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Research article

Enzyme Immunoassay Detection of Antibody in Dogs with Blastomycosis: Comparison of Four *Blastomyces dermatitidis* Animal Isolate Antigens

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Abstract

The misdiagnosis of *Blastomyces dermatitidis* as a viral or bacterial infection has become an increasingly apparent problem in endemic areas. The proper and exact immunodiagnostics of the organism has been the center of much research in our lab. A majority of our focus is on the reactivity of numerous lysate antigens against serum specimens from various animals. In this study we used the ELISA technique to compare the reactivity of four lysate antigens [polar bear:T-27, sea lion:81, bat:56920 and cat:104] with regard to antibody detection in 24 serum specimens from dogs with blastomycosis. Absorbance values for each of the four lysate antigens with the different sera [three trials] indicated that the lowest mean absorbance value was 1.399 (bat, 56920) and the maximum value was 1.775 (polar bear, T-27). The lysate antigen isolated from sea lion (81) had a mean absorbance value of 1.674, and the lysate antigen isolated from cat (104) was 1.631. All four of the lysate antigens were able to detect antibody in the dog sera.

Keywords: *Blastomyces dermatitidis*, immunodiagnostic, antibody detection, lysate antigens, ELISA, dog serum

Introduction

Blastomyces dermatitidis is a dimorphic fungal agent that causes the systemic fungal disease blastomycosis. *B. dermatitidis* is endemic in the southeastern and north-central regions of the United States and also in areas of Canada bordering the states of Wisconsin and Minnesota. Its primary niche is moist soil and decaying matter [1,3]. Found in a mycelial state in nature, the spores of *B. dermatitidis* may become airborne and inhaled into the lungs, which initiates the conversion into a broad-based yeast cell. Without proper treatment, *B. dermatitidis* can disseminate to the central nervous system to cause meningitis, as well as to various other organs in the body. Blastomycosis can have fatal repercussions, especially for the immunocompromised; therefore, it is critical for blastomycosis to be diagnosed early in the infection process to avoid the misdiagnosis as a bacterial or viral infection. Although culturing and histologic diagnosis of the pathogen have been effective, they are expensive and often delay the identification of the disease until after dissemination [4,10]. The development of an immunodiagnostic technique allowing for the early and reliable identification of blastomycosis may prevent the misdiagnosis of blastomycosis.

The focus of our lab has been centered on the utilization of immunoassays to test the reactivity of lysate antigens against various serum specimens of infected and immunized animals. Although significant progress has been made, more research is needed to improve the specificity and sensitivity of *B. dermatitidis* lysate antigens [11-14]. Our current study uses the ELISA technique to test the reactivity of four lysate antigens against 24 different sera from dogs infected with *B. dermatitidis.*

Materials and Methods

Lysate antigens

Four yeast lysate antigens were prepared from sea lion (81), polar bear (T-27), bat (56920), and cat (104) isolates of *B. dermatitidis*. Each of the isolates was prepared by a method similar to one that was 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 [11]. 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, re-suspended in distilled water and allowed to lyse for 7 days at 37°C in water with shaking. The preparations were centrifuged, filter sterilized, merthiolate added (1:10,000) and stored at 4°C. Protein determinations were performed on the lysates using the BCA Protein Assay Kit (Thermo-Fisher, Pierce Chemical Company, Rockford, IL) and dilutions of the antigenic reagents used in the ELISA assays were based on protein concentration.

Serum specimens

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

Enzyme linked immunosorbent assay (ELISA)

The ability of each yeast lysate reagent to detect antibodies in the above serum specimens was determined using the indirect enzyme-linked immunosorbent assay (ELISA) as previously described [11-14]. Each 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 µl) 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 µl) 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 µl of goat anti-dog IgG (H & L) or anti-rabbit IgG (H&L) peroxidase conjugate (Kirkegaard and Perry, Gaithersburg, MD, KPL) was added to each well and incubated for 30 min at 37°C. The plates were again washed as above and 100 µl of Sure Blue Reserve TMB peroxidase substrate (KPL) was added to each well and incubated for approximately 2 min at room temperature. The reaction was stopped by the addition of Stop Solution (KPL) and the absorbance was read at 450 nm using s Bio-Tek Synergy HT reader.

