Comparative Study on Cross-Reaction of Leptospiral Antibodies in Several Serological Tests to Detect Antibodies to *Borrelia burgdorferi* in Dogs

Yoshishiro SUGIYAMA, Fumihiro SUGIYAMA, and Ken-ichi YAGAMI

*Laboratory Animal Research Center, University of Tsukuba, Tennodai, Tsukuba, Ibaraki 305, Japan*

(Received 13 February 1992/Accepted 9 October 1992)

**ABSTRACT.** The effects by the leptospiral antibody are described at several serological tests for the detection of the antibody to *B. burgdorferi* in dogs. The titers at ELISA used with the ultrasonicated bacterial antigen (US-ELISA) were related with IFA. But cross reaction with the leptospiral antibody were shown in an agglutination test. Non-specific and cross reactions were observed in ELISA used with formalin-killed bacterial antigen. This indicates that US-ELISA is a reliable serodiagnosis method for Lyme disease in dogs in eliminating the cross reaction with leptospiral antibody.—**KEY WORDS:** *B. burgdorferi*, cross reaction, Leptospira.


*Borrelia (B.) burgdorferi* [9], a causative spirochetal agent for Lyme disease [3], was first isolated in the eastern United States. Lyme disease is known as a major tick-bone zoonosis, and recently many researchers have studied this disease. Precise detection of antibody to *B. burgdorferi* is important for epidemiology and diagnosis of this disease. Mammals can be natural hosts of this spirochete. Other species of spirochetes besides *B. burgdorferi* also infect these animals, and antibodies to these spirochetes often cross-react with spirochetes including *B. burgdorferi* [11, 12].

Lyme disease in dogs infected with *B. burgdorferi* through ticks was clinically reported [5, 6, 10, 14]. They also described antibodies to *B. burgdorferi* and some researchers have recently reported the positive rate of antibodies to *B. burgdorferi* in Japanese dogs [1, 8]. In a test with dog serum, leptospiral antibody is considered most likely to be cross-reactive with *B. burgdorferi* antigen. Although leptospirosis is one of the major diseases in dogs and its vaccine is commonly used for dogs in Japan, the cross-reactivity between *B. burgdorferi* and *Leptospira* in several serological tests was not sufficiently designated.

In this study we obtained detailed data on cross-reactivity by the leptospiral antibody in several serological tests of the detection of the antibody to *B. burgdorferi* in dogs.

A standard strain, *B. burgdorferi* strain B31, was propagated in BSKII medium [2] at 30°C for 5 days and killed with 0.5% formalin. The killed organisms, washed three times with phosphate buffered saline (PBS) and resuspended in PBS, were used as the antigen for indirect immunofluorescent assay (IFA) and agglutination test (AG-test). For enzyme linked immunosorbent assay (ELISA) two types of antigens were used and each ELISA was presented as F-ELISA and US-ELISA in this study. One was the formalin-killed antigen applied to F-ELISA. And the other was the ultrasonicated antigen for US-ELISA, which was prepared from the formalin-killed organisms treated by ultrasonication three times for 30 seconds each time at 60 W output (Ultrasonic homogenizer US-150, Nissei, Japan).

The serum samples obtained from 30 dogs were used in our study. Serum samples (B+ serum) reacting positively to *B. burgdorferi* B31 by US-ELISA were selected from 35 wounded mongrel dogs, which seemed to be naturally infected with *B. burgdorferi*. Other serum samples (L+ serum), containing antibodies to *Leptospira*, were obtained from the 15 mongrel dogs for experimental use. Three out of the 15 dogs were experimentally infected with *Leptospira interrogans* serovar *icterohaemorrhagiae*, four other dogs were vaccinated with *Leptospira interrogans* serovar *icterohaemorrhagiae* and the remaining 8 with *canicola*. Each L+ serum indicated 16–4,096 titers to *Leptospira interrogans* serovar *icterohaemorrhagiae* or *canicola* in an agglutination test.

The methods previously described [4, 7] were modified for the present study. A summary of the changes is shown below.

1) **ELISA:** Each antigen (bacterial wet volume: 10 μg) was fixed on 96-well immuno-plates (NUNC) by overnight incubation at 4°C in carbonate buffer. The serum sample diluted in buffer (1% tween 80-PBS containing 0.2% bovine serum albumin) was added to the antigen and incubated at 37°C for 1 hr. Peroxidase labeled anti-dog Ig G goat serum (CAPPEL) was applied on top and the same incubation was performed. PBS containing 0.5% tween 20 was used for washing of the free antigen and sera. O-phenylenediamine was used as a substrate and its coloring reaction was measured in a microplate spectrophotometer (492 nm) and the positive reaction was taken as 0.2 greater optical density than that of the negative control.

