JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1994, p. 777–782
0095-1137/94/$04.00+0

Vol. 32, No. 3

Detection of *Borrelia burgdorferi* in Urine of *Peromyscus leucopus* by Inhibition Enzyme-Linked Immunosorbent Assay

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Received 27 September 1993/Returned for modification 5 November 1993/Accepted 14 December 1993

An inhibition enzyme-linked immunosorbent assay was developed to detect *Borrelia burgdorferi*, the etiologic agent of Lyme borreliosis, in urine from white-footed mice (*Peromyscus leucopus*). Of the 87 urine specimens tested from 87 mice collected in widely separated tick-infested sites in Connecticut, 57 (65.5%) contained detectable concentrations of spirochetal antigens. Forty-seven (62.7%) of 75 serum samples analyzed contained antibodies to *B. burgdorferi*. In culture work with tissues from bladders, kidneys, spleens, or ears, 50 of 87 mice (57.5%) were infected with *B. burgdorferi*. Thirty-eight (76%) of 50 infected mice had antigens of this spirochete in urine, while 36 (72%) individuals had infected bladders. Of those with infected bladders, 24 (66.7%) mice excreted subunits or whole cells of *B. burgdorferi* into urine. Successful culturing of *B. burgdorferi* from mouse tissues, the presence of serum antibodies to this bacterium, and detection of antigens to this spirochete in urine provide further evidence that multiple assays can be performed to verify the presence of *B. burgdorferi* in *P. leucopus*.

The diagnosis of human Lyme borreliosis is accomplished by evaluating clinical, epidemiological, and laboratory findings (23, 24). However, the best clinical marker, erythema migrans, is sometimes lacking or undetected. Moreover, indirect fluorescent antibody (IFA) staining, enzyme-linked immunosorbent assay (ELISA), and immunoblotting methods sometimes fail to confirm *Borrelia burgdorferi* infections because of low sensitivity or specificity (22, 23). Culturing *B. burgdorferi* from skin tissues and mammalian bodily fluids is direct evidence of infection, but this method is laborious and expensive. To aid laboratory diagnosis and epizootiological studies of this disease, there is a need for additional antigen detection assays. Whole cells or subunits of *B. burgdorferi* have been detected in the urine, blood, and skin tissues of persons and mice by dark-field microscopy and immunofluorescence staining methods, dot-blot ELISA (11), immunoelectron microscopy (8), or by application of PCR methods and molecular probes (10, 17, 19, 20). Assays to detect amplified DNA are highly sensitive and specific, but the expertise required to perform the tests, the expense, and the laborious nature of the analyses limit the usefulness of this approach in many research and clinical laboratories. Our objective was to develop and evaluate an inhibition ELISA, a less-expensive and simpler method than PCR procedures, to detect *B. burgdorferi* in urine specimens. The white-footed mouse (*Peromyscus leucopus*), a forest-dwelling rodent and main reservoir of *B. burgdorferi* (2, 7, 12, 13), was used for experimental purposes.

MATERIALS AND METHODS

Sampling. White-footed mice were captured from May through November of 1988 to 1990 and in 1992 in Sherman box traps placed in or near woodlands. Widely separated tick-infested areas of Connecticut were chosen as part of an ongoing surveillance program in 18 towns. Human borreliosis is considered to be endemic for the following 11 towns in south central and southeastern parts of the state: Chester, Durham, East Haddam, East Lyme, Lyme, Montville, Mystic, North Stonington, Old Lyme, Salem, and Stonington (6, 25). Four additional towns were included from southwestern Connecticut (Stamford, Wilton, Weston, and Westport), where the prevalence of human cases of Lyme borreliosis is lower (6). The tick vector *Ixodes scapularis*, formerly known as *Ixodes dammini* (18), and human infections with *B. burgdorferi* are uncommon in the remaining towns of Barkhamsted, Warren, and West Hartford in northern Connecticut.

