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**Research article**

# The Detection of *Blastomyces dermatitidis* Antibodies in Dog Serum Specimens with Yeast Lysate Antigens Prepared from Isolates of the Fungus from a Human Outbreak of Blastomycosis

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## Abstract

Medical mycology investigators have continued concentrating their research on the development of improved immunodiagnostic assays for the detection of antibodies or antigens in the systemic fungal disease blastomycosis. Our laboratory has developed *Blastomyces dermatitidis* yeast phase lysate antigens and has performed comparative evaluations on the antigens for antibody detection in serum specimens from immunized rabbits or infected dogs. The objective of this present study was to use lysate antigens prepared from 9 *B. dermatitidis* isolates from a human outbreak of blastomycosis for antibody detection in serum specimens from dogs with diagnosed blastomycosis. Results using the indirect ELISA indicated that three of the preparations (B5896,B5929, B5927) were highly reactive with a mean absorbance value of 1.803. Five of the reagents (B5895,B5934,B5931,B5898,B5926) were moderately reactive (mean absorbance value of 1.302) and one antigen was less reactive (absorbance vaslue: 1.036). All of the 9 reagents were able to detect antibodies in the dog sera, but the three antigens were optimal. This study provides evidence that lysate antigens prepared from human isolated may be used in the ELISA to detect blastomycosis in dogs.

**Keywords:** *Blastomyces dermatitidis, Lysate antigens, enzyme-linked immunosorbent assay (ELISA), Serum specimens, blastomycosis*

## Introduction

The systemic fungal disease blastomycosis is caused by *Blastomyces dermatitidis.* This disease of humans and other animals is an endemic disease found in the Southeastern and upper Midwestern including highly endemic areas of Wisconsin and Minnesota and in regions of lower Canada. Evidence has indicated that this organism exists in areas with an abundance of moisture and decaying organic matter [1,2]. *B. dermatitidis* is a thermally dimorphic fungus acquired by inhalation of the mycelial infectious particles into the lung where it has the ability to convert to a large broad-based yeast cell. This is a primary pulmonary acute infection, but the organism may disseminate into other organs of the body including the central nervous system and cutaneous lesions may develop as the disease progresses. It may be fatal if a proper diagnosis is not made or if the disease is misdiagnosed as a bacterial or viral infection, especially in an immunosuppressed individual [3-6].

With regard to diagnosis, culturing or histologic identification of the organisms have been successful in certain instances, but in some situations these methods may not provide a reliable diagnosis or take a considerable amount of time which may delay treatment. During the past several years investigators have made considerable progress with regard to improved laboratory diagnosis by developing immunodiagnostic assays for the detection of antibodies or antigens in patients with blastomycosis [3-7].

Efforts in our laboratory have been concerned with the preparation and utilization in assays of *B. dermatitidis* yeast lysate antigens, prepared from various isolates of the fungus, for the detection of antibodies in sera from immunized and infected animals [8-15]. Encouraging results have been obtained with the *B. dermatitidis* lysate antigens, but additional studies are desired to further evaluate the reagents with regard to sensitivity and specificity of the reagents prepared from isolates obtained from human, animal and environmental sources. The aim of this present study was to perform a comparative evaluation of yeast phase lysate antigens, prepared from 9 *B. dermatitidis* isolates from a human outbreak of blastomycosis in Minnesota, for the ELISA detection of antibodies in dogs with diagnosed blastomycosis.

## Materials and Method

Lysate antigen preparation

Nine *B. dermatitidis* yeast phase lysate reagents, from a human outbreak of blastomycosis in Mountain Iron, Minnesota, (B5926, B5927, B5898, B5931, B5929, B5934, B5894, B5895, B5896 ) were prepared by a method similar to one that was previously used for the production of yeast lysate antigen from *Histoplasma capsulatum* [16-18] and modified in our laboratory for *B. dermatitidis* lysate antigen production [8]. 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 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 for up to 22 years. 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

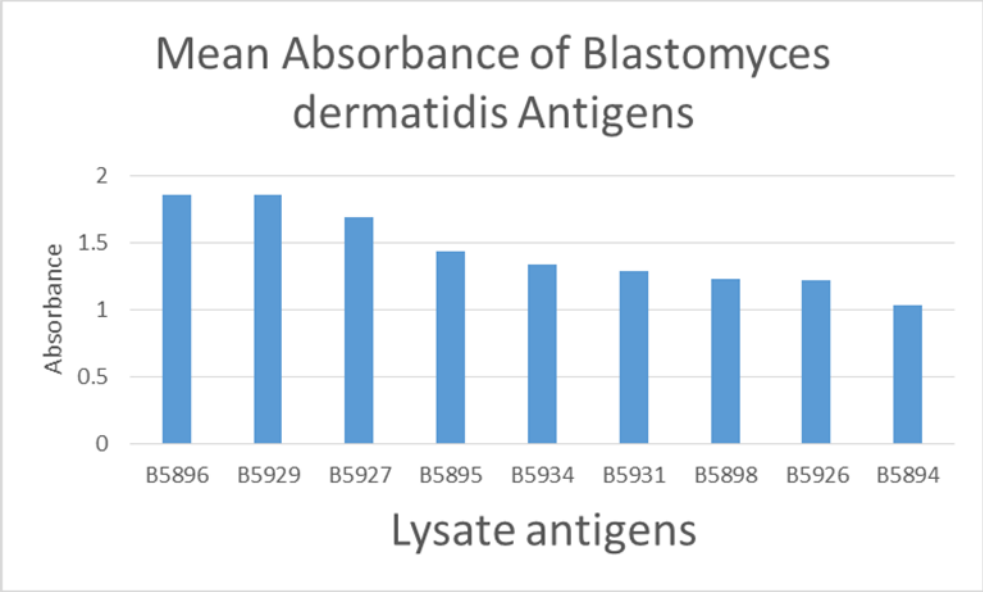
Fifteen serum specimens from dogs with diagnosed blastomycosis were provided by Dr. A.M. Legendre (University of Tennessee College of Veterinary Medicine, Knoxville, Tennessee)

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 [12-15]. 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 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 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 ul 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 read at 450 nm using a BIO-RAD 2550 EIA reader.

## Results and Conclusion

## Figure 1




As shown in Figure 1, the mean absorbance values obtained when the 9 lysate antigens, prepared from human *B. dermatitidis* isolates, when used to detect antibody in te 15 dog sera ranged from 1.860 to 1.036. The three lysates exhibiting the greatest immunoreactivity were B5896,B5929 and B5927. In contrast, the B5894 lysate showed the least amount of reactivity, but all of the reagents were able to detect antibody in the sera in a very reliable manner. Therefore optimal reactivity was evidenced with the B5896 reagent with lesser reactivity associated with the other preparations (Figure 2). This comparative evaluation illustrates the efficacy of the yeast lysate antigens as

immunodiagnostic reagents for the ELISA laboratory diagnosis of blastomycosis in dogs with blastomycosis. Studies are continuing in an effort to further optimize the reagents and the immunoassay

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