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SERUM ANTIBODIES TO WHOLE-CELL AND RECOMBINANT ANTIGENS OF *BORRELIA BURGDORFERI* IN COTTONTAIL RABBITS

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ABSTRACT: Archived serum samples, from 95 eastern cottontail rabbits (*Sylvilagus floridanus*) captured in New York, New York, USA and Millbrook, New York, USA, during 1985–86, were analyzed in solid-phase enzyme-linked immunosorbent assays (ELISA) for total and class-specific immunoglobulin (Ig) M antibodies to whole-cell or recombinant antigens of *Borrelia burgdorferi* sensu stricto. Using a polyvalent conjugate, rabbit sera contained antibodies to whole-cell and recombinant antigens (protein [p]35, p37, or VlsE) during different seasons, but there was no reactivity to outer surface protein (Osp)A or OspB. Seventy-six of the 102 sera (75%) analyzed were reactive with one or more of the antigens; 61 of the positive samples (80%) reacted to whole-cell antigens, followed by results for the p35 (58%, 44/76), VlsE (43%, 33/76), and p37 (29%, 22/76) antigens. Fifty-eight sera (76%) contained antibodies to the VlsE or p35 antigens with or without reactivity to whole-cell antigens. High antibody titers (≥1:2,560) recorded for 52 sera indicate robust antibody production. In analyses for IgM antibodies in an ELISA containing whole-cell antigens, there were 30 positive sera; titers ranged from 1:160 to 1:640. There was minimal cross-reactivity when rabbit antisera to *Treponema pallidum* or four serovars of *Leptospira interrogans* were screened against *B. burgdorferi* antigens. Based on more-specific results, VlsE and p35 antigens appear to be useful markers for detecting possible *B. burgdorferi* infections.

Key words: Antibodies, *Borrelia burgdorferi*, ELISA, *Sylvilagus floridanus*.

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INTRODUCTION

Rabbits and other lagomorphs, heavily parasitized by ticks and exposed to several species of hematophagous insects, have worldwide importance as known or suspected reservoirs for the agents of Crimean-Congo hemorrhagic fever, Rocky Mountain spotted fever, tick-borne relapsing fever, tularemia, and Lyme borreliosis (Dennis and Piesman, 2005). The isolation of a *Babesia* species from eastern cottontail rabbits (*Sylvilagus floridanus*) in tick-infested areas of Nantucket Island, Massachusetts, USA (Holman et al., 2005), further illustrates the diversity of etiologic agents associated with these hosts. By harboring an array of pathogens, rabbits might be serving as reservoirs of multiple infectious agents. Extensive field and laboratory studies in northeastern United States have revealed that cottontail rabbits and the ticks that feed on these hosts harbor antigenically variable strains of *Borrelia burgdorferi* sensu lato (Anderson et al., 1989). Some of these isolates are genetically similar to those recovered from *Ixodes scapularis* ticks and cause human infections. However, another bacterium (*Borrelia andersonii*), isolated from an engorged larval *Ixodes dentatus* tick removed from a rabbit, is not known to cause human disease (Marconi et al., 1995). It is reported that cottontail rabbits can perpetuate the Lyme disease agent (*B. burgdorferi*) in an enzootic cycle where *I. dentatus* abounds (Telford and Spielman, 1989a, b).

In studies of cottontail rabbits, antibody-detection assays have been used as a preliminary step to characterize isolates of *B. burgdorferi*, to determine prevalence of particular antibodies in animal populations, and to provide information on comparative reactivities of sera with

