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Research article

Blastomyces dermatitidis Antibody Detection in Dogs with Blastomycosis: Comparative Evaluations with Two Indirect ELISA Methods

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Abstract

Blastomyces dermatitidis is the causative agent of the systemic disease blastomycosis. Disease diagnosis has proven difficult, however the indirect enzyme-linked immunosorbent assay shows promise as a rapid and specific diagnosis tool for use in the clinical laboratory. In this study two commercial anti-dog IgG conjugates, manufactured by Kirkegaard & Perry and Santa Cruz Biotechnology, were used in the indirect ELISA for detection of IgG dog antibodies against *B. dermatitidis*. The two conjugates were tested with sera from 25 dogs diagnosed with blastomycosis. The conjugate/substrate from Kirkegaard & Perry produced a mean absorbance of 1.212 whereas the conjugate/substrate from Santa Cruz Biotechnology produced a mean absorbance of 0.790. Both commercial anti-dog IgG conjugates detected *B. dermatitidis* antibodies in all of the infected dogs.

Keywords: *Blastomyces dermatitidis*, Blastomycosis, Anti-body detection, lysate antigens, ELISA, dog serum

Introduction

Blastomycosis is a systemic disease, caused by the opportunistic dimorphic fungus *Blastomyces dermatitidis*, which typically infects canines and humans. *B. dermatitidis* is endemic in the Southeastern United States, the Mississippi River basin and up to the Great Lakes region. It grows best in warm, moist soil that is rich in decaying organic

material. Isolating the organism directly from the soil has proven difficult, therefore the endemic region has been defined through reports of infection [1-3].

At 25^{o}C, *B. dermatitidis* grows in the mycelial phase, producing the infectious spores that may be inhaled. Once inhaled, the spores can infect the lungs and convert to the yeast phase which grows optimally at 37^{o}C. In the lungs, *B. dermatitidis* may cause acute or chronic pneumonia, while some individuals may be entirely asymptomatic. For many, the infection may resolve on its own with or without treatment. However, once the lungs have been infected, *B. dermatitidis* may disseminate, causing disease within other organs, especially the skin, bones and genitourinary system [4-6].

Blastomycosis is often misdiagnosed as a bacterial or viral respiratory infection in human patients, leading to delayed treatment. In addition, culturing the organism from patient samples may take several weeks, and fungal disease is often not suspected by clinicians. For most canines, infection is not suspected until the disease has already disseminated to the epidermis presenting as red lesions across the body [4-8].

Diagnosing blastomycosis has proved quite challenging in the clinical laboratory. Techniques such as microscopy, culture, and histopathologic methods have been used in the detection of *B. dermatitidis* infections with varying levels of specificity. One of the most sensitive and rapid methods for detecting *B. dermatitidis* infections is the enzyme-linked immunosorbent assay (ELISA), with a specificity ranging from 77% to 100% [7-14]

Numerous different commercial anti-dog conjugates are available on the market. In this study we tested two different anti-dog IgG conjugates; the first from Kirekegaard & Perry, and the second from Santa Cruz Biotechnology. Indirect ELISA was performed using *B. dermatitidis* yeast phase lysate antigen and 25 dog sera.

Materials and Method

Lysate antigen preparation

The *B. dermatitidis* yeast phase lysate antigen (ERC-2; dog isolate; Wisconsin) used to coat the microdilution plates was prepared by a method similar to one previously used for the production of yeast lysate antigen from *Histoplasma capsulatum* [15-17] and modified in our laboratory for *B. dermatitidis* lysate antigen production [9]. The yeast phase cells were grown for 7 days at 37°C in a chemically defined medium in an incubator shaker. They were then harvested by centrifugation (700 x g; 5 min), washed with distilled water, resuspended in distilled water and allowed to lyse in water for 7 days at 37°C with shaking. The preparations were centrifuged, filter sterilized, and merthiolate was added (1:10,000) before storage at 4^{o}C. Protein determinations were performed on the lysate using the BCA Protein Assay Kit (Thermo-Fisher, Pierce Chemical Company, Rockford, IL) and dilutions of the antigen used in the ELISA assays was based on protein concentration.

