeastern United States (Mahnke et al., 1993; Magnarelli et al., 2001c), but seroprevalence appears to be much lower there. Regional and local differences in seropositivity values for *B. burgdorferi* are probably due, in part, to variable sample sizes, numbers of infected ticks feeding on hosts, variations in host immune responses among subjects to the same or different strains of the spirochete (Lane et al., 1994; Luttrell et al., 1994), and the timing of antibody responses relative to sampling (Gallivan et al., 1998). In experimentally challenged deer, antibody responses to *B. burgdorferi* occurred rapidly after inoculation (Luttrell et al., 1994) and peaked 6 to 7 wk later. However, it is unknown how long these immunoglobulins persist over several months. There is less information on humoral responses to *A. phagocytophilum* and persistence of antibodies in deer. Therefore, interpretation of seropositivity rates is difficult. High prevalences of seropositive specimens may not necessarily correlate with prevalence of infected ticks at selected sites.

In analyses for antibodies to *A. phagocytophilum,* seroprevalence for the p44 re-

combinant antigen in an ELISA exceeded that for whole-cell antigens determined by IFA staining methods. Earlier studies demonstrated the value of this antigen in analyses of human and horse sera (IJdo et al., 1999; Magnarelli et al., 2001c) where Western blot analyses confirmed ELISA results. In the present study, there was relatively high concordance when results of immunoblotting and ELISA were compared. Detection of *A. phagocytophilum* DNA for some samples supports evidence of infection. Therefore, reactivity of deer sera to the p44 antigen appears to be an important indicator of exposure to *A. phagocytophilum.*

A lower concordance value was noted when antibody test results for *A. phagocytophilum* were compared with PCR findings. In earlier studies of deer (Belongia et al., 1997; Arens et al., 2003), which focused on *B. burgdorferi, Ehrlichia chaffeensis,* or *Ehrlichia ewingii,* and of horses with *A. phagocytophilum* infections (Van Andel et al., 1998; Magnarelli et al., 2001c), investigators found that DNA findings sometimes do not correlate well with antibody test results. Pathogens may be present in blood for relatively short periods (i.e., a few weeks) following initial infection via tick bites. During early infection, antibody concentrations are usually too low to be detected by an ELISA, regardless of the antigen used. With time and an ensuing expansion in humoral responses, antibody concentrations rise and, therefore, along with mounting cell-mediated immune responses, may be sufficient to depress pathogen concentrations. For laboratory diagnosis of equine granulocytic ehrlichiosis, it was concluded that DNA analyses were most suitable during the early days of acute infection, while antibody tests were more practical several days later when horses were convalescing (Van Andel et al., 1998; Magnarelli et al., 2001c). Therefore, different methods should be used to confirm *A. phagocytophilum* infections. In the present study, it is unknown when deer were infected with this agent, but, as observed for horse infections (Van Andel et al., 1998), it is possible that the positive DNA results were indicating recent infections associated with the bites of *I. scapularis* females during the fall.

Results of specificity testing indicated minor cross-reactivity when cattle sera with *A. marginale* or *Brucella* antibodies were screened against *B. burgdorferi* and *A. phagocytophilum* antigens. In general, heterologous antibody titers were relatively low, and the false positives were probably a result of normal assay variability. In tests of reproducibility, up to fourfold variation of antibody titers was recorded. In earlier studies (Magnarelli et al., 1986), low-titered reactions (1:64 to 1:128) were noted when deer sera containing antibodies to *B. burgdorferi* were screened by IFA staining methods against *Leptospira interrogans* serovars (pomona, hardjo, and icterohemorrhagiae) and *Treponema denticola.* In southern states where lone star ticks (*Amblyomma americanum*) are abundant, *Borrelia lonestari* may geographically coexist with *B. burgdorferi* in some areas. Serologic cross-reactivity in antibody tests is possible because many antigens are shared among *Borrelia* species. Therefore, caution should be used when interpreting low-titered reactions. Also, it is important to know the geographic distributions and host records for pathogens; *B. lonestari* and *A. marginale* are not known to occur in the northeastern United States. Although predominantly a pathogen of cattle, the latter has been reported infecting deer and elk (*Cervus elaphus*) in other regions of the United States (Keel et al., 1995; Zaugg et al., 1996).

