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Research article

Blastomyces dermatitidis Antibody Detection with Yeast Lysate Antigens Prepared from Isolates from Two Human Outbreaks of Blastomycosis

Tyler J. Kennedy, Austin C. Boyd, Jeremy W. Hegman, Bryn C. Kennell, Blake W. Martin and Gene M. Scalarone.

Department of Biological Sciences, Idaho State University, Pocatello, Idaho, USA,

E-mail: boydaust@isu.edu

Abstract

Blastomyces dermatitidis, the causative agent of blastomycosis, is difficult to diagnose due to sensitivity and specificity concerns that have been associated with the nature of the diagnostic tests used in clinical laboratories. For this study six yeast phase lysate antigens prepared from human isolates of *B. dermatitidis* (three prepared from Mt. Iron, Minnesota and three from Eagle River, Wisconsin) were compared for their ability to detect antibody in dog and rabbit sera. This was determined using the enzyme-linked immunosorbent assay [ELISA] with sixteen serum specimens from dogs that were diagnosed with blastomycosis and sixteen from rabbits that were immunized with *B. dermatitidis* in our laboratory. All six antigenic reagents were able to detect antibody in each of the sera with variations in the mean absorbance values. Reactivity mean absorbance of the dog sera with the Mt. Iron outbreak antigens was 0.828 and ranged from 0.738 to 0.877. The mean absorbance value of the rabbit sera with the same antigens was 0.823, and ranged from 0.776 to 0.914. Reactivity mean absorbance of the dog sera with the same antigens was 1.02 and ranged from 0.927 to 1.153. Reactivity mean absorbance of the rabbit sera for the Eagle River outbreak, was 1.18 and ranged from 0.879 to 1.698. This study demonstrates that although the antigenic

lysates displayed variances in reactivity, each lysate had the capacity to detect antibody in both dog and rabbit sera with antigen 597 being the most reactive of all six antigens.

Further studies will continue to compare a large number of *B. dermatitidis* yeast phase lysate antigens prepared from human isolates.

Keywords: *Blastomyces dermatitidis*, lysate antigens, antibody detection, ELISA, blastomycosis

Introduction

Blastomycosis, a pulmonary and potentially systemic fungal disease caused by *Blastomyces dermatitidis*, is a dimorphic organism that infects humans and other animals. This disease has been associated with regions of the United States where there is an abundance of water and decaying vegetation including states that border the Mississippi and Ohio Rivers and also states like Minnesota, Wisconsin, areas of lower Canada, and even in certain regions of Africa and India. [1,2].

Infection takes place when an individual inhales mycelial spores into the lung which then convert to broad-based budding yeast cells. This primary acute infection may progress to a chronic state or even disseminate to other organs including the production of cutaneous lesions or infection of the central nervous system. These infections may be fatal depending if the patient is immunocompromised, and the amount of spores inhaled. Often times the disease is misdiagnosed, and anti-microbial agents are prescribed to combat a bacterial or a viral infection which have no effect on the eukaryotic fungal cells [3-6].

Various techniques have been used in the clinical laboratory for the diagnosis of blastomycosis including microscopy, culturing and histopathologic methods. In some cases these methods have provided a reliable diagnosis, but in other cases a diagnosis may not be achieved or the time period required for the diagnosis may be lengthy. Investigators have done a lot of research during the past several years in attempting to develop better immunodiagnostic assays for antibody and antigen detection in blastomycosis [7-10]. For many years our laboratory has developed and evaluated yeast phase lysate antigens prepared from a variety of *B. dermatitidis* isolates and the utilization of such reagents for the detection of antibodies in serum specimens from immunized and infected animals [11-18].

Materials and Method

Lysate antigen preparation

Six yeast lysate antigens were prepared from two separate *B. dermatitidis* outbreaks among human populations (591, 597, and 598 from Eagle River, Wisconsin; and B5894, B5934, and B5929 from Mountain Iron, Minnesota). 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* [19-21] and modified in our laboratory for *B. dermatitidis* lysate antigen production [17]. 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

Sixteen serum specimens from dogs diagnosed with blastomycosis were provided by Dr. A.M. Legendre (University of Tennessee College of Veterinary Medicine, Knoxville, TN). Sixteen serum specimens from rabbits that were previously immunized with *B. dermatitidis* antigens were available in our laboratory.