Mice were euthanized with carbon dioxide and dissected under sterile conditions to remove bladders, kidneys, spleens, or urine. Ear-punch biopsy tissues were collected and included in many instances as additional samples for isolation of *B. burgdorferi*. Urine was collected from the bladders of mice whenever possible with a hypodermic needle and syringe prior to the processing of tissues for isolations and was held for at least 2 days at 5°C to allow particulate matter to settle. The solution lying directly above the precipitated contents was used in antigen-detection analyses. Collection of pelleted material for urine samples was minimized.

An additional 10 female white-footed mice, captured in Connecticut during 1992 from Barkhamsted (n = 2), Deep River (n = 4), Haddam (n = 2), Killingworth (n = 1), and Madison (n = 1), were allowed to produce offspring in the laboratory. Fifteen newborn mice were reared to adulthood and individually placed into a metabolism cage (Maryland Plastics, Inc., Federalsburg, Md.) to obtain urine specimens. Ear-punch biopsy and internal tissues of these mice were processed for culture work. Findings for the reared mice were used to evaluate cutoff values for positive results by urine analyses with an inhibition ELISA. Sampling areas in Deep River, Haddam, Killingworth, and Madison are heavily infested with *I. scapularis*.

Isolations. Tissues of mouse bladders, kidneys, spleens, and (or) ears were introduced into duplicate tubes of Barbour-Stoenner-Kelly medium with or without 0.1% agarose (1). Samples of culture media were checked for spirochetes by dark-field microscopy after 3 to 6 weeks of incubation at 31°C. Spirochetes in positive culture tubes were passed into fresh tubes of media and tested with IFA staining methods with murine monoclonal antibody H5332, which is specific for outer surface protein A (OSPA) of *B. burgdorferi* (3, 4). Fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulins

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(Organon Teknika Corp., West Chester, Pa.) were diluted to 1:40 in phosphate-buffered saline (PBS) solution and used as the second antibody.

Antibody tests. An ELISA was used to detect total serum immunoglobulins to *B. burgdorferi* (15, 16). To shorten the time of analysis, a commercially prepared affinity-purified horseradish peroxidase-labeled goat anti-*P. leucopus* immunoglobulin (H- and L-chain specific) replaced the unconjugated rabbit anti-*P. leucopus* and peroxidase-conjugated goat anti-rabbit reagents used before. The newly developed reagent (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) was diluted in PBS solution to 1:2,000. Net optical density (OD) values of 0.18, 0.15, and 0.11 were considered positive for the respective serum dilutions of 1:160, 1:320, and ≥1:640. These critical regions were established by statistically analyzing net absorbance readings for 38 normal serum samples (3 standard deviations + mean) obtained from 38 *P. leucopus* samples collected in earlier studies (16) from northwestern and other areas of Connecticut where *I. scapularis* and human cases of Lyme borreliosis are uncommon. Freshly prepared washed whole cells of *B. burgdorferi* (Connecticut strain 2591) and newly purchased reagents were standardized before mouse serum samples were analyzed to ensure consistent assay performance. The same positive and negative serum controls were used on each plate along with the appropriate controls for antigen, conjugates, and diluents. Positive serum controls were from *P. leucopus* caught in Connecticut and found harboring *B. burgdorferi* (16). Application of murine monoclonal antibody H5332 in IFA staining of slide preparations of spirochetes isolated from these and other rodents confirmed the identity of *B. burgdorferi.*