An ELISA with recombinant VlsE or p44 antigens is suitable for testing deer sera for antibodies to *B. burgdorferi* and *A. phagocytophilum* in the eastern United States. However, depending on genotypic differences of the pathogen strains present in widely separated regions and immune responses of hosts, key outer surface proteins (relied on as markers in laboratory

tests) may be variably expressed or differentially recognized immunologically. Studies are needed to determine whether both antigens are acceptable for analyses of deer sera in the western United States or Europe where related *Ixodes* ticks occur. Previous work has demonstrated that a synthetic peptide based on the VlsE IR6 of *Borrelia garinii* (strain Ip90) has an epitope that frequently reacts with human serum antibodies produced in Lyme borreliosis infections in the United States and Europe (Liang et al., 1999, 2000, 2001), but consistency of results sometimes varied. Nonetheless, in heavily tick infested areas of the northeastern United States, an ELISA with VlsE or p44 antigens can be used as an adjunct procedure along with assays containing whole-cell antigens for general screening purposes to determine whether deer are exposed to *B. burgdorferi* and *A. phagocytophilum,* while PCR analyses can be relied on to provide more direct evidence of infection.

ACKNOWLEDGMENTS

We are grateful to T. Blevins for technical assistance and acknowledge financial support from the Centers for Disease Control and Prevention including the Emerging Infections Program (HR8/CCH113382-01; U5O/CCU111188-01) and Hatch funds administered by the United States Department of Agriculture. We also thank S. J. Norris of the University of Texas Medical School in Houston for kindly providing the VlsE antigen and for his helpful comments on an early draft of the manuscript. J.W.I. was supported by a fellowship from the L.P. Markey Charitable Trust. E.F. is the recipient of a Burroughs Wellcome Clinical Scientist Award in Translational Research.

LITERATURE CITED

ANONYMOUS. 2002. Notification that new names and new combinations have appeared in volume 51, part 6 of the IJSEM. International Journal of Systematic and Evolutionary Microbiology 52: 5–6.

ARENS, M. Q., A. M. LIDDELL, G. BUENING, M. GAUDREAULT-KEENER, J. W. SUMNER, J. A. COMER, R. S. BULLER, AND G. A. STORCH. 2003. Detection of *Ehrlichia* spp. in the blood of wild white-tailed deer in Missouri by PCR assay and serologic analysis. Journal of Clinical Microbiology 41: 1263–1265.

BACON, R. M., B. J. BIGGERSTAFF, M. E. SCHRIEFER, R. D. GILMORE JR., M. T. PHILIPP, A. C. STEERE, G. P. WORMSER, A. R. MARQUES, AND B. J. B. JOHNSON. 2003. Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with the 2-tiered testing using whole-cell lysates. Journal of Infectious Diseases 187: 1187–1199.

BELONGIA, E. A., K. D. REED, P. D. MITCHELL, C. P. KOLBERT, D. H. PERSING, J. S. GILL, AND J. J. KAZMIERCZAK. 1997. Prevalence of granulocytic *Ehrlichia* infection among white-tailed deer in Wisconsin. Journal of Clinical Microbiology 35: 1465–1468.

DUMLER, J. S., A. F. BARBET, C. P. J. BEKKER, G. A. DASCH, G. H. PALMER, S. C. RAY, Y. RIKIHISA, AND F. R. RURANGIRWA. 2001. Reorganization of the genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales:* Unification of some species of *Ehrlichia* with *Anaplasma, Cowdria* with *Ehrlichia,* and *Ehrlichia* with *Neorickettsia,* descriptions of six new species combinations and designation of *Ehrlichia equi* and "HGE agent" as subjective synonyms of *Ehrlichia phagocytophila.* International Journal of Systematic and Evolutionary Microbiology 51: 2145–2165.

GALLIVAN, G. J., I. K. BARKER, H. ARTSOB, L. A. MAGNARELLI, J. T. ROBINSON, AND D. VOIGT. 1998. Serologic survey for antibodies to *Borrelia burgdorferi* in white-tailed deer in Ontario. Journal of Wildlife Diseases 34: 411–414.

