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Cross-reactivity between *B. burgdorferi* and other spirochetes affects specificity of serotests for detection of antibodies to the Lyme disease agent in dogs

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ABSTRACT

Western immunoblots, the kinetics-based enzyme-linked immunosorbent assay (KELA), and the microagglutination test were used to evaluate cross-reactivity among antibodies to serovars of *Leptospira interrogans* (leptospiral serovars), and *B. burgdorferi* from naturally infected dogs, and to *Serpulina (Treponema) hyodysenteriae* from vaccinated rabbits. Whole-cell lysates from *Borrelia* spp., leptospiral serovars, and *Serpulina* spp. were used for SDS-PAGE, western blots, and KELA. Cross-reactivity occurred between the antibodies to *B. burgdorferi* and leptospiral serovars when tested on the heterologous antigens. Antibodies to leptospiral serovars tended to cross-react more strongly with antigens of *B. burgdorferi* spp. than did antibodies to *B. burgdorferi* when tested against antigens of leptospiral serovars. The antibodies against *B. burgdorferi* showed a lesser degree of cross-reactivity to the antigens of *S. hyodysenteriae* and *S. innocens* than they did to leptospiral serovars. We conclude that cross-reactivity occurs between *B. burgdorferi* and leptospiral serovars. Validation and interpretation of ELISA tests for detection of antibody activity to whole cell lysates of the Lyme agent must take this cross-reactivity into consideration. Conversely, dogs infected with the Lyme agent do not show significant cross-reactivity in the microagglutination test for antibody to the leptospiral serovars.

INTRODUCTION

Lyme disease caused by *Borrelia burgdorferi*, is one of the most common tick-transmitted zoonotic diseases in the United States, Europe, and other parts of the world (Anderson, 1989). This disease, first discovered in human patients from Lyme, Connecticut, (Steere, et al., 1977), was later found in dogs and horses (Lissman, et al., 1984; Kornblatt, et al., 1985; Burgess, et al., 1986). Since dogs inhabit almost the same environment as people, they are

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probably useful as sentinel animals for investigations of the epidemiology of Lyme disease (Magnarelli, et al., 1985; Schulze, et al., 1986).

Dogs may develop arthritis after infection (Lissman, et al., 1984; Kornblatt, et al., 1985; Roush, et al., 1989) or kidney disease (Grauer, et al, 1988). It has also been reported that febrile spirochetemic dogs showed migratory lameness, and pain and swelling in the carpal joints (Kornblatt, et al., 1985). Recently, surveys using blood samples from dogs with limb/joint disorders or clinical signs of unknown etiology such as fever, anorexia, or fatigue, indicated that 76.3% and 66.5% of sera were Lyme-antibody-positive for the dogs living in the lower Hudson Valley of New York state and southern Connecticut, respectively (Magnarelli, et al., 1987). Schulze et al. (1987) reported that 34.7% of Lyme sero-positive dogs from New Jersey were symptomless. The prevalence rate is estimated at approximately 2.7% in North Carolina (Greene et al., 1988) and 5.5% of sera from dogs in Texas were positive for Lyme antibodies (Cohen, et al., 1990).

Several common serologic techniques have been used for presumptive confirmation of *B. burgdorferi* infection in dogs in our laboratory and others; these include the indirect immunofluorescence assay (IFA), kinetics-based enzyme-linked immunosorbent assay (KELA), and Western blot (Lindenmayer et al., 1990; Greene et al., 1988; Karlsson, 1990; Karlsson et al., 1989; Zoller et al., 1991). The specificity of serotest results for dogs, both in surveillance and clinical applications, thus becomes an important issue. In this paper, we evaluate the protein profiles of *Borrelia* spp., leptospiral serovars, and *Serpulina* spp. using the Western blot technique in an attempt to assess the extent and degree of cross reactions among antibodies produced against these organisms. Correlation of Western blot results with those of KELA are also reported.

MATERIALS AND METHODS

Bacterial isolates and cultivation

Sixteen isolates of *Borrelia burgdorferi*, one isolate each of *B. anserina*, *B. hermsii*, *Leptospira interrogans* serovars *hardjo*, *grippotyphosa*, *pomona*, *icterohaemorrhagiae*, and *canicola* (leptospiral serovars), three isolates of *Serpulina (Treponema) hyodysenteriae* and two isolates of *Serpulina (Treponema) innocens* were used in these studies (Table 1). All *Borrelia* species were cultured in BSK medium (Barbour, 1984). Leptospiral serovars were cultured in leptospira broth (Bovuminar microbiological media solution, PLM5, Intergen Company, Purchase, NY). *S. hyodysenteriae* and *S. innocens* were cultured in trypticase soy broth with glucose, containing 5% horse serum, 0.5% yeast extract, 2.0% each of VPI A (0.04% $CaCl_2$, 0.04% $MgSO_4$) and B (0.2% K_2HPO_4, 2.0% $NaHCO_3$) salt solutions and 0.05% L-cysteine; these orga-