Serum specimens

Twenty-five serum specimens from dogs with diagnosed blastomycosis were provided by Dr. A.M. Legendre (University of Tennessee College of Veterinary Medicine, Knoxville, TN).

Enzyme-linked immunosorbent assay (ELISA)

Two different ELISA systems were used to assay the dog serum specimens. In Trial 1 a KPL (Kirkegaard & Perry) anti-dog IgG conjugate, TMB peroxidase substrate and stop solution were used and in Trial 2 a Santa Cruz Biotechnology anti-dog conjugate, TMB peroxidase substrate and stop solution were used to determine the reactivity of each serum specimen. The ability of each ELISA method to detect antibodies in the above serum specimens was determined using the indirect enzyme-linked immunosorbent assay (ELISA). The ERC-2 lysate antigen was diluted (2000 ng/ml of protein) in a carbonate-bicarbonate coating buffer (pH 9.6) and then added to triplicate wells (100 ul) of a NUNC 96-well microplate (Fisher-Thermo). The plates were then incubated overnight at 4 C in a humid chamber followed by washing three times with phosphate buffered saline containing 0.15% Tween 20 (PBS-T). The serum specimens (1:2000 dilution; 100 ul) were added to the microplate wells in triplicate and incubated for 30 min at 37°C in a humid chamber. Following this incubation, the wells were washed as above and 100 ul of goat anti-dog (H & L) peroxidase conjugate (Trial 1 and Trial 2) was added to each well and incubated for 30 min at 37°C. Initial dilutions were performed and determined that the optimal dilution for the KPL conjugate was a 1:2000 dilution and the optimal dilution for the Santa Cruz conjugate was a 1:1500 dilution. The plates were again washed as above and 100 ul of the above substrates (Trial 1 and Trial 2) was added to each well and incubated for approximately 2-5 min at room temperature. The reaction was stopped by the addition of Stop Solution (KPL) and the absorbance read at 450 nm using a BIO-RAD 2550 EIA reader.

Results and Conclusion

In trial 1, using the KPL anti-dog IgG conjugate, antibody was detected in all 25 tested dog serum specimens. Figure 1 displays the mean absorbance result for each of the 25 dog sera tested. The 25 specimens varied in mean absorbance values from 0.409 to 2.819. The mean absorbance for all specimens tested with the KPL conjugate/substrate was 1.212.

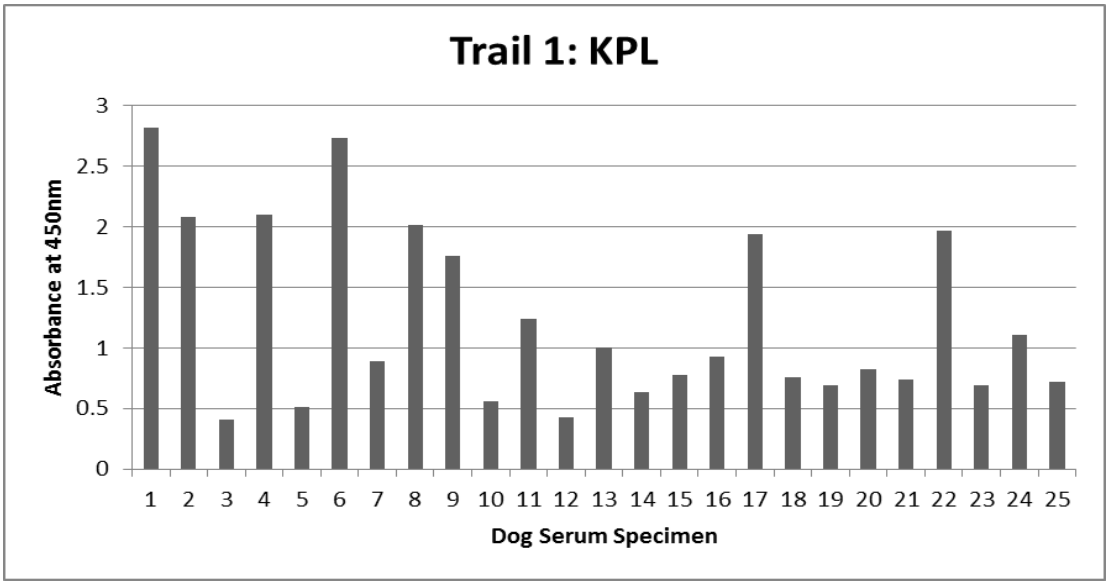

Figure 1. Mean absorbance results from Trial 1 ELISA using KPL anti-dog IgG conjugate/substrate and the serum specimen from 25 dogs with diagnosed Blastomycosis.