IJDO, J. W., C. WU, L. A. MAGNARELLI, AND E. FIKRIG. 1999. Serodiagnosis of human granulocytic ehrlichiosis by a recombinant HGE-44-based enzyme-linked immunosorbent assay. Journal of Clinical Microbiology 37: 3540–3544.

KEEL, M. K., W. L. GOFF, AND W. R. DAVIDSON. 1995. An assessment of the role of white-tailed deer in the epizootiology of anaplasmosis in the southeastern United States. Journal of Wildlife Diseases. 31: 378–385.

LANE, R. S., D. P. BERGER, L. E. CASHER, AND W. BURGDORFER. 1994. Experimental infection of Columbian black-tailed deer with the Lyme disease spirochete. Journal of Wildlife Diseases 30: 20–28.

LAWRENZ, M. B., J. M. HARDHAM, R. T. OWENS, J. NOWAKOWSKI, A. C. STEERE, G. P. WORMSER, AND S. J. NORRIS. 1999. Human antibody responses to VlsE antigenic variation protein of *Borrelia burgdorferi.* Journal of Clinical Microbiology 37: 3997–4004.

LIANG, F. T., A. C. STEERE, A. R. MARQUES, B. J. B. JOHNSON, J. N. MILLER, AND M. T. PHILIPP. 1999. Sensitive and specific serodiagnosis of Lyme disease by enzyme-linked immunosorbent assay with a peptide based on an immunodominant conserved region of *Borrelia burgdorferi*

VlsE. Journal of Clinical Microbiology 37: 3990–3996.

———, E. ABERER, M. CINCO, L. GERN, C. M. HU, Y. N. LOBET, M. RUSCIO, P. E. VOET JR., V. E. WEYNANTS, AND M. T. PHILIPP. 2000. Antigenic conservation of an immunodominant invariable region of the VlsE lipoprotein among European pathogenic genospecies of *Borrelia burgdorferi* SL. Journal of Infectious Diseases 182: 1455–1462.

———, L. BOWERS, AND M. T. PHILIPP. 2001. C-terminal invariable domain of VlsE is immunodominant but its antigenicity is scarcely conserved among strains of Lyme disease spirochetes. Infection and Immunity 69: 3224–3231.

LITTLE, S. E., D. E. STALLKNECHT, J. M. LOCKHART, J. E. DAWSON, AND W. R. DAVIDSON. 1998. Natural coinfection of a white-tailed deer population with three *Ehrlichia* spp. Journal of Parasitology 84: 897–901.

LUTTRELL, M. P., K. NAKAGAKI, E. W. HOWERTH, D. E. STALLKNECHT, AND K. A. LEE. 1994. Experimental infection of *Borrelia burgdorferi* in white-tailed deer. Journal of Wildlife Diseases 30: 146–154.

MAGNARELLI, L. A., J. F. ANDERSON, C. S. APPERSON, D. FISH, R. C. JOHNSON, AND W. A. CHAPPELL. 1986. Spirochetes in ticks and antibodies to *Borrelia burgdorferi* in white-tailed deer from Connecticut, New York State, and North Carolina. Journal of Wildlife Diseases 22: 178–188.

———, A. DENICOLA, K. C. STAFFORD III, AND J. F. ANDERSON. 1995. *Borrelia burgdorferi* in an urban environment: White-tailed deer with infected ticks and antibodies. Journal of Clinical Microbiology 33: 541–544.

———, J. W. IJDO, J. F. ANDERSON, S. J. PADULA, R. A. FLAVELL, AND E. FIKRIG. 1998. Human exposure to a granulocytic *Ehrlichia* and other tick-borne agents in Connecticut. Journal of Clinical Microbiology 36: 2823–2827.

———, ———, K. C. STAFFORD III, AND E. FIKRIG. 1999. Infections of granulocytic ehrlichiae and *Borrelia burgdorferi* in white-tailed deer in Connecticut. Journal of Wildlife Diseases 35: 266–274.

———, ———, S. J. PADULA, R. A. FLAVELL, AND E. FIKRIG. 2000a. Serologic diagnosis of Lyme borreliosis by using enzyme-linked immunosorbent assays with recombinant antigens. Journal of Clinical Microbiology 38: 1735–1739.