TABLE 1

Borrelia spp. and leptospiral serovars

Strains	Reference(s) or source	Host[a]	Passage[b]
L. hardjo (Hardjoprejitno)	Dr. D. Miller[c]		
L. grippotyphosa (Andaman)	Dr. D. Miller		
L. pomona (Pomona)	Dr. D. Miller		
L. icterohaemorrhagiae (M20, Copenhageni)	Dr. D. Miller		
L. canicola (Hond Utrecht IV)	Dr. D. Miller		
B. burgdorferi ES1, 7, 14, 17, 23, YFC1, AH2	This study[d],	*Ixodes dammini*	4 or 5
20004	St. Peron, France[e],	*I. ricinus*	7
22921	Pocantico, NY[e],	*I. dammini*	5
26815	Stonington, Connecticut[e]	*Tamais straitus* (Chipmunk 114)	3
2591	E. Haddan, Connecticut[e]	*Peromyscus leucopus*	137
21305	E. Haddan, Connecticut[e]	*Peromyscus leucopus*	5
21721	Ft. McCoy, Wis[e]	*I. dammini* larvae	6
21343	Ft. McCoy, Wis[e]	*P. leucopus*	4
25550	Westchester, NY[e]	*P. leucopus*	44
26826	Patience Island, RI[e]	*Microtus pennsylvanicus* (vole)	4
B. anserina #12	Dr. John F. Anderson[e]		6
B. hermsii #32	Dr. John F. Anderson[e]		7
S. hyodysenteriae			
B169	Dr. M.J. Wannemuehler[f]		
ACK300/8	Dr. M.J. Wannemuehler[f]		
S. innocens			
B256	Dr. M.J. Wannemuehler[f]		
B657	Dr. M.J. Wannemuehler[f]		

[a]Species from which *B. burgdorferi* were isolated.
[b]Number of passages in BSK-II media.
[c]National Reference for Leptospirosis, NVSL, P.O. BOX. 844. Ames IA 50010.
[d]Isolated in our laboratory from *I. dammini* which were collected from North Salem, New York.
[e]Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504.
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nisms were the gifts of Dr. M. Wannemuehler (Veterinary Medical Research Institute, Iowa State University, Ames, IA).

Preparation of antigens

Whole-cell lysates of all of the bacterial isolates were prepared. Sixteen isolates of *B. burgdorferi* were initially isolated from *Ixodes dammini* ticks and rodents (Table 1). *B. anserina* and *B. hermsii* were grown in 20 ml of BSK liquid media at 34°C incubator without CO_2, washed with phosphate buffer saline solution (PBS; 0.01 M, pH 7.2) three times, and the final pellet was

resuspended in SDS-sample buffer (0.01% Tris hydrochloride, 10% glycerol, 2% sodium dodecyl sulfate, 0.5% 2-mercaptoethanol, 0.1% bromphenol blue, pH 8.0), boiled for 3 minutes, and spun 5 min in an Eppendorf centrifuge (13000 rpm). The supernatants were either used immediately or stored at −20°C until used.

Leptospiral serovars were initially obtained from the National Reference Center for Leptospirosis, NVSL (Ames, IA), and have been maintained in our laboratory in PLM5 media. Whole cell lysates were prepared by boiling in the SDS-sample buffer and treated as described above for *B. burgdorferi.*

Lyophilized killed whole cells of *S. hyodysenteriae* and *S. innocens* were prepared by boiling in the SDS-sample buffer as described for the *B. burgdorferi* antigen.

Kinetics enzyme-linked immunosorbent assay (KELA)

Serum samples from dogs were tested for *B. burgdorferi* antibodies using a computer-assisted, kinetics-based ELISA (KELA). Microdilution plates (Maxisorp F16, Nunc, Denmark) were coated for three hours in a moist chamber at 37° using 100 μl/well of supernatant of French pressed *B. burgdorferi* strain 25550 (16000 psi, 3×) diluted 1:1200 in carbonate buffer (pH 9.6). Wells exposed to carbonate buffer and the washing steps served as background control for each sample. Antigen (Ag) coated plates were stored in sealed plastic bags at −20°C and used within a few days of preparation. Prior to testing, Ag coated plates were thawed and the wells were washed 4 times with 0.1 M phosphate buffered saline containing 0.05% Tween (PBST). Serum samples and controls were diluted 1:100 in freshly prepared 0.1 M PBST containing 2% nonfat dry milk (Carnation Co., Los Angeles, California) and dispensed (100 μl) into antigen coated plates in quadruplicate. Plates were incubated at 37°C for one hour and washed with PBST. One hundred μl of conjugate consisting of horseradish peroxidase (HRP) linked to goat anti-dog serum (heavy and light chain-specific) was added to each well and incubated for 30 min at room temperature. (The antibody concentration in the conjugate was 8.0 mg/ml diluted 1:2000 in PBST with 2% milk.) The plate was then washed with PBST and 100 μl of freshly prepared substrate (3,3′,5,5′-tetramethylbenzidine, 0.4 mg/ml, mixed with equal volume of 0.02% H_2O_2 in a citric acid buffer) was added to each well. Plates were placed on a Bio-Tek EL312 reader and three readings (650 nm) were taken at 1 min intervals with continuous shaking between readings. Results were calculated as the slope of the reaction rate for each sample (Barlough et al., 1986). Several hundred samples from dogs that were either symptomatic or asymptomatic for Lyme disease, were evaluated previously by KELA, IFA, and Western blot. Also, SPF dogs exposed to infected ticks developed antibody responses which, upon Western blotting, had patterns identical to those of naturally-infected dogs (Jacobson et al., 1992). Western blot confirmed that

samples having KELA slopes ranging from 0–100 units were negative. Slopes from 101–200 were consistent with low positive antibody responses to the Lyme agent while slopes >200 were considered strongly positive. The highest slope values observed approached 600 units, a level equivalent to >20000 IFA titer units.