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 [11-18]. 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) 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 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 Discussion

Figure 1 indicates that each of the six *B. dermatitidis* yeast lysate antigens from these isolates were reactive with the dog serum specimens. Reactivity mean absorbance of the dog sera ranged from 1.153 (597) to 0.738 (B5934) with a mean value for all six antigens equal to 0.926. Figure 2 shows that the reactivity mean absorbance of the rabbit sera ranged from 1.698 (597) to 0.776 (B5934) with a mean absorbance value for all six antigens equal to 0.999.

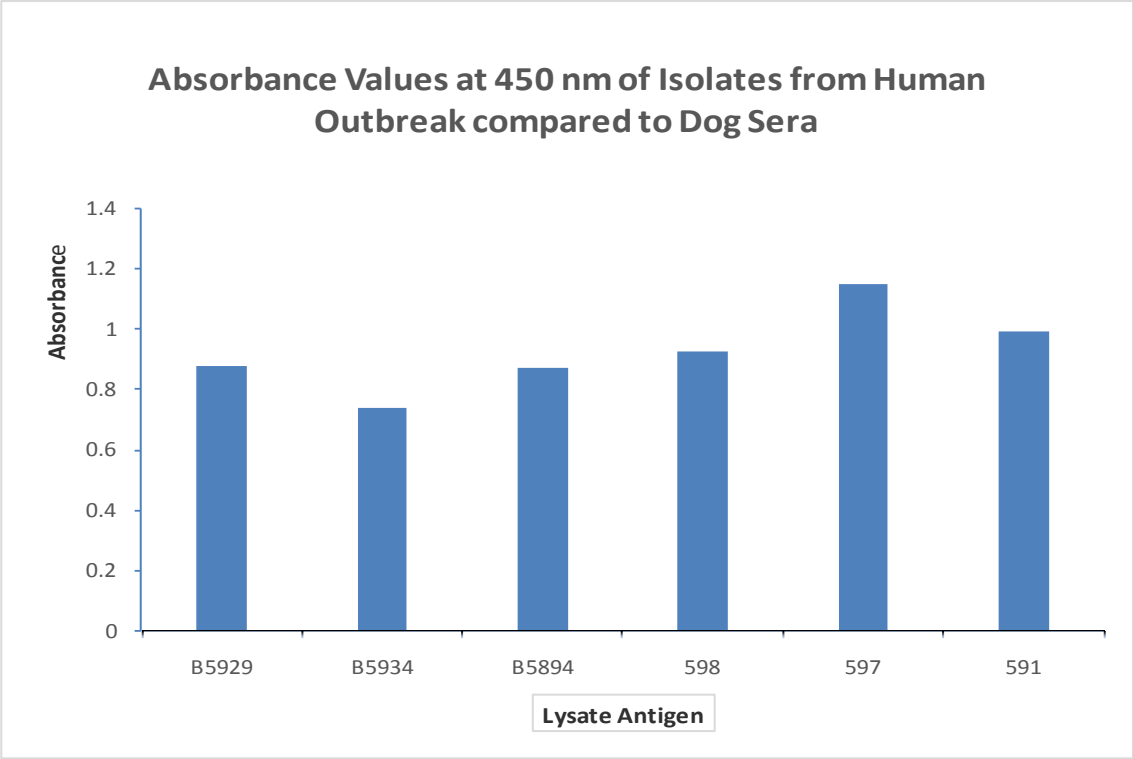

Figure 1: Mean absorbance values obtained form 16 specimens from dogs diagnosed with blastomycosis.