whole-cell lysates or a selected group of recombinant antigens (Anderson et al., 1989; Telford and Spielman, 1989b; Magnarelli et al, 1990; Fikrig et al., 1993) in enzyme-linked immunosorbent assays (ELISA) or Western blot analyses. These studies revealed that the use of whole-cell antigens or flagellin in seroanalyses led to false-positive reactions. Since then, more-specific antigens, such as VlsE of *B. burgdorferi*, have been developed. Use of this antigen in analyses of human, canine, and deer sera yielded highly sensitive and specific results (Lawrenz et al., 1999; Liang et al., 1999, 2000; Magnarelli et al., 2010). Therefore, it was important to test key recombinant (fusion) proteins of this etiologic agent to identify which antigens were most suitable for a conventional ELISA to detect possible *B. burgdorferi* infections. Our objectives were to measure antibody titers for *B. burgdorferi* in cottontail rabbits by applying noncompetitive ELISA methods with separate preparations of whole-cell and recombinant antigens and to determine which of the recombinant proteins may be suitable antigens for preliminary laboratory verification of past or current *B. burgdorferi* infections.

MATERIALS AND METHODS

Cottontail rabbits were captured in wooden box traps, located in the New York Botanical Gardens in New York, New York, USA (40°51′N, 73°52′W) and in the Mary Flagler Cary Arboretum in Millbrook, New York, USA (41°47′N, 73°42′W), during 1985–86. Whole-blood samples were obtained from 95 rabbits, seven of which (7%) were recaptured within 3 wk of the original sampling date. Following centrifugation, 102 serum samples were stored at −60 C at the Connecticut Agricultural Experiment Station (CAES, New Haven Connecticut, USA), as previously reported (Anderson et al., 1989; Magnarelli et al., 1990), until antibody testing with new highly specific antigens could be conducted.

A polyvalent, solid-phase ELISA (Magnarelli et al., 1984, 1990, 2004) was used initially to test sera separately with whole-cell lysates of *B. burgdorferi* (strain 2591) or with the following recombinant antigens: VlsE, outer surface protein (Osp)A, OspB, protein (p)35, and p37. In subsequent analyses, class-specific ELISA, containing whole-cell antigens, were used to measure immunoglobulin (Ig) M antibodies. The recombinant VlsE1-His was produced at the University of Texas Medical School at Houston (Houston, Texas, USA). When incorporated in an ELISA, this His_6-tagged version of the full-length, surface-exposed VlsE protein has improved laboratory diagnosis of *B. burgdorferi* infections for different mammalian hosts (Lawrenz et al., 1999; Liang et al., 2000, 2001; Bacon et al., 2003; Magnarelli et al., 2010) in the United States. In immunoblot analyses of human sera for class-specific antibodies to *Borrelia garinii* (Goettner et al., 2005), sensitive and specific results were also obtained when a VlsE antigen was used. The OspA and OspB antigens, produced at Yale University, are specific markers for *B. burgdorferi* and *Borrelia* species, respectively. Finally, p35 and p37, also produced at Yale University, were included in analyses because they were highly reactive when tested with human and dog sera (Fikrig et al., 1997; Magnarelli et al., 2001), they are synthesized in the early stages of mammalian *B. burgdorferi* infections, and they elicited protective immunity in experimentally challenged mice (Fikrig et al., 1997). The VlsE antigen was coated to flat-bottom polystyrene plates (NUNC A/S, Roskilde, Denmark) at a concentration of 1 μg/ml, whereas all other antigens were coated at 5 μg/ml. Polyvalent, horseradish peroxidase–labeled goat anti-rabbit immunoglobulins (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA) were diluted to 1:15,000 and 1:12,000 in phosphate-buffered saline (PBS) for the VlsE and other antigens, respectively. Rabbit sera also were tested for class-specific IgM (μ-chain specific) antibodies by ELISA methods. This peroxidase-labeled goat anti-rabbit reagent was commercially prepared (SouthernBiotech, Birmingham, Alabama, USA) and diluted in PBS to 1:4,000. In all tests, 60 μl of commercially prepared 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate) substrate (Kirkegaard and Perry) were delivered to each well. Absorbance values (optical densities [OD]) of all preparations were measured by a microplate reader at 414 nm, as described earlier (Magnarelli et al., 1984). A reference rabbit antiserum to *B. burgdorferi* sensu stricto, included in earlier studies (Magnarelli et al., 1990; Fikrig et al., 1993), served as a positive control. The homologous antibody titers were 1:20,480 and 1:5,120, as determined by polyvalent and class-specific IgM assays, respectively. Positive dog sera,