Microagglutination test (MAT)

For the detection of antibodies to leptospiral serovars, the microscopic microagglutination technique (MAT) was used as described (USAHA, 1987).

Sera

Anti-*B. burgdorferi* sera from dogs naturally exposed to the Lyme disease agent were evaluated individually by KELA and were pooled for use in Western blot. Group 1 consisted of a pool from 32 dogs, group 2 from 18 dogs, group 3 from 14 dogs, group 4 from 16 dogs, and group 5 from 18 dogs.

Antibodies to leptospiral serovars were derived from dogs inhabiting areas that were non-endemic for Lyme disease. These dogs had no possible exposure to ticks harboring the *B. burgdorferi*. The titers were evaluated individually by MAT and sera were pooled for use in Western blots. Three groups of leptospira seropositive samples were pooled. First group containing 12 dogs, the second group containing 15 dogs and the third group containing 19 dogs. Titers of the pooled sera are shown in Table 2.

Rabbit antisera to *S. hyodysenteriae* were the gift of Dr. Wannemuehler. All sera were kept in −20°C until use.

TABLE 2

Characterization of serum pools used in cross-reactivity studies

Lyme pool	n[a]	KELA slope units *B. burgdorferi*[b]	*Leptospira* MAT titers				
1	32	458	negative				
2	18	478	negative				
3	14	467	negative				
4	16	455	negative				
5	18	467	negative				
Leptospira pool			L.P.	L.h.	L.i.	L.g.	L.c.[c]
1	12	113	6,400	400	100	400	100
2	15	143	3,200	200	100	12,800	100
3	19	277	12,800	800	100	400	200

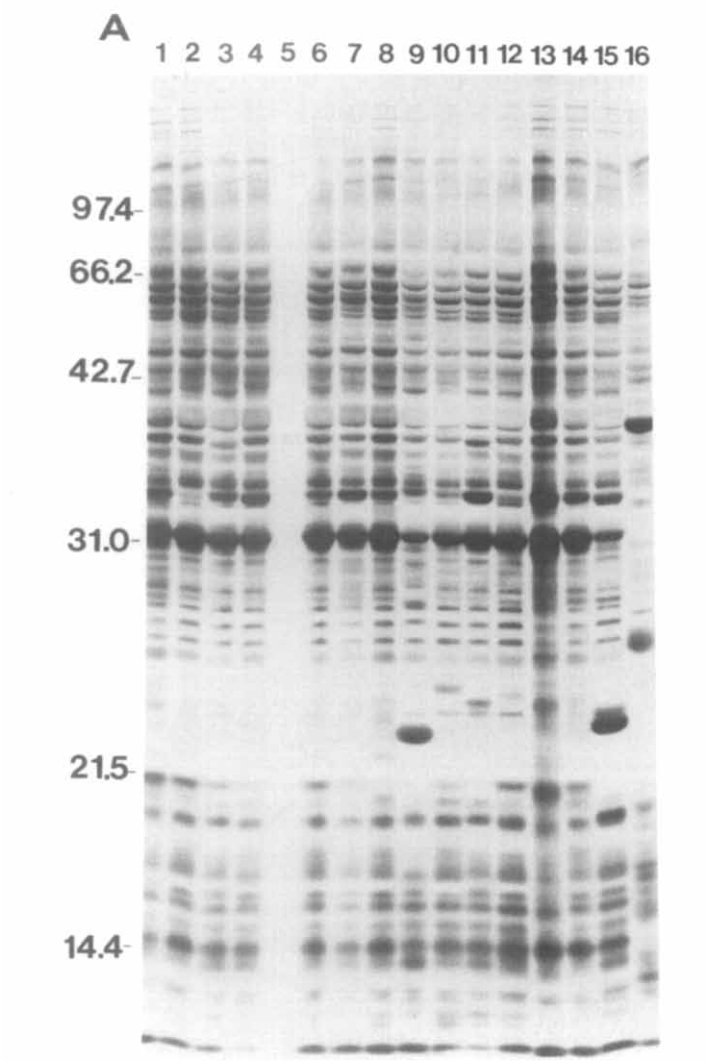
[a]Number of dogs represented in the serum pool.

[b]KELA slope units×1000. KELA values below 100 are negative for *B. burgdorferi*-specific antibody. Values from 100–200 are interpreted as low-positive while values exceeding 200 are moderate-to high-positive.

[c]L.P.=*L. pomona*, L.h.=*L. hardjo*, L.i.=*L. icterohaemorrhagiae*, L.g.=*L. grippotyphosa*, L.c.=*L. canicola*.