Results

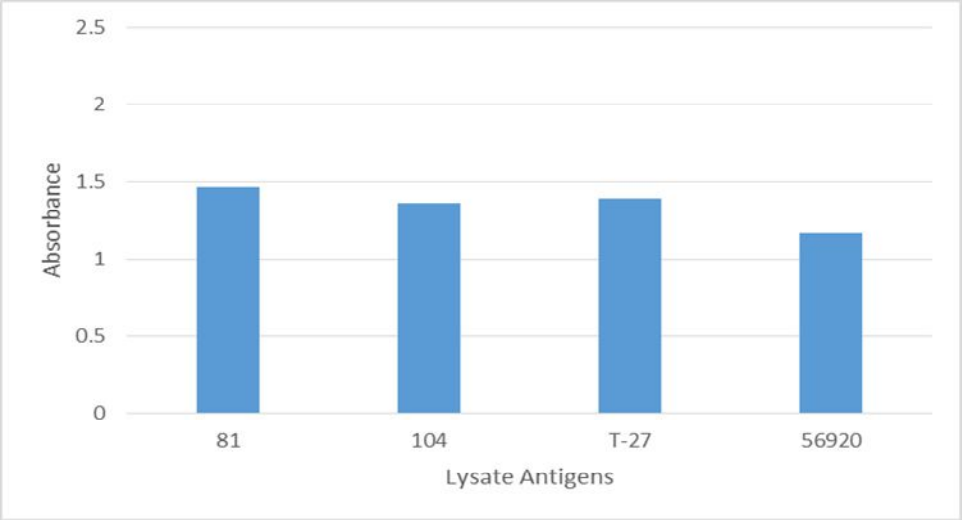

Figure 1 shows the reactivity of the four lysates in detecting antibody in 8 dog serum specimens (Trial 1).

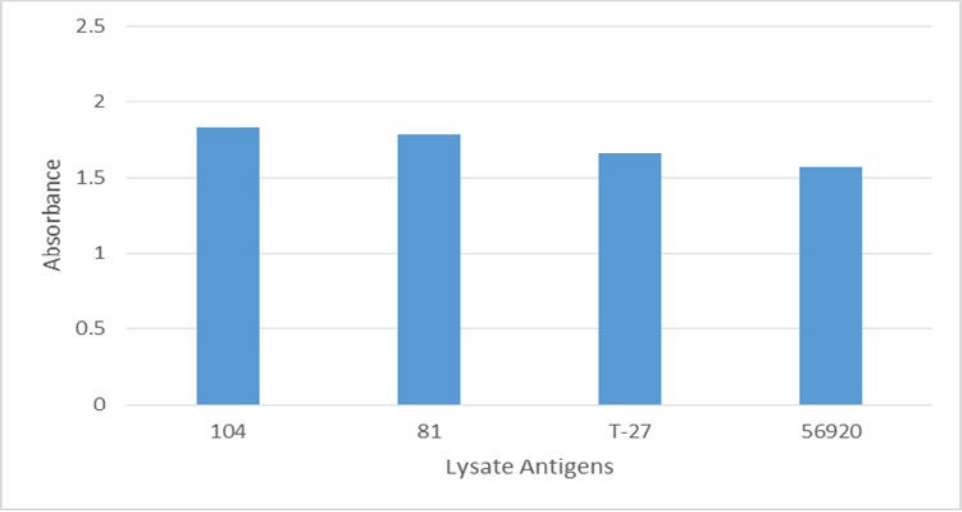

Figure 2 shows the reactivity of the four lysates in detecting antibody in 8 dog serum specimens (Trial 2).