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analyzed earlier (Magnarelli et al., 1987, 2001) with horseradish peroxidase-conjugated anti-dog immunoglobulins, were included to further verify antigen reactivity. Negative control sera were obtained from New Zealand white rabbits (*Oryctolagus cuniculus*) not exposed to ticks or *B. burgdorferi*. All plates contained positive and negative sera to ensure standardization, plus controls for PBS, conjugated antibodies, and affinity-purified, nonconjugated glutathione *S*-transferase. The latter was included as an additional check because it is a component of some of the fusion proteins.

To determine critical regions for positive results, 14 negative rabbit sera were tested with each antigen. Net absorbance values, the differences in OD readings with or without antigen for serum dilutions of 1:160, 1:320, and 1:640, were calculated. For each serum dilution, cutoff values were established by statistical analyses (i.e., the mean±3 SD of the net absorbance values for the negative control sera). Critical regions for serum dilutions of 1:640 were used to define reactivity at higher serum dilutions when test sera were titrated to determine end points. In a polyvalent ELISA with whole-cell antigens, net OD values of 0.30, 0.19, and 0.16 were considered positive for the respective serum dilutions of 1:160, 1:320, and ≥1:640. Lower cutoff values for the VlsE antigen (0.09, 0.05, and 0.04) reflect minimal background readings in the control plate wells. Similar critical regions were computed for OspA (0.13, 0.09, and 0.04) and OspB (0.07, 0.03, and 0.03) antigens. Cutoff values for the p35 (0.19, 0.16, and 0.07) and p37 (0.37, 0.21, and 0.13) antigens were comparable to those defined for whole-cell antigens. In tests for IgM antibodies, a net OD value of 0.08 was considered positive at serum dilutions of 1:160, 1:320, and ≥1:640 when whole-cell antigens were used. Test sera were diluted in PBS to 1:160, 1:320, and ≥1:640. If positive at ≥1:640, the sera were retested on another day to determine titration end points and to check reproducibility of results. Antibody titers of <1:160 were considered negative.

A group of reference rabbit antisera, stored at the CAES and available from previous studies (Magnarelli et al., 1990; Fikrig et al., 1993), were used to assess cross-reactivity in an ELISA. Even though prior testing of *B. burgdorferi* recombinant antigens with human and dog sera (Magnarelli et al., 2001, 2002) revealed little or no cross-reactivity with VlsE, p35, and p37 antigens, it was necessary to check for possible cross-reactivity of the reference rabbit sera in our assays with these antigens. Antisera to *Treponema pallidum* and *Leptospira interrogans* serovars canicola (strain Mouton), grippotyphosa (strain SC4397), icterohemorrhagiae (strain CF-1), and pomona (strain MLS) were tested in comparative analyses.

A z-test was used to determine whether percentages of positive results were statistically different. The software program, SigmaStat (formerly SPSS Inc., now IBM Corporation, Armonk, New York, USA) includes the Yates' correction in the analyses.

RESULTS

Cottontail rabbits had antibodies to whole-cell or recombinant antigens of *B. burgdorferi* during all months and seasons of sampling (Table 1). There were no detectable differences in antibody titers or reactivity of sera representing animals captured in the two study sites. Seventy-six of the 102 sera (75%) tested were reactive with one or more of the antigens tested; of these 76 positive samples, 61 (80%) reacted in a polyvalent ELISA containing whole-cell antigens, followed by results for the p35 (58%), VlsE (43%), or p37 (29%) antigens. The difference in antibody prevalences determined for the VlsE and p35 antigens was not statistically significant ($z=1.478$, $P=0.139$). However, the difference in antibody prevalence determined for an ELISA with whole-cell antigens versus the p35 antigen was statistically significant ($z=2.289$, $P=0.022$). Thirty-five sera were collected in March 1985 and again in May 1986. When results were compared for each of these months, antibody prevalences paralleled those of overall rates, with the highest percentages recorded for whole-cell antigens and the lowest percentages for the p37 antigen. The difference in antibody prevalence for whole-cell antigen in March 1985 (77%) compared with May 1986 (34%) was statistically significant ($z=3.379$, $P<0.001$), but the difference for the VlsE antigen in March 1985 (37%) versus May 1986 (20%) was not ($z=1.311$, $P=0.190$). There was no reactivity of rabbit sera with OspA or OspB antigens. A reference antiserum from a cottontail rabbit inoculated with culture-derived *B. burgdorferi* sensu stricto was reactive to all antigens

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TABLE 1. Seasonal prevalence of total antibodies to whole-cell (WC) or recombinant antigens (VlsE, and proteins p35 and p37) of *Borrelia burgdorferi* in sera of cottontail rabbits (*Sylvilagus floridanus*), as determined by polyvalent enzyme-linked immunosorbent assays, in samples from two sites in New York State, USA, 1985–86.

Month and year	No. sera tested	No. (%) of sera with antibodies to *B. burgdorferi*[a]			
		WC	VlsE	p35	p37
March 1985	35	27 (77)	13 (37)	23 (66)	11 (31)
April 1985	9	8 (89)	2 (22)	3 (33)	3 (33)
October 1985	3	2 (67)	2 (67)	2 (67)	0 (0)
November 1985	8	5 (63)	3 (38)	5 (63)	3 (38)
May 1986	35	12 (34)	7 (20)	8 (23)	4 (11)
June 1986	7	4 (57)	4 (57)	1 (14)	1 (14)
November 1986	5	3 (60)	2 (40)	2 (40)	0 (0)
Totals	102	61 (60)	33 (32)	44 (43)	22 (21)

[a] Numbers across the rows do not equal numbers tested because 50 sera reacted to multiple antigens.

except OspA and OspB. The positive dog sera, used as controls, verified reactivity of OspA and OspB antigens. Thirty rabbit sera contained IgM antibodies to whole-cell antigens. Antibody prevalence (29%) was relatively low. During 1985, positive sera were obtained in March ($n=16$), April ($n=1$), and November ($n=2$), whereas in 1986, IgM antibodies were detected in samples collected in May ($n=7$), June ($n=2$), and November ($n=2$).

Antibody titers varied. The highest antibody titer (1:20,480) was recorded in analyses with whole-cell or VlsE antigens (Table 2). Correspondingly elevated geometric means were noted. Results for an ELISA with the p35 antigen incorporated, however, revealed marked skewness toward lower antibody titers; 22 (50%) of the 44 positive sera had an antibody titer of 1:160. In whole-cell tests for IgM antibodies, titers ranged from 1:160 to 1:640.

Ten or more positive and three negative sera were reanalyzed on different days to check reproducibility of antibody titers. In tests with whole-cell antigens, positive titers were unchanged for three sera, varied by twofold ($n=4$), or differed by fourfold ($n=3$). Titration end points for total immunoglobulins were unchanged for four sera, varied by twofold for two sera, fourfold for three sera, and eightfold for one sample when the VlsE antigen was incorporated in a polyvalent ELISA. Similar results were recorded when the p35 antigen was tested; titers varied by twofold ($n=6$), fourfold ($n=2$), or eightfold ($n=2$). Antibody results were likewise reproducible when positive sera were retested with the p37 antigen. Antibody titers were unchanged ($n=6$ samples), or

TABLE 2. Frequency distributions of reciprocal antibody titers and geometric mean titers for sera from cottontail rabbits (*Sylvilagus floridanus*) in New York State, USA, positive by a polyvalent enzyme-linked immunosorbent assay for antibodies to *Borrelia burgdorferi*.

Antigens[a]	No. of sera with antibody titers								Geometric mean titers[b]
	160	320	640	1,280	2,560	5,120	10,240	20,480	
WC	1	4	11	13	17	10	1	4	1,893
VlsE	0	2	15	7	0	4	2	3	1,483
p35	22	5	5	7	5	0	0	0	387
p37	1	2	9	4	3	2	1	0	1,059
Totals	24	13	40	31	25	16	4	7	

[a] Whole-cell (WC), VlsE, and proteins p35 and p37 antigens of *B. burgdorferi*.

[b] Geometric mean titers computed for positive results only. Antibody titers <1:160 were considered negative.

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TABLE 3. Summary of results of polyvalent enzyme-linked immunosorbent assay for total antibodies to whole-cell or recombinant antigens of *Borrelia burgdorferi* in sera of cottontail rabbits (*Sylvilagus floridanus*) in New York State, USA.

Reactive antigen(s)	No. (%) of sera[a] with antibodies to indicated antigens
Whole cells only	13 (13)
Whole cells and VlsE	7 (7)
VlsE only	5 (5)
VlsE and p35	1 (1)
Whole cells, VlsE, and p35	11 (11)
Whole cells, VlsE, and p37	2 (2)
Whole cells, VlsE, p35, and p37	7 (7)
Whole cells, p35, and p37	6 (6)
Whole cells and p35	11 (11)
Whole cells and p37	4 (4)
P35 and p37	2 (2)
P35 only	6 (6)
P37 only	1 (1)
None	26 (25)

[a] Total of 102 sera tested (76 positive and 26 negative).

varied by twofold ($n=3$) or eightfold ($n=1$) in triplicate testing. In duplicate tests for IgM antibodies, titers were unchanged for two positive sera or varied by two-fold ($n=10$) or fourfold ($n=3$). Results for the negative sera were unchanged in all trials, regardless of the conjugates or antigens used.

Serum antibody prevalences to one or more antigens in polyvalent assays are summarized in Table 3. There were 76 positive and 26 negative reactions. Although 25 sera reacted to only one antigen, the remaining 51 sera were positive to two or more antigens. Seven sera reacted to all four antigens, whereas 19 other sera were positive to three antigen preparations. Reactivity to only whole-cell antigens was most frequent ($n=13$). Fifty-eight sera contained antibodies to the VlsE or p35 antigens with or without reactivity to whole-cell antigens.

Paired sera were analyzed for seven rabbits that had been recaptured within 3 wk of the original sampling dates. Results for rabbits (1, 3, and 6) revealed greater than fourfold changes in antibody

TABLE 4. Reciprocal antibody titers to whole-cell (WC) or recombinant antigens (VlsE and proteins p35 and p37) of *Borrelia burgdorferi*, as determined by applying polyvalent enzyme-linked immunosorbent assays in analyses of paired sera from cottontail rabbits (*Sylvilagus floridanus*) recaptured in New York State.

	B. burgdorferi[b]			
Sample No[a]	WC	VlsE	p35	p37
1A	640	640	N	2,560
1B	5,120	640	N	1,280
2A	2,560	640	2,560	N
2B	2,560	320	160	N
3A	2,560	N	2,560	N
3B	N	N	N	N
4A	20,480	5,120	160	N
4B	5,120	5,120	640	N
5A	N	N	N	N
5B	N	N	N	N
6A	640	N	2,560	N
6B	20,480	N	1,280	N
7A	N	640	N	N
7B	N	N	N	N

[a] Rabbits recaptured within 3 wk of original sampling (A = first capture; B = second capture).
[b] N = negative.

titers when whole-cell antigens were tested by a polyvalent ELISA (Table 4). For example, in rabbits 1 and 6, antibody titers increased from 1:640 to 1: 5,120 and to 1:20,480, respectively. A decline in antibody titer to the VlsE antigen was recorded for rabbit 7. Similar decreases in antibody titers to the p35 antigen were noted for rabbits 2 and 3. In analyses for IgM antibodies with whole-cell antigens, positive results were recorded for paired sera representing rabbits 1, 2, and 5. Titration end points for these sera varied by twofold or less and ranged between 1:160 and 1:640.

We tested rabbit antisera to *T. pallidum* and four serovars of *L. interrogans* to determine whether antibodies cross-react in polyvalent and class-specific ELISAs with whole-cell or recombinant antigens of *B. burgdorferi*. Although reactive with whole-cell *B. burgdorferi* antigens, homologous antibodies to *T. pallidum* did not react with any recombinant antigens of the Lyme disease agent, including OspA or

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OspB in a polyvalent ELISA. Similar results were recorded when homologous antibodies to *L. interrogans* serovars grippotyphosa, icterohemorrhagiae, and pomona were screened against these antigens. However, in analyses of a rabbit serum with antibodies to *L. interrogans* serovar canicola, there was a reproducible false-positive reaction to only the VlsE antigen (titer = 1:1,280). In analyses for IgM antibodies, there were no false-positive reactions when these antisera were tested with whole-cell antigens; findings are not reported for the recombinant antigens because of inconsistent results.

DISCUSSION

Cottontail rabbit sera contained antibodies to whole-cell and recombinant antigens of *B. burgdorferi* during different seasons. These animals are commonly parasitized by *Ixodes scapularis*, *Ixodes dentatus*, *Haemaphysalis leporispalustris*, or *Dermacentor variabilis* ticks in northeastern United States. Although *I. scapularis* is a known vector of *B. burgdorferi* to mammals and birds, this tick is not usually as prevalent on rabbits as *I. dentatus*. Although the latter feeds mainly on these lagomorphs and occasionally on humans, this tick is an efficient vector of *B. burgdorferi* (Telford and Spielman, 1989a) and is probably more important in maintaining Lyme disease spirochetes in an enzootic cycle mainly independent of the zoonotic cycle in white-footed mice (*Peromyscus leucopus*; Telford and Spielman 1989b). Antigenically variable isolates of *B. burgdorferi* sensu lato have been isolated from cottontail rabbits and *I. dentatus* feeding on these animals (Anderson et al., 1989). Moreover, presence of IgM antibodies to *B. burgdorferi* in rabbits, such as the positive sera collected in March 1985, may reflect recent exposure of these animals to infected ticks. High titers of total antibodies indicate robust immune responses, an expected result considering frequent exposure of rabbits to ticks over several months. These findings parallel those reported for jackrabbits (*Lepus californicus*) in Western USA, where *Ixodes pacificus* and other ticks abound (Lane and Burgdorfer, 1988). Although *I. pacificus* and *I. scapularis* do not coexist at sites, larvae and nymphs of these species and *I. dentatus* parasitize birds. The corresponding widespread dispersal of infected ticks via bird movement is important in establishing new foci for Lyme disease.

Wide ranges of antibody titers were noted for cottontail rabbits when whole-cell or recombinant antigens of *B. burgdorferi* were incorporated into an ELISA. Titration end points for IgM antibodies were relatively lower. These results are similar to those reported for human sera (Magnarelli et al, 2002). Varying titers are probably due, in part, to different frequencies of host exposure to infected ticks, variable host immune responses, and, possibly, to minor technical factors associated with the assay performance. Detection of low-titer reactions shows that our assays are sensitive enough to measure minimal concentrations of antibodies.

Based on analyses with *T. pallidum* antisera, our use of recombinant antigens yielded more-specific results, compared with assays from whole-cell antigens. It is unclear, however, why rabbit serum to *L. interrogans* serovar canicola cross-reacted in a polyvalent ELISA with the VlsE antigen. There may be some sequence similarity between variable large proteins expressed by these bacteria. Rabbit antisera to *Borrelia andersonii* and *Borrelia bissettii*, spirochetes known to occur in northeastern United States (Marconi et al., 1995; Schneider et al, 2008), were unavailable in the present study. Based on overall numbers of isolations, these spirochetes appear to be less prevalent than *B. burgdorferi* sensu stricto in ticks and mammals in most sites in the northeastern United States. Nonetheless, we recognize that there is likely to be cross-reactivity of

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antibodies with whole-cell *B. burgdorferi* lysate preparations and some of the recombinant antigens because of the many other common antigens shared among these bacteria (Schneider et al., 2008). Similarly, if *Treponema paraluiscuniculi* was present in cottontail rabbits, cross-reactive antibodies would tend to elevate the proportion of positive results for the polyvalent ELISA containing whole-cell antigens because of cross-reactivity with heat-shock, flagellar, and other similar antigens. Prevalence of *T. paraluiscuniculi* in our sampling sites is unknown. Further, in tests of human sera containing IgM antibodies to *Borrelia recurrentis* ($n=11$), the etiologic agent of louse-borne relapsing fever, *T. pallidum* ($n=24$), oral infections ($n=6$), and rheumatoid arthritis ($n=7$), there was no cross-reactivity with the VlsE or p35 antigens (Magnarelli, et al., 2002). In polyvalent assays with the VlsE antigen, false-positive reactions are rare. Likewise, in analyses of dog sera with the p35 antigen (Magnarelli, et al., 2001), results indicated relatively high sensitivity and specificity as well. Therefore, the separate use of these recombinant antigens in an ELISA should help facilitate field and laboratory studies designed to detect possible *B. burgdorferi* infections in rabbits. Supportive evidence on the isolation and molecular identification of *B. burgdorferi* sensu lato isolates in these lagomorphs is ultimately required to confirm the presence of specific pathogens in suspected foci.

There were some notable differences in the reactivities of cottontail rabbit sera when tested with recombinant antigens by polyvalent ELISA. For example, all 102 test sera (100%) lacked antibodies to OspA and OspB, even though positive dog control sera verified the reactivity of both antigens. These negative results agree with earlier findings (Fikrig et al., 1993) where cottontail rabbit sera were analyzed by immunoblotting methods. Antibodies to OspA and OspB have been detected, however, in white-footed mice, raccoons (*Procyon lotor*), canines, and equids (Magnarelli, et al., 1995, 1997). In tests of sera from persons who had Lyme disease, humoral responses to these surface proteins are typically weak or absent during the early weeks of *B. burgdorferi* infection and may or may not become more pronounced during later stages of disease (Wilske et al., 1986, 1994; Fikrig et al., 1992; Dressler et al., 1994). When *B. burgdorferi* is in the midguts of unfed *I. scapularis* ticks, OspA is expressed as a prominent antigen but is subsequently down-regulated or lost when these bacteria migrate from the midgut to the salivary glands during the tick-feeding process (de Silva et al., 1996). In other studies where *B. burgdorferi* or closely related *Borrelia* organisms were characterized (Anderson et al., 1989, 1990, 1996), OspA and OspB were sometimes absent, even in some isolates from *I. scapularis*. Clearly, these outer surface proteins are not always expressed. Further, results for the VlsE and p35 antigens may indicate early *B. burgdorferi* infections. Based on analyses of human sera from persons who had erythema migrans and of immune sera from mice infected with *B. burgdorferi*, IgM antibodies were detected (Fikrig et al., 1997, Lawrenz et al., 1999; Liang et al., 1999; Magnarelli et al., 2002), thus reinforcing that VlsE and p35 are expressed during early periods of mammalian infections. Therefore, differential expression of key antigenic proteins of different strains of *B. burgdorferi* in mammalian hosts can dramatically affect serologic test results when certain recombinant antigens are used.

Rabbits can harbor unrelated tick-transmitted pathogens, such as *Rickettsia rickettsii*, *B. burgdorferi*, *Babesia* species, and *Francisella tularensis*, which can cause disease in humans, domesticated animals, and other vertebrate hosts. *Anaplasma phagocytophilum* may also be present in these hosts (Zhan et al., 2009). Considering the wide range of tick species associated with cottontail rabbits and the high

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parasitism rates, this mammalian host and related lagomorphs should be monitored more closely and included in ecologic and epidemiologic studies of Lyme borreliosis and other zoonotic infections.

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