SDS-polyacrylamide gel electrophoresis and western blotting

Antigens prepared as aforementioned were subjected to SDS-polyacrylamide gel electrophoresis (12% acrylamide) at 200 V for 4–6 hours (Chang et al., 1987). The gels were either silver stained or proteins were transferred to nitrocellulose membranes electrophoretically. Immunoreactive proteins were detected by Western blot analysis (Towbin et al., 1979) as previously described (Chang et al., 1989). Antibody pools, as described above, were used as the first antibody, followed by a second antibody conjugate consisting of



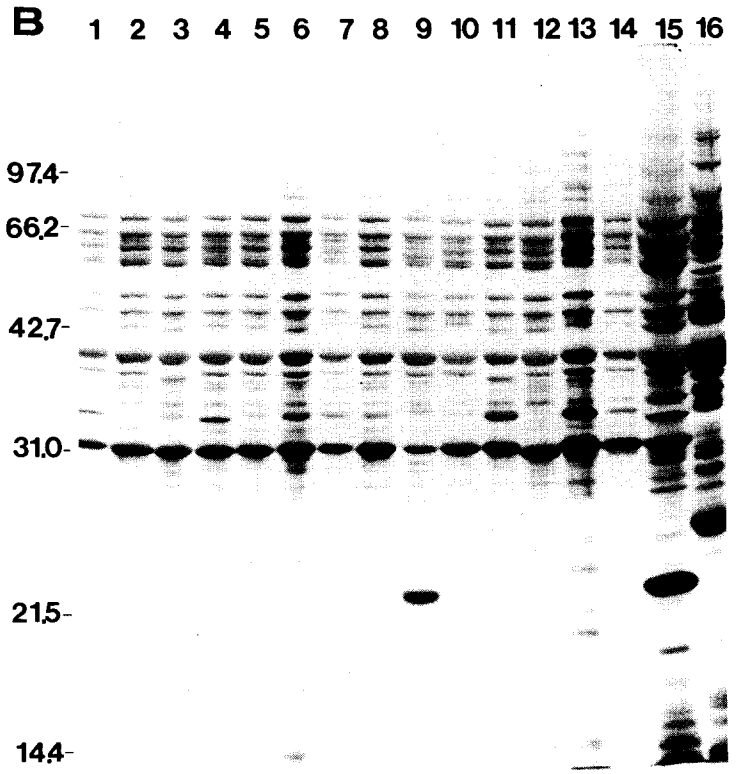


Fig. 1. Silver stained proteins of whole cell lysates of *Borrelia* species. long gel (14×24 cm), Lanes 1–14, *Borrelia burgdorferi* isolates from different areas: lane 1, ES1; lane 2, 22921; lane 3, AH2; lane 4, ES17; lane 5, no protein; lane 6, 21721; lane 7, 25550; lane 8, ES7; lane 9, 20004; lane 10, ES23; lane 11, 26815; lane 12, 21343; lane 13, 26816; lane 14, 21305; lane 15, *B. anserina* #12; and lane 16, *B. hermsii* #32.

goat anti-dog IgG linked to horse-radish peroxidase (Cappel Laboratories, Inc. lot #31340).

RESULTS

SDS-PAGE

SDS-PAGE patterns of the spirochetes were compared (Fig. 1). All *B. burgdorferi* isolates showed many major protein components with patterns, particularly of the major bands (60, 47, 41, 39 and 20 kDa), that are consistent with previously published data (Greene et al. 1988). Several isolates (lane 1, 3, 4, 6, 7, 8, 11, 13 and 14) showed strong bands at 34 kDa which may be the outer surface protein B (OspB). Other isolates (represented in lanes 2 and 10) had a limited amount of this protein. One isolate (lane 9) showed a 23

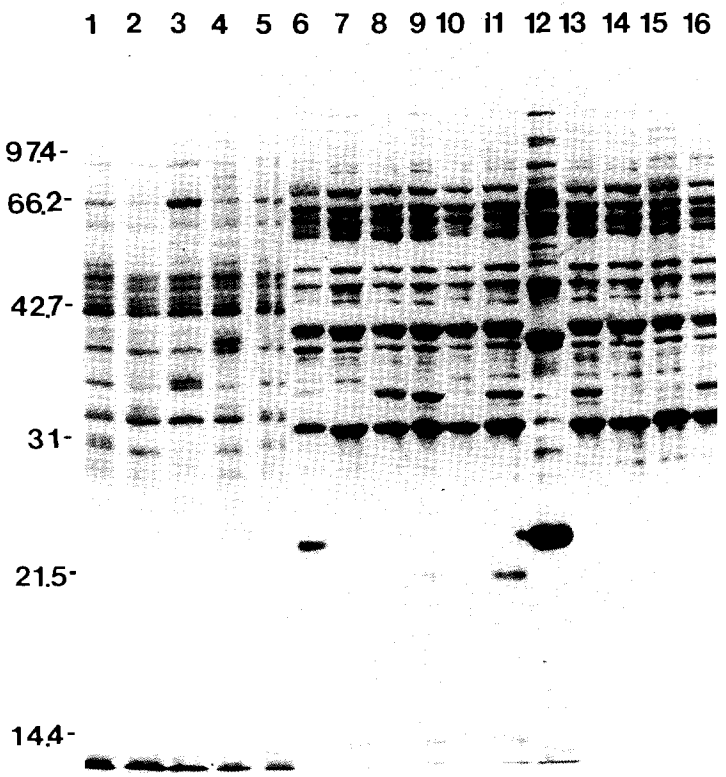


Fig. 2. Silver stained proteins of whole cell lysates of leptospiral serovars and *Borrelia* spp. Lane 1, serovar *pomona*; lane 2, serovar *hardjo*; lane 3, serovar *icterohaemorrhagiae*; lane 4, serovar *grippotyphosa*; lane 5, serovar *canicola*; Lanes 6–11 and 13–16:a *B. burgdorferi*, lane 6, 20004; lane 7, ES23; lane 8, ES14; lane 9, ES17; lane 10, 22921; lane 11, *B. anserina* #12, lane 12, *B. anserina* #32; lane 13, YFC1; lane 14, 25550; lane 15, ES14; and lane 16, 26815.