———, ———, A. E. VAN ANDEL, C. WU, S. J. PADULA, AND E. FIKRIG. 2000b. Serologic confirmation of *Ehrlichia equi* and *Borrelia burgdorferi* infections in horses from the northeastern United States. Journal of the American Veterinary Medical Association 217: 1045–1050.

———, ———, C. WU, AND E. FIKRIG. 2001a. Recombinant protein-44-based class-specific enzyme-linked immunosorbent assays for serologic diagnosis of human granulocytic ehrlichiosis. European Journal of Clinical Microbiology and Infectious Diseases 20: 482–485.

———, S. A. LEVY, J. W. IJDO, C. WU, S. J. PADULA, AND E. FIKRIG. 2001b. Reactivity of dog sera to whole-cell or recombinant antigens of *Borrelia burgdorferi* by ELISA and immunoblot analysis. Journal of Medical Microbiology 50: 889–895.

———, J. W. IJDO, A. E. VAN ANDEL, C. WU, J. H. OLIVER JR., AND E. FIKRIG. 2001c. Reactivity of serum samples of dogs and horses tested by use of class-specific recombinant-based enzyme-linked immunosorbent assays for detection of granulocytic ehrlichiosis. American Journal of Veterinary Research 9: 1365–1369.

———, ———, B. A. SHERMAN, S. L. BUSHMICH, S. A. LEVY, AND E. FIKRIG. 2002a. Antibodies to granulocytic ehrlichiae in cattle from Connecticut. Journal of Medical Microbiology 51: 326–331.

———, M. LAWRENZ, S. J. NORRIS, AND E. FIKRIG. 2002b. Comparative reactivity of human sera to recombinant VlsE and other *Borrelia burgdorferi* antigens in class-specific enzyme-linked immunosorbent assays for Lyme borreliosis. Journal of Medical Microbiology 51: 649–655.

MAHNKE, G., D. E. STALLKNECHT, C. E. GREENE, V. F. NETTLES, AND M. A. MARKS. 1993. Serologic survey for antibodies to *Borrelia burgdorferi* in white-tailed deer in Georgia. Journal of Wildlife Diseases 29: 230–236.

SCHULTE-SPECHTEL, U., G. LEHNERT, G. LIEGL, V. FINGERLE, C. HEIMERL, B. J. B. JOHNSON, AND B. WILSKE. 2003. Significant improvement of the recombinant *Borrelia*-specific immunoglobulin G immunoblot test by addition of VlsE and DbpA homologue derived from *Borrelia garinii* for diagnosis of early neuroborreliosis. Journal of Clinical Microbiology 41: 1299–1303.

THOMAS, V., J. ANGUITA, S. W. BARTHOLD, AND E. FIKRIG. 2001. Coinfection with *Borrelia burgdorferi* and the agent of human granulocytic ehrlichiosis alters murine immune responses, pathogen burden, and severity of Lyme arthritis. Infection and Immunity 69: 3359–3371.

VAN ANDEL, A. E., L. A. MAGNARELLI, R. HEIMER, AND M. WILSON. 1998. Development and duration of antibody response against *Ehrlichia equi* in horses. Journal of the American Veterinary Medical Association 212: 1910–1914.

WALKER, D. H. AND J. S. DUMLER. 1996. Emergence of the ehrlichioses as human health problems. Emerging Infectious Diseases 2: 18–29.

———, A. G. BARBOUR, J. H. OLIVER, R. S. LANE, J. S. DUMLER, D. T. DENNIS, D. H. PERSING, A. F. AZAD, AND E. MCSWEEGAN. 1996. Emerging bacterial zoonotic and vector-borne diseases: Ecological and epidemiological factors. Journal

of the American Medical Association 275: 463–469.

ZAUGG, J. L., W. L. GOFF, W. FOREYT, AND D. L. HUNTER. 1996. Susceptibility of elk (*Cervus elaphus*) to experimental infection with *Anaplasma marginale* and *A. ovis*. Journal of Wildlife Diseases 32: 62–66.

Received for publication 14 July 2003.