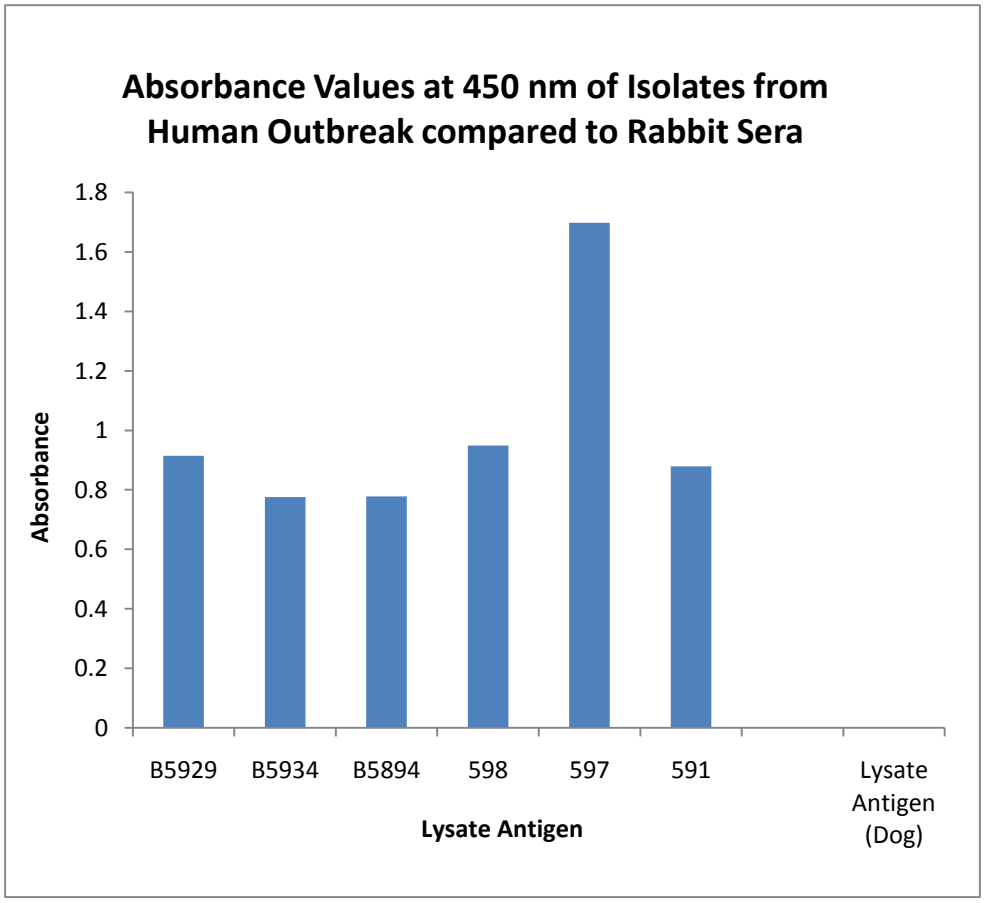

Figure 2: Mean absorbance values obtained from 16 serum specimens from immunized rabbits.

Conclusion

The focus of this study was to compare the antibody detection capability of six different yeast lysate antigens of *B. dermatitidis* from two separate human outbreaks. This was accomplished by reacting the lysate antigens with sera acquired from dogs and rabbits that had been immunized with *B. dermatitidis* as illustrated in Figure 1 and Figure 2, lysate antigen 597 was the most reactive antigen in both dog and rabbit sera. Inversely, antigen B5934 in both dog and rabbit sera exhibited the lowest reactivity. The rabbit sera displayed overall higher antibody with all but two of the six lysate antigens (B5894 and 591). The ability of some yeast lysate antigens to detect antibody better than others provides evidence that needs to be considered when using such antigens as immunodiagnostic tools in clinical settings. This research is important to consider in the production and use of such preparations for the laboratory diagnosis of fungal infections. This study provides evidence that the yeast lysate antigens from these two human

outbreaks had the ability to detect *B. dermatitidis* antibodies in both dog and rabbit sera. The ultimate aim of such comparative studies is to develop immunoassays that are sensitive and specific for the diagnosis of blastomycosis in humans and in animals.

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