Antigen detection. An inhibition ELISA was developed to detect *B. burgdorferi* antigens in mouse urine. Flat-bottomed, polystyrene plates were coated with whole-cell *B. burgdorferi* (3 to 5 μg of protein per ml) or PBS solution (negative control wells) as described previously (15). The volume of blocking solution (diluted horse serum) was increased to 300 μl per well, and the incubation period for blocking was extended to 1 h and 30 min at 37°C. In a second plate (i.e., microtiter plate), 60 μl of undiluted mouse urine was mixed in equal volume with either commercially prepared horseradish peroxidase-labeled rabbit anti-*B. burgdorferi* immunoglobulins (Kirkegaard and Perry Laboratories), diluted to 1:1,000 in PBS solution, or 60 μl of a 1:1,280 dilution of mouse ascitic fluid containing monoclonal antibodies (H5332) directed to OSPA of *B. burgdorferi*. In separate analyses with IFA staining methods, homologous antibody titers to *B. burgdorferi* for both reagents (i.e., unlabeled antisera) were ≥1:20,480. The urine-antibody solutions were incubated for 1 h at 37°C in the microtiter plate to allow for antigen-antibody complex formation. The blocking solutions were washed out of the polystyrene plates, and 50 μl of the test solutions from the microtiter plate was then added per well to the polystyrene plates and incubated for 1 h at 37°C. These plates were subsequently washed to remove unbound antibodies. When the polyvalent peroxidase-labeled conjugate was used, 50 μl of substrate (2,2′-azino-di-3-ethyl-benzthiazoline sulfonate) was delivered to each well and incubated for 1 h at 37°C before absorbance values were determined. In this assay, all procedures can be completed within 5 h. In the flat-bottomed, polystyrene plates that contained mouse ascitic fluid and urine, 50 μl of diluted (1:4,500) horseradish peroxidase-conjugated goat $F(ab')_2$ anti-mouse immunoglobulins (H- and L-chain specific), purchased from Tago, Inc. (Burlingame, Calif.), was added to each well after the antibody-urine solutions were incubated for 1 h and removed by washing. The conjugated antibodies likewise were incubated for 1 h at 37°C and removed by washing. The addition of substrate and subsequent procedures were the same as described above. With the additional 1 h of incubation required for tests with ascitic fluid, all steps can be completed within 6 h.

To determine cutoff values for positive results and the range of assay sensitivity, urine from 15 culture-negative white-footed mice, born and reared in the laboratory and lacking antibodies to *B. burgdorferi*, was analyzed. In addition, standard curves were constructed after different concentrations of *B. burgdorferi* antigens were added to urine from three mammalian species and analyzed with an inhibition ELISA. Urine specimens were obtained from *P. leucopus* captured in the Warren (n = 1) and Barkhamsted (n = 4) areas of northern Connecticut, where Lyme borreliosis is uncommon, healthy dogs from Wyoming (another area where this spirochetosis is rare), and persons who had no histories of *Leptospira*, *Borrelia*, or *Treponema pallidum* infections. In replicated trials, serial dilutions of washed *B. burgdorferi* cells, derived from cultures and diluted in PBS solution, were added to urine in concentrations ranging from 0.8 to 2.8 μg of protein per ml and tested. The stock reagent consisted of *B. burgdorferi* 2591 and was the same antigen used to coat the polystyrene plates for standard ELISA to detect serum antibodies. Use of a commercially available assay (Bio-Rad Laboratories, Richmond, Calif.) revealed that the mean protein content of the stock antigen was 90 μg of protein per ml. On the basis of replicated analyses of negative urine specimens and tests of positive standards, net OD values of 0.02 to 0.15 were considered positive for test samples by an inhibition ELISA. Urine specimens from mice, dogs, or humans reacted similarly. In short, when a urine specimen lacked *B. burgdorferi*, no antigen-antibody complex formed in the microtiter plate, and the full amount of unbound peroxidase-labeled antibody or ascitic fluid delivered to the polystyrene plates reacted with *B. burgdorferi* coated to the solid phase. Following the addition of substrate, this resulted in the production of a dark green color in wells. Conversely, if whole-cell *B. burgdorferi* or shed subunit antigens of this bacterium were present in urine specimens, they combined with the peroxidase-conjugated antibody or ascitic fluid during the initial incubation phase in the microtiter plates and formed an antigen-antibody complex. Depending on the concentration of spirochetal antigens and the amount of complex produced, there was a corresponding decrease in enzyme-substrate reactivity in the antigen-coated polystyrene plates. The peroxidase-labeled antibodies bound to urine antigens in the form of the complex did not react with the spirochetes coated to the solid phase. When such inhibition of reactivity occurred, solutions with a light green color in plate wells were considered positive if net OD values were within the acceptable range of the standard curve. Plates contained known reference standards (i.e., urine mixed with known concentrations of *B. burgdorferi*) for positive controls, negative urine samples, and controls for conjugates and diluents.