2) **IFA:** One μl of antigen was put onto a glass slide (Multitest slide: FLOW) and dried for fixation. A diluted serum sample and FITC labeled anti-dog Ig G goat serum (CAPPEL) were applied to the spot of antigen on the slide glass for reaction at 37°C for 1 hr. PBS was used for dilution and washing of the sera. The slide glass was observed under a fluorescence microscope.

3) **AG-test:** The antigen and diluted serum sample were mixed in a U-bottomed 96-well microplate (FALCON) and incubated at 37°C for 2 hr. After incubation, the microplate was kept overnight at 4°C and then the agglutination was observed under a stereomicroscope.

Each test was preliminarily carried out to decide the optimal concentrations of the antigen and secondary serum with the reliable positive (ELISA titer; ≥20,480).
and negative dog sera to *B. burgdorferi* which were collected from the hyper-immunized and non-immunized beagle dogs, respectively. The titer in each test was expressed as a reciprocal of the highest serum dilution.

The results are shown in Fig. 1. US-ELISA titers were closely related with IFA titers (r=0.817), as shown in Fig. 1A. All the L+ serum had low levels of antibodies to *B. burgdorferi*; below 400 titer in US-ELISA and below 20 titer in IFA. In contrast, the B+ serum demonstrated high titers, 800-6,400 in US-ELISA and 40-320 in IFA. The minimal positive levels of antibodies in US-ELISA and IFA were estimated to be 800 and 40 titers, respectively. These results indicated that cross-reaction of leptospiral antibodies was virtually eliminated in detecting antibodies to *B. burgdorferi* in dog serum samples in these two tests. However, in practice we had a little difficulty in assessing the positive reaction of the sera with low titers by IFA. We therefore recommend that US-ELISA rather than IFA be used to detect the antibodies to *B. burgdorferi*.

The titer in F-ELISA showed a low degree of correlation (r=0.186) with that in US-ELISA (Fig. 1B). Most of the B+ serum samples (32/35) showed a titer below 400, which was a negative level in F-ELISA. These findings suggest that the reaction of F-ELISA is interfered with by the antigen itself. Masuzawa et al. [13] reported that the outer surface protein A of the Japanese strain was different from that of other strains of *B. burgdorferi*. We suppose that the weak activity of the antibody in this study can be attributed to this difference in the outer surface protein of the antigen. On the other hand, 2 out of 15 L+ serum samples had high titers (3,200) in F-ELISA. This high level might be produced by the cross-reaction of leptospiral antibody or another non-specific reaction. As F-ELISA antigen was more insoluble and more unevenly distributed on the well wall than US-ELISA antigen, these non-specific reactions might have been caused. In the AG-test (Fig. 1C), it was estimated that the agglutination titers of all the B+ serum samples were over 40. They also seemed to have a relatively high correlation with US-ELISA titers. But 6 out of 15 L+ serum samples cross-reacted with *B. burgdorferi* antigen. This suggests that further treatments of bacterial antigen in F-ELISA and AG-test, such as the absorption with leptospiral antibody, are required to minimize the cross-reaction and non-specific reaction.

Magnarelli et al. [12] reported that 2 out of 14 serum samples in human leptospirosis showed signs of cross-reactivity with *B. burgdorferi* by IFA. Isogai et al. [8] carried out the first serological surveillance of Lyme disease on Japanese dogs and briefly discussed the cross-reaction of leptospiral antibody but there were no data. Arashima [1] demonstrated that the history of leptospiral vaccination did not affect the serological assay of *B. burgdorferi* in dogs. However, there were no data showing the titer of leptospiral antibody in these vaccinated dogs. On the other hand, Schulze et al. [14] indicated that the low titer (<16) of leptospiral antibody did not interfere with the interpretation of the levels of the

Fig. 1. Titers of the antibody to *B. burgdorferi* in US-ELISA were compared with those in IFA (A), F-ELISA (B) and AG-test (C). Closed and open circles indicate the B+ serum titer and L+ serum titer, respectively. Dotted lines indicate the borders between positive and negative titers.
antibody to *B. burgdorferi*. In this study, L+ serum which showed signs of reliable agglutination titers (≥16) to *Leptospira* was used and several serological tests for Lyme disease in dogs were compared at the point of the cross-reactivity of L+ serum. The results indicated that the cross-reaction of L+ serum seemed to be virtually eliminated in US-ELISA, but not in F-ELISA or the AG-test. Therefore, a high titer of leptospiral antibody also did not interfere with the interpretation of antibody levels to *B. burgdorferi* in US-ELISA used with the antigen of strain B31. In conclusion, US-ELISA with the antigen of strain B31 is a more reliable method to use for serodiagnosis of Lyme disease than F-ELISA or AG-test from the standpoint of the elimination of cross-reaction with leptospiral antibody.

ACKNOWLEDGEMENTS. We are grateful to Dr. M. Mori (National Institute of Health, Tokyo, Japan) for providing the bacteria and also for his helpful advice.

REFERENCES