kDa band not seen in the other isolates which was similar to a major band of *B. anserina* (lane 15, approximately 23.5 kDa). It is uncertain, however, whether these two bands are distinct. *B. hermsii* (lane 16) showed two additional bands at approximately 27 and 40 kDa, respectively.

SDS-PAGE of whole-cell lysates of serovars *pomona*, *hardjo*, *icterohaemorrhagiae*, *grippotyphosa*, and *canicola* lysates (lanes 1–5, Fig. 2) showed a common pattern (62, 46, 43, 39.5 and 32 kDa) and several different bands among different serovars (40 kDa for serovar *grippotyphosa* – lane 4; 36 kDa for serovar *icterohaemorrhagiae* – lane 3; 30 kDa for serovar *pomona* – lane 1). However, the general SDS-PAGE pattern of leptospiral serovars was distinctly different from *Borrelia* spp. (Fig. 2).

Cross reactivity in KELA and MAT

The five groups of pooled sera from dogs naturally infected with the Lyme agent all had strong antibody activity in KELA but had no detectable anti-

body to the leptospiral serovars antigens (Table 2). The three groups of pooled sera from dogs naturally infected with leptospiral serovars developed antibody to the 4 leptospiral serovars as expected. These sera also had some reactivity against *B. burgdorferi* in the KELA test (Table 2).

Western blot analysis for detection of cross reacting antibodies to antigens among leptospiral serovars, Borrelia spp., S. hyodysenteriae and S. innocens

Five leptospiral serovars, 8 isolates of *B. burgdorferi*, and one isolate each of *B. anserina* and *B. hermsii* were subjected to SDS-PAGE and western blot analysis. The western blots were developed by subjecting one blot (Fig. 3) to group 2 pooled sera from dogs infected with the five different leptospiral serovars while the other blot (Fig. 4) was developed by using the group 5 pooled sera from dogs naturally exposed to the Lyme agent. The protein patterns between leptospiral serovars and *Borrelia* spp. were easily distinguishable.

When developing the immunoblots with an antibody pool consisting of an-

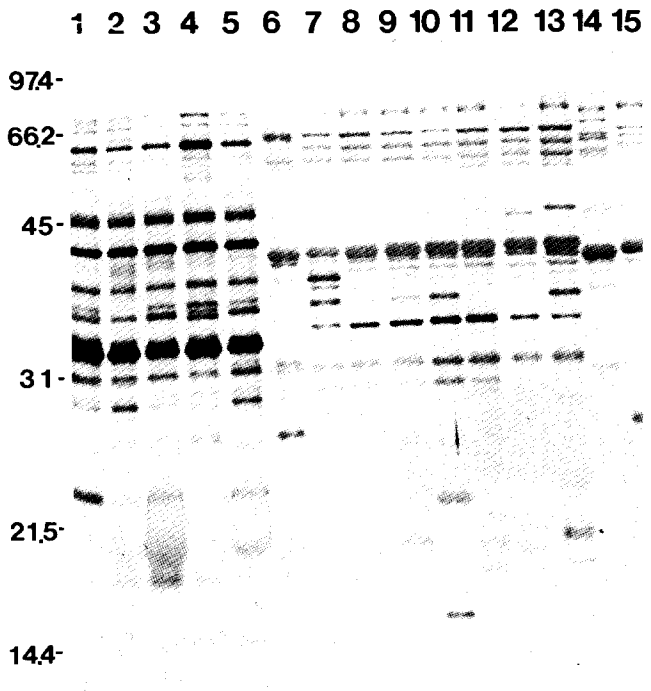


Fig. 3. Immunoblotting of leptospiral serovars and *Borrelia* spp. antigens developed using sera of the leptospiral pool #2. The molecular weight standards are noted in the left column of the figure. serovar *hardjo* (lane 1), *grippotyphosa* (lane 2), *pomona* (lane 3), *icterohaemorrhagiae* (lane 4), *canicola* (lane 5). Lanes 6–13 are isolates of *B. burgdorferi*: 22921 (lane 6), YFC1 (lane 7), ES12 (lane 8), ES10 (lane 9), 20004 (lane 10), ES23 (lane 11), 26815 (lane 12), 2591 (lane 13), *B. anserina* #12 (lane 14), and *B. hermsii* #32 (lane 15).