Specificity testing. Concentrations of *Leptospira interrogans* serovars and *T. pallidum* were mixed separately with diluted peroxidase-conjugated rabbit anti-*B. burgdorferi* immunoglobulins or ascitic fluid (H5332) and tested with an inhibition ELISA to determine specificity. The sources and preparation of antigens have been reported previously (14). In duplicate trials, a wider range of concentrations (0.4 to 3.2 μg of protein per ml) than that used for *B. burgdorferi* standards was tested. The following *L. interrogans* serovars were included: *canicola* (strain Moulton), *icterohemorrhagiae* (strain CF-1), and *pomona* (strain ML5). To verify antigen reactivity, the *Lepto-*

TABLE 1. Results of serologic testing for serum antibodies to *B. burgdorferi* and for spirochetal antigens in urine from *P. leucopus*

County	Antibodies			*B. burgdorferi*		
	Total serum samples tested	No. (%) positive[a]	Titer range	Total urine samples tested	No. (%) positive[b]	OD range[c]
Fairfield	16	9 (56.3)	320–10,240	16	7 (43.8)	0.02–0.15
Hartford	3	2 (66.7)	320–640	3	0	
Litchfield	4	0		4	0	
Middlesex	14	6 (42.9)	1,280–10,240	18	15 (83.3)	0.04–0.14
New London	38	30 (79.0)	160–20,480	46	35 (76.1)	0.05–0.15
Total	75	47 (62.7)	160–20,480	87	57 (65.5)	0.02–0.15

[a] Positive ELISA titer ≥ 1:160.
[b] Whole cells or subunits of antigens detected by a polyvalent inhibition ELISA.
[c] Values for positive results (OD ≤ 0.15).

spira and *Treponema* spirochetes were tested with homologous rabbit antisera with IFA staining methods (14).

Challenged mice. Five white-footed mice, born in the laboratory and not exposed to ticks, were inoculated intradermally with about 1.4×10^4 cultured *B. burgdorferi* N40 cells per ml. An additional mouse with no exposure to ticks or *B. burgdorferi* was held as a normal control and, similar to the other mice, was kept in a separate cage. Urine specimens were collected from each animal in the metabolism cage during a period of 2 months before and 3 months after inoculation and were analyzed with an inhibition ELISA. Ear-punch biopsy tissues were acquired 1 month postinoculation and processed for culturing spirochetes. At the conclusion of the experiment, 6 months after inoculations, all animals were euthanized by application of carbon dioxide. Tissues from the bladders, kidneys, and spleens were processed for isolation of *B. burgdorferi*, and blood was collected from each animal to test for antibodies to this bacterium.

RESULTS

White-footed mice were exposed to *B. burgdorferi* at widely separated sites in Connecticut. On the basis of serum antibody analyses, there were prior or current infections with this spirochete in mice from at least four counties; 47 (62.7%) of 75 serum specimens were positive (Table 1). The highest antibody titers (≥1:10,240) were noted for animals captured in Fairfield, Middlesex, and New London Counties in southern Connecticut. Urine specimens, collected from 57 (65.5%) of 87 mice, contained subunits or whole cells of *B. burgdorferi*. The prevalence of positive urine was relatively high in Fairfield, Middlesex, and New London Counties (43.8 to 83.3% positive).

Shed antigens of *B. burgdorferi* and antibodies to this spirochete were detected in urine and serum samples, respectively, during each month of sampling (Table 2). Although the prevalence of infected mice, as determined by culturing methods (n = 50 positive), was slightly lower (57.5% of 87 mice) than the prevalence of positive serum samples (62.7%) or positive urine samples (65.5%), there was verification of living *B. burgdorferi* infection in mouse tissues collected from May through November. Of the 50 mice shown to be infected with *B. burgdorferi* by culturing methods, 38 (76%) contained antigens of this bacterium in urine. *B. burgdorferi* was isolated from the bladders of 36 mice, 24 of which (66.7%) contained detectable amounts of subunits or whole cells in urine specimens.