In trial 2, using the Santa Cruz anti-dog IgG and substrate conjugate, antibody was detected in all 25 tested dog serum specimens. Figure 2 displays the mean absorbance value results. The 25 specimens varied in mean absorbance from 0.328 to 1.502. The mean absorbance for all specimens tested with the Santa Cruz conjugate/substrate was 0.790.

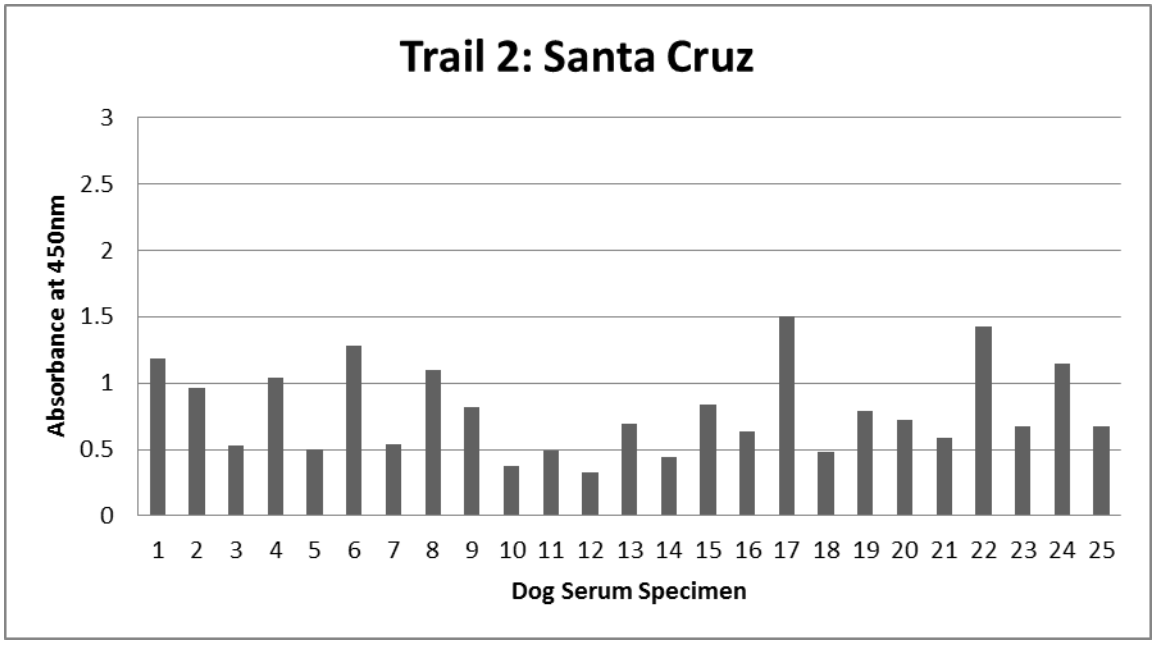

Figure 2. Mean absorbance results from Trial 2 ELISA using Santa Cruz anti-dog IgG conjugate/substrate and the serum specimens from 25 dogs with diagnosed Blastomycosis.

Both the KPL and Santa Cruz IgG conjugates/substrate were capable of detecting measurable antibody in all 25 dog sera. The KPL conjugate/substrate reacted in approximately 1 minute whereas the Santa Cruz conjugate/substrate reacted in approximately 3 minutes. Even after the longer reaction time, the Santa Cruz conjugate/substrate had lower mean absorbance values for the majority of the serum specimens than the KPL conjugate. Perhaps with a different dilution of the Santa Cruz conjugate, the two trials could have produced more similar mean absorbance values. Despite differences in mean absorbance values, this study shows that both the KPL and Santa Cruz conjugates/substrates could serve as useful reagents in the detection of dog *B. dermatitidis* antibodies in the ELISA.

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