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Research article

Blastomyces dermatitidis Antibody Detection in Dogs with Blastomycosis: Comparison of Lysate Antigens Prepared from Human, Dog, and Soil Isolates

Tyler J. Kennedy, Austin C. Boyd, Charles W. Puschak, Katrina A. Chapman, Sydney J. Brower, Michael T. Haldorson and Gene M. Scalarone

Idaho State University, 921 South 8th Avenue, Pocatello, ID 83209

Phone: (208) 716-1772, Fax: (208) 282-4570

E-mail Address: TJK: Kenntyle@isu.edu, GMS: scalgene@isu.edu

Abstract

Blastomyces dermatitidis, the causative agent of blastomycosis, is extremely difficult to diagnose due to the sensitivity and specificity problems of the diagnostic tests used in clinical laboratories. In this study nine yeast lysate antigens of *B. dermatitidis*, three prepared from soil isolates, three from dog isolates and three from human isolates to determine the efficiency of these antigens for detecting antibody in dog sera. This was determined using the enzyme-linked immunosorbent assay [ELISA] in thirty serum specimens from dogs with diagnosed blastomycosis. All nine reagents were able to detect antibody in each of the sera with only slight variations in the mean absorbance values. Reactivity mean absorbance values ranged from 1.345 (T-66, dog from Tennessee) to 0.948 (soil from Eagle River Wisconsin) with a mean value for all nine antigens equal to 1.146. This study demonstrates that although the antigenic lysates display variances in reactivity, each lysate has capacity to detect antibody in dog sera.
Further studies will continue to compare a large number of *B. dermatitidis* yeast phase lysate antigens prepared from human, animal and soil 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 converts 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 [3-10].

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 too 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 [9-11]. 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 [12-18]. This study compares nine new lysate preparations (three each: dog isolates, human isolates and soil isolates) for antibody detection in serum specimens from dogs with blastomycosis.

Materials and Method

Lysate antigen preparation

Nine yeast lysate antigens were prepared from *B. dermatitidis* isolates (T-66: dog, Tennessee; ERC-2: dog, Wisconsin; T-97: dog, Tennessee; ER 591: human, Eagle River Wisconsin; ER 597: human, Wisconsin; ER 598: human, Wisconsin; ER-3: woodpile, Wisconsin; 394: soil, Georgia; 248: soil, Eagle River Wisconsin). 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 [12]. 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

Thirty different 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)

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 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 Discussion

Figure 1 indicates that each of the nine *B. dermatitidis* yeast lysate antigens from these isolates were reactive with the dog serum specimens. Mean absorbance values ranged from 1.345 (T-66, dog from Tennessee) to 0.948 (soil from Eagle River Wisconsin) with a mean value for all nine antigens equal to 1.146. The mean absorbance value difference between the lysate showing the greatest value and the lysate with the lowest value was 0.397. Figure 2 shows the mean value of every class of lysate antigens (human, dog, soil) based on their reactivity with a total of 30 different dog sera. B. dermatitidis lysates prepared from dog isolates showed the greatest range of reactivity among the dog sera with a range of 3.0-0.283. While B. dermatitidis lysates prepared from soil