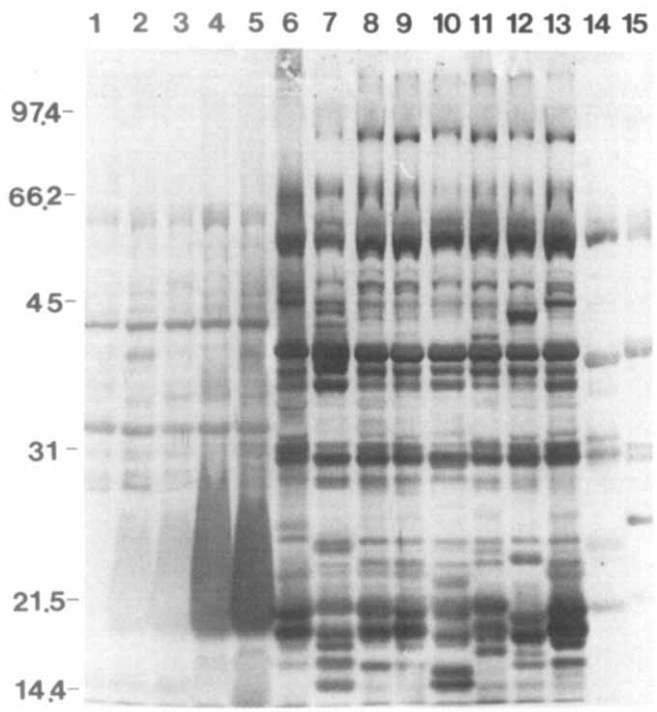


Fig. 4. Immunoblotting of leptospiral and *Borrelia* spp. antigens developed using sera from Lyme pool #5. The lanes contain the same antigens as indicated in Fig. 3.

tibodies to the 5 leptospiral serovars, the protein profile of the 5 serovars of *Leptospira interrogans* (Fig. 3) showed very similar major protein bands (60, 45, 43, 41, 33.5 and 31 kDa). There were, however, minor differences: serovar *grippotyphosa* (lane 2) did not show the 35 kDa band and serovar *icterohaemorrhagiae* (lane 4) showed an additional 34 kDa band. A band appeared at approximately 30 kDa for serovar *grippotyphosa* (lane 2) and *canicola* (lane 5) while this band was very faint in serovar *hardjo* (lane 1), *pomona* (lane 3), and *icterohaemorrhagiae* (lane 4). Three major bands (60, 41, and 33 kDa) among the eight isolates of *B. burgdorferi* appeared when using antibodies to the leptospiral serovar pool. Also, there was only one major band of 41.5 kDa in *B. anserina* (lane 14) and two major bands of 41.5 and 25 kDa for *B. hermsii* (lane 15).

When sera from dogs infected with the Lyme disease agent were used, two distinct bands appeared for the 5 leptospiral serovars with molecular weight of 32 and 44 kDa (Fig. 4). The smear staining of 30–20 kDa for lysates of serovar *grippotyphosa*, *pomona*, *icterohaemorrhagiae*, and *canicola* (lanes 2–5) probably represents carbohydrate (Fig. 4).

The other four pools of serum from dogs infected with the Lyme agent, and

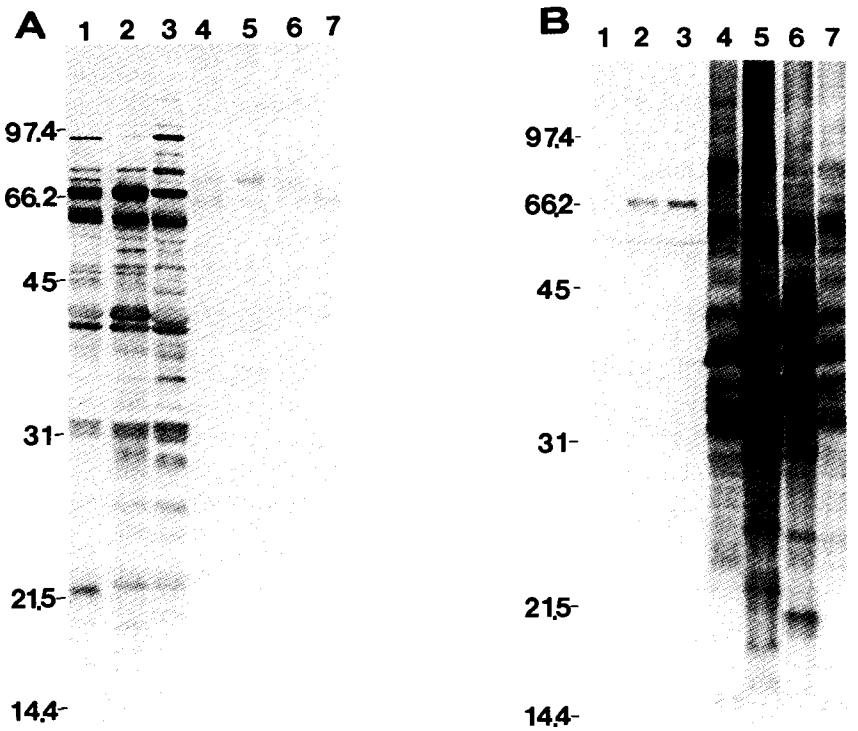


Fig. 5. Immunoblotting of *Borrelia burdgorferi* and *Serpulina* antigens by the sera of Lyme pool #5 (blot A) and anti-*S. hyodysenteriae* serum (blot B). Lanes 1–3 contain *B. burdgorferi* antigens, 21305, 25550, and AH2; Lanes 4 and 5 contain *S. hyodysenteriae* B169 and ACK300/8; Lane 6 and 7 contain *S. innocens*, B256 and B657.

the two other pools of sera from dogs infected with leptospiral serovars spp. showed western blot patterns very similar to those shown in Figs 2–4 (data not shown).

When using Lyme infection-specific antibody for immunoblotting, several faint bands with molecular weights varying from 40, 60 and 70 kDa appeared for isolates of *S. hyodysenteriae* (lanes 4 and 5), and bands of 42, 60, and 68 kDa in the 2 strains of *S. innocens* (Fig. 5A). Two faint bands (55 and 52 kDa) appeared in three of the *B. burgdorferi* strains and another 68 kDa band developed in strain 25550 (lane 2) and AH2 (lane 3) when anti-*S. hyodysenteriae* antibody was used in immunoblotting (Fig. 5B).

DISCUSSION

Western immunoblotting has been used to analyze the antibody response or to confirm Lyme disease infection in human patients (Karlsson et al., 1989;

Karlsson, 1990; Zoller et al., 1991). Western blotting has confirmed KELA results in our laboratory and has also been used elsewhere as a confirmatory test for dog Lyme disease (Greene et al., 1988; Lindenmayer et al., 1990; Jacobson, et al., 1992).

SDS-PAGE of the total lysate of the five leptospiral serovars species showed distinctly different protein profiles when compared to the *Borrelia* species antigens (Fig. 2). The *B. anserina* strain also showed a different SDS-PAGE pattern from that of *B. burgdorferi* isolates. Based on the protein profiles from SDS-PAGE, differentiation of leptospiral serovars, *B. burgdorferi*, and *B. anserina* was not difficult.

In western immunoblotting, antibody to *B. burgdorferi* cross-reacted slightly with leptospiral antigens and antibody to the leptospiral serovars reacted somewhat more vigorously with antigens of *B. burgdorferi*. The protein profiles were qualitatively different and staining patterns were less intensive in the heterologous systems indicating relatively limited quantities of cross reacting antibodies (Fig. 3 and Fig. 4). When reacted with *Borrelia* antigen, antibodies from the leptospiral pool showed dominant bands at approximately 41 kDa, which is consistent with the flagellin antigen, and at 60 kDa, which may reflect a heat shock protein of *Borrelia*; some other protein bands also were developed by antibody to leptospiral serovars (Fig. 3). When we tested sera from the Lyme pool against antigens of the leptospiral serovars, two main bands appeared with molecular weight of approximately 32 and 44 kDa, respectively. A smear between 30–18 kDa is probably a carbohydrate; further identification of this substance is necessary. These results indicate that the antibody to the leptospiral serovars resulted in stronger cross reactions to *B. burgdorferi* antigens than did sera from the *B. burgdorferi* antibody pool when tested against leptospiral antigens.

These observations suggest that dogs exposed to leptospiral antigens may have antibodies that will cross-react in serotests for the antibody to the Lyme agent. In KELA, the range of activity observed for several thousand clinical samples and dogs exposed experimentally to infected ticks ranged from 0 to about 600 slope units (Jacobson et al., 1992). Activity from 0 to 100 units could not be distinguished from background activity and samples in this range have been considered negative in KELA while antibody activity ranging from 100 to 200 units is considered as low-positive based on western immunoblotting confirmation. From Table 2, it is clear that antibody to the leptospiral serovars is detected as low positive in the KELA serotest for antibody to *B. burgdorferi*. It is, therefore, probable that the same cross-reactivity occurs in other ELISAs that use whole-cell lysates of *B. burgdorferi*.

Western blot also showed a weak cross reaction between *B. burgdorferi* and *S. hyodysenteriae* or *S. innocens* (Fig.5. A and B). Such reactions may not be a problem in the diagnosis of canine Lyme disease, since *S. hyodysenteriae* or *S. innocens* cause disease only in pigs.

Since leptospirosis is an important disease in dogs in the USA, most dogs have received a leptospiral vaccine. Because western blots confirm serological cross-reactions between leptospiral serovars and *Borrelia* species, they constitute a useful tool in validating the specificity of ELISA tests. Furthermore, since it is not difficult to differentiate between antibodies to the leptospiral serovars and *B. burgdorferi* in western blots, the technique can be a useful aid in the diagnosis of canine Lyme disease. Because validation of the KELA assay is based on western immunoblotting of sera of known Lyme agent infection status, the cutoff between seropositive and seronegative dogs in the KELA Lyme test is based on objective criteria. The KELA is thus a valuable serotest for the confirmation of canine Lyme disease.

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REFERENCES

Anderson, J.F., 1989. Epizootiology of *Borrelia* in Ixodes tick vectors and reservoir hosts. Rev. Infect. Dis., 11: S1451–1459.

Barbour, A.G., 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med., 54: 521–525.

Barlough, J.E., Jacobson, R.H., Sorresso, G.P., Lynch, T.J., and Scott, F.W., 1986. Coronavirus antibody detection in cats by computer-assisted kinetics-based enzyme-linked immunosorbent assay (KELA): Field studies. Cornell Vet., 76: 227–235.

Burgess, E.G., Gillette, D., and Pickett, J.P., 1986. Arthritis and panuveitis as manifestations of *Borrelia burgdorferi* in a Wisconsin pony. J. Am. Vet. Med. Assoc., 189: 1340–1342.