Concentrations of *B. burgdorferi* in urine varied greatly. The majority (52.6%) of the 57 positive specimens (n = 30) had moderate amounts of antigen (OD = 0.07 to 0.12). Relatively low (OD = 0.13 to 0.15) or very high (OD = 0.02 to 0.06) concentrations of *B. burgdorferi* were recorded for 17 (29.8%) and 10 (17.5%) urine specimens, respectively. The latter occurred in mice captured from August through November.

TABLE 2. Seasonal prevalence of white-footed mice with serum antibodies to *B. burgdorferi* or harboring *B. burgdorferi* in bladder tissues and urine

Month	Antibodies		Mice infected with *B. burgdorferi*			
	Total serum samples tested	No. (%) positive[a]	Total tested	No. (%) culture positive[b]	No. (%) with infected bladders[c]	No. (%) with positive urine[d]
May	6	3 (50.0)	6	1 (16.7)	0	1 (16.7)
June	12	6 (50.0)	14	6 (42.9)	4 (66.7)	5 (35.7)
July	5	4 (80.0)	7	6 (85.7)	6 (100)	5 (71.4)
August	13	9 (69.2)	17	13 (76.5)	6 (46.2)	13 (76.5)
September	10	8 (80.0)	12	10 (83.3)	8 (80)	10 (83.3)
October	17	13 (76.5)	19	10 (52.6)	9 (90)	12 (63.2)
November	12	4 (33.3)	12	4 (33.3)	3 (75)	11 (91.7)
Total	75	47 (62.7)	87	50 (57.5)	36 (72)	57 (65.5)

[a] Positive ELISA titer ≥ 1:160.
[b] Culture-positive kidney, spleen, blood, ear, or bladder tissues. Twenty-one (8.8%) of 238 cultures were contaminated with other bacterial or fungal growths.
[c] The percent positive was calculated as the number of mice with an infected bladder divided by the total number of mice which were culture positive in that month.
[d] The percent positive was calculated as the number of mice with positive urine samples divided by the total number of mice tested by culture methods and (or) urine analysis in that month.

Analyses of serum antibodies to *B. burgdorferi*, the presence of this pathogen in mouse tissues, and *B. burgdorferi* antigens in urine were conducted to assess the sensitivity of the urine antigen assay. Of the 75 field-caught mice tested by all three methods, there was agreement in results for 42 (56.0%) animals. Twenty-eight rodents were positive by all three tests, while 14 mice were negative by each method. In an additional four mice, the results of isolation attempts and urine analyses were negative, but mouse sera contained antibodies to *B. burgdorferi*. There were detectable amounts of serum antibodies and urine antigens without a culture-positive confirmation for three mice. The results for the remaining 26 animals indicated either positive (n = 15) or negative (n = 11) urine analyses lacking supportive data from antibody assays and culturing methods. Results for the 15 white-footed mice born and reared to adulthood in the laboratory were negative by culture work, tests for serum antibodies, and the urine antigen assay. However, one of 10 field-caught mothers of these rodents contained *B. burgdorferi* in ear tissues and in the spleen, kidneys, and bladder. Cultures for the other nine field-caught mothers were negative. The infected mouse, captured in Haddam, had a serum antibody titer of 1:10,240. Serum antibodies to *B. burgdorferi* also were detected at concentrations of 1:1,280 to 1:10,240 in two other mother mice in this study group. Of the remaining seven mother mice, six lacked antibodies to *B. burgdorferi*. Serum was unavailable for one mouse. Urine specimens were not collected from any of the mother mice because the results of culturing were used as a primary means of determining infectivity.