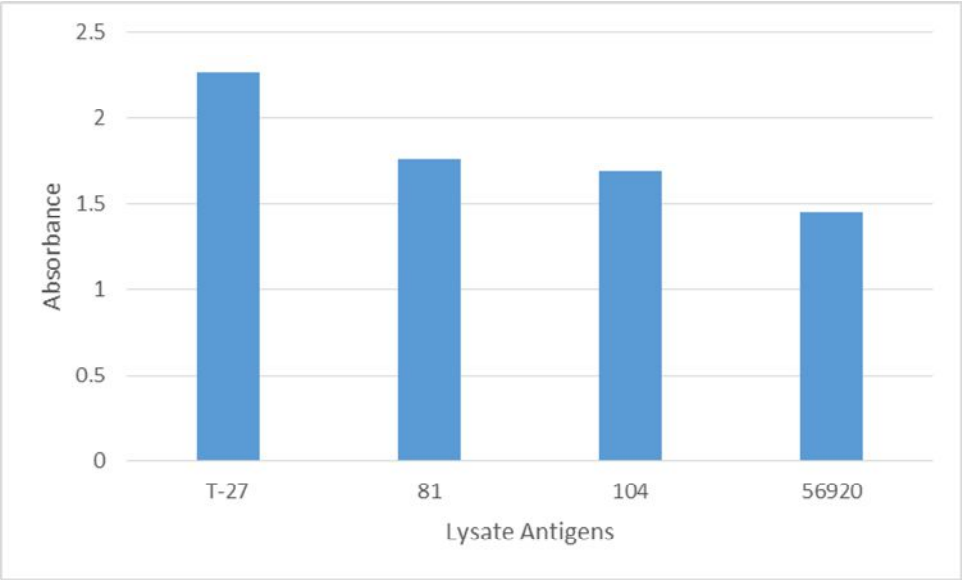

Figure 3 shows the reactivity of the four lysates in detecting antibody in 8 dog serum specimens (Trial 3).

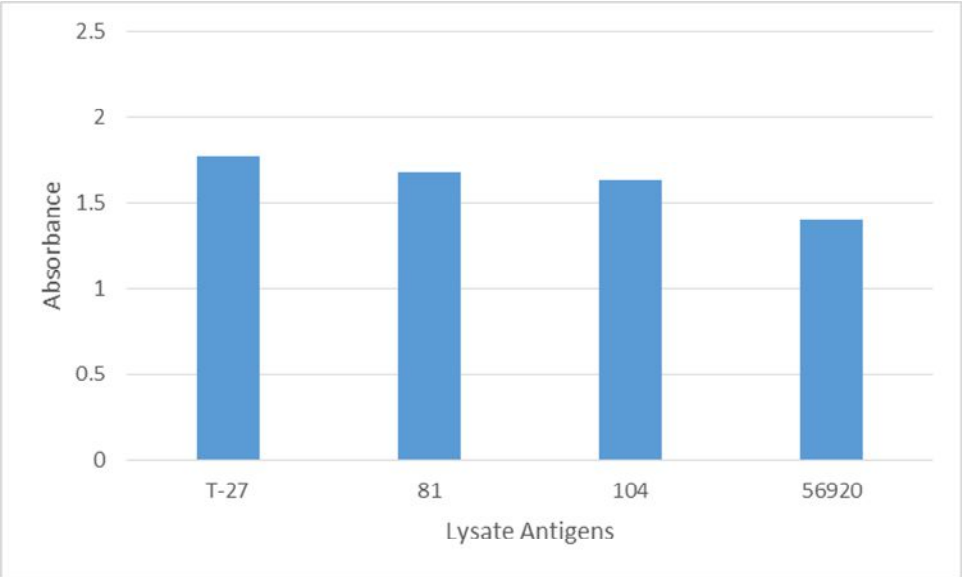

Figure 4 shows the overall reactivity of the lysates with the dog sera (mean absorbance values from all three trials)..

Conclusion

All four antigens T-27, 81, 104, and 56920 were able to detect *B. dermatitis* antibodyin the 24 dog sera with varying efficacy. In Trial 1, 81 had the highest mean absorbance value (1.467), followed by 104 (1.367), T-27 (1.394), and 56920 (1.172). In Trial 2, 104 displayed the highest mean absorbance (1.832), followed by 81 (1.792), T-27 (1.665), and 56920 (1.571). In Trial three, T-27 had the highest mean absorbance value of 2.266, followed by 81 (1.764), 104 (1.693), and 56920 (1.445). The mean absorbance values ranged from 1.399 (56920) to 1.775 (T-27). Overall, T-27 was the most reactive with a mean absorbance value (1.775), followed by 81 (1.674), 104 (1.631), and 56920 (1.399). The current study demonstrates the efficacy of these four lysates in detecting *B. dermatitis* antibody in the 24 dog serum specimens. Further research is recommended to confirm the sensitivity and specificity of these lysates and to demonstrate their effectiveness at detecting antibody in human sera.

Acknowledgement

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