Chang, Y.F., Young, R., Post, D., and Struck, D.K., 1987. Identification and characterization of the *Pasteurella haemolytica* leukotoxin. Infect. Immun., 55: 2348–23564.

Chang, Y.F., Young, R., and Struck, D.K., 1989. Cloning and characterization of a hemolysin gene from *Actinobacillus (Haemophilus) pleuropneumoniae*. DNA, 8: 635–647.

Cohen, N. D, Carter, C. N, Thomas, M.A. Jr., Angulo, A.B. and Eugster, A.K., 1990. Clinical and epizootiologic characteristics of dogs seropositive for *Borrelia burgdorferi* in Texas: 110 cases (1988). J. Am. Vet. Med. Assoc., 197: 893–898.

Grauer, G.F., Burgess, E. C, Cooley, A. J, and Hagee, J.H., 1988. Renal lesions associated with *Borrelia burgdorferi* infection in a dog. J. Am. Vet. Med. Assoc., 193: 237–239.

Greene, R.T., Levine, J.F, Breitschwerdt, E.B., and Berkhoff, H.A., 1988. Antibodies to *Borrelia burgdorferi* in dogs in North Carolina. Am. J. Vet. Res., 49: 473–476.

Greene, R.T., Walker, R.L., Nicholson, W.L., Heidher, H.W., Levine, J.F., Burgess, E.C., Wyand, M., Breitschwerdt, E.B., and Berkhoff, H.A., 1988. Immunoblot analysis of immunoglobulin G response to the Lyme disease agent (*Borrelia burgdorferi*) in experimentally and naturally exposed dogs. J. Clin. Microbiol., 26: 648–653.

Jacobson, R.H.., Shin, S.J., Appel, M.J., Lauderdale, T.-L., and Chang, Y.-F., 1992. Canine antibody responses to *Borrelia burgdorferi* induced by exposure to infected ticks, injection of viable organisms, or vaccination. V Intl. Conf. Lyme Borreliosis, Arlington, Virginia, May, 1992. Abstract No., 202.

Karlsson, M., Mollegard, Stiernstedt, G., and Wretlind, B., 1989. Comparison of western blot and enzyme-linked immunosorbent assay for diagnosis of Lyme borreliosis. Eur. J. Clin. Microbiol. Infect. Dis., 8: 871–877.

Karlsson, M., 1990. Western immunoblot and flagellum enzyme-linked immunosorbent assay for serodiagnosis of Lyme borreliosis.J. Clin. Microbiol., 28: 2148–2150.

Kornblatt, A.N., Urband, P.H., and Steere, A.C., 1985. Arthritis caused by *Borrelia burgdorferi* in dogs. J. Am. Vet. Med. Assoc., 186: 960–964.

Lindenmayer, J., Weber, M., Bryant, J., Marquez, E., and Onderdonk, A., 1990. Comparison of indirect immunofluorescent-antibody assay, enzyme-linked immunosorbent assay, and western immunoblot for the diagnosis of Lyme disease in dogs. J. Clin. Microbiol., 28: 92–96.

Lissman, B.A., Bosler, E.M., Camay, M., Ormiston, B.G. and Benach, J.L., 1984. Spriochete-associated arthritis (Lyme disease) in a dog. J. Am. Vet. Med. Assoc., 185: 219–220.

Magnarelli, L.A., Anderson, J.F., Kaufmann, A.F., Lieberman, L.L., and Whitney, G.D., 1985. Borreliosis in dogs from southern Connecticut. J. Am. Vet. Med. Assoc., 186: 955–959.

Magnarelli, L.A., Anderson, J.F., Schreier, A.B., and Ficke, C. M., 1987. Clinical and serologic studies of canine borreliosis. J. Am. Vet. Med. Assoc., 191: 1089–1092.

Roush, J.K., Manley, P.A., and Dueland, R.T., 1989. Rheumatoid arthritis subsequent to *Borrelia burgdorferi* infection in two dogs. Am. Vet. Med. Assoc., 195: 951–953, 1989.

Schulze, T.L., Bosler, E.M., and Shisler, J.K., 1986. Prevalence of canine Lyme disease from an endemic area as determined by serosurvey. Zentralbl. Bakteriol. Paraasitenkd. Infectionkr. HyG. Abt. Orig. Reihe A 263: 427–434.

Schulze, T.L., Bosler, E.M., Shisler, J.K., Ware, I.C., Lakat, M.F. and Parkin, W.E., 1987. Prevalence of canine Lyme disease from an endemic area as determined by serosurvey. Zentralbl. Bakteriol. Mikrobiol. Hyg. [A]., 263: 427–434, 1987.

Steere, A.C., Malawista, S.E., Snydman, D.R., Shope, R.E., Andiman, W.A., Ross, M.R., and Steele, F.M., 1977. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum., 20: 7–17.

Towbin, H., Staehelin, T., and Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Proc. Natl. Acad. Sci. USA, 76: 4350–4354.

Zoller, L., Burkard, S. and Schafer, H., 1991. Validity of Western immunoblot band patterns in the serodiagnosis of Lyme borreliosis. J. Clin. Microbiol., 29: 174–182.

USAHA, 1987. Proc., 91st Annual Meeting of the USAHA, 1987, pp 63–73.