When adequate amounts of urine were available, parallel tests were conducted to compare the reactivities of mouse ascitic fluid (H5332) and the peroxidase-labeled polyvalent antiserum in an inhibition ELISA. In analyses of 27 urine specimens, *B. burgdorferi* antigens were detected (n = 3 samples) or undetected (n = 7) by both tests. With the exception of three specimens that were negative by both assays and positive by culture, the results of antigen detection by ELISA and isolation work were in agreement. For the remaining 17 urine specimens, *B. burgdorferi* antigens were detected by an ELISA when the polyvalent antiserum was used, but assays with mouse ascitic fluid failed to detect antigen. *B. burgdorferi* was cultured from the tissues of 15 mice in this group.

To assess the specificity of the urine assay, concentrations of three *L. interrogans* serovars and *T. pallidum* were added separately to urine and tested independently with an inhibition ELISA with mouse ascitic fluid (H5332) or the peroxidase-labeled polyvalent antiserum. The results of all trials were negative, regardless of the concentration of antigen used. Positive controls with whole-cell *B. burgdorferi* (0.8 to 1.2 μg of protein per ml), however, were reactive when mouse ascitic fluid or polyvalent antiserum was used. Similarly, the *Leptospira*, *T. pallidum*, and *B. burgdorferi* antigens reacted positively to homologous rabbit antisera by IFA staining methods in replicated controls, verifying antigen reactivity.

In laboratory experiments with challenged mice, culturing procedures revealed no *B. burgdorferi* in ear tissue samples from animals before inoculation but verified infection in five mice 1 month postinoculation. Ear tissues from the normal mouse were uninfected throughout the 8-month study. Similarly, there were no detectable antigens in urine of mice preinoculation or in the normal mouse at any time. Urine specimens from four inoculated mice contained *B. burgdorferi* antigens 3 to 11 weeks postinoculation. OD values ranged from 0.03 to 0.15. In analyses of a series of urine samples from each mouse, positive reactions for three mice were noted at about 8 weeks after inoculation. In each case, there were negative urine specimens that preceded and followed the positive urine samples. Four urine specimens from the remaining challenged mouse contained no detectable *B. burgdorferi* antigen. At the conclusion of the experiment, *B. burgdorferi* was isolated from the bladders or spleens of four challenged mice. Cultures of tissues from the remaining infected mouse were contaminated. Each of these rodents contained antibodies to *B. burgdorferi* at concentrations of 1:5,120 to 1:20,480. Similar to earlier test results, the normal mouse had uninfected tissues and no antibodies to this bacterium.

DISCUSSION

White-footed mice excrete subunits or whole cells of *B. burgdorferi* in urine. These findings support those of earlier studies (5, 8, 11), including laboratory experiments (19, 21), which showed that urinary bladder tissues can harbor *B. burgdorferi*. Being an important reservoir of this bacterium in nature, white-footed mice can be infected for several months and serve to infect ticks that parasitize them. On the basis of the results of an inhibition ELISA, the concentration of shed antigens of *B. burgdorferi* in mouse urine sometimes is relatively high and can be detected when mice are collected in different seasons. The geographic distribution of white-footed mice with components of *B. burgdorferi* in urine correlates with the distribution of human cases of Lyme borreliosis (6), isolation of the spirochete from mice (1, 2), and the presence of antibodies to this bacterium in dogs and wildlife (14–16) in widely separated sites.

Three parameters were used to judge whether or not a given mouse harbored or had been exposed to *B. burgdorferi*. For more than half of the field-collected mice tested, there was concordance in the results of the serum antibody, culture, and urine analyses. Similar results were recorded for four of five laboratory-bred mice inoculated with *B. burgdorferi*. However, there were discrepancies. *B. burgdorferi* antigens sometimes were detected in urine from field-collected mice without supportive data from antibody assays or culture work. This was particularly noticeable in animals captured during October and November, a period after peak nymphal *I. scapularis* population levels have been reached. Populations of *I. scapularis* nymphs, the chief vectors of *B. burgdorferi*, are highest in June to mid-July in the northeastern United States. The prevalence of antibody-positive serum specimens and antibody titers in mice can be low (15, 16), especially during early infection. Moreover, successful culturing of *B. burgdorferi* depends, in part, on the number of spirochetes present in host tissues. Therefore, it is not surprising to have mixed test results. Despite encouraging findings in tests on specificity, one must still acknowledge the possibility of some false-positive reactions (i.e., cross-reactivity with other spirochetes). In other instances, serum antibody analyses and culturing results were positive, while urine antigen test results were negative. *B. burgdorferi* antigens may not always be released into urine. The time when components or whole cells of *B. burgdorferi* are most likely to be detected in mouse urine relative to onset and duration of infection should be determined for mice infected via tick feedings. These experiments would provide comparative results and would more closely reflect natural infections. Use of a metabolism cage in these studies would enable one to obtain more adequate amounts of urine from infected mice without sacrificing the animal. Finally, PCR methods (19) could be employed in future studies to help further verify the identity of *B. burgdorferi* antigens in mouse tissues or urine.

The peroxidase polyvalent antiserum was more effective in

detecting antigens of *B. burgdorferi* than the mouse ascitic fluid directed to OSPA. The latter verified the presence of *B. burgdorferi* in urine, but the results of the ELISA with mouse ascitic fluid correlated poorly with the culture data. These findings might be due to low amounts of OSPA antigen present in urine. Compared with concentrations of flagellin and several other immunogenic antigens, such as OSPB and polypeptides with molecular masses of about 39, 83, and 110 kDa, concentrations of OSPA would be expected to be comparatively much lower. Therefore, the sensitivity of an inhibition ELISA designed to detect and measure only OSPA in urine would be far less than that of a polyvalent assay structured to detect multiple antigens. Application of PCR methods developed to detect amplified genetic components of OSPA, however, might help improve the sensitivity while maintaining the high specificity of this urine assay. Ultimately, the most practical diagnostic tool for antigen detection would have to be easy to perform, highly sensitive, and highly specific. Aside from the expense and time required to obtain results, culturing *B. burgdorferi* from rodent tissues remains the best method for determining active *B. burgdorferi* infection.

The inhibition ELISA for white-footed mice described in this study is a modification of conventional antibody assays used extensively in research and clinical laboratories and requires less time than DNA detection or culturing methods. With further work, an inhibition ELISA might have application in human diagnostic testing, epizootiologic studies, and surveillance programs. Similar methods have been successfully applied to detect *Rickettsia tsutsugamushi*, the etiologic agent of scrub typhus fever (9), in human blood specimens. However, unlike most rickettsial infections, the concentration of *B. burgdorferi* in human tissues and bodily fluids appears to be low, particularly during the early weeks of infection. Moreover, little is known about the amounts or discharge rates of *B. burgdorferi* antigens from the kidneys and bladder into human urine. The specific times for maximal discharge of these antigens into urine relative to duration of disseminated infection, stage of disease, and antibiotic therapy are unknown. Therefore, further studies are required to determine whether the inhibition ELISA described for white-footed mice can be reliably used to confirm the presence of *B. burgdorferi* in human urine. If *B. burgdorferi* concentrations in human urine are consistently low, then development of a highly sensitive and specific inhibition ELISA is likely to be more challenging than the methods used to analyze urine specimens from mice. Nonetheless, when mice are captured to identify foci for Lyme borreliosis, analyses of urine for shed components of this spirochete could provide additional evidence of infection.

ACKNOWLEDGMENTS

We thank Niel Infante, Bonnie Hamid, Cynthia Moore, and Tia Blevins for technical assistance and are grateful to Alan G. Barbour of the University of Texas (San Antonio) and Russell C. Johnson of the University of Minnesota (Minneapolis) for providing the mouse ascitic fluid, *Leptospira* antigens, and rabbit antisera used in this study.

This work was supported in part by a grant from the Centers for Disease Control (U50/CCU106598) and funding from the Connecticut State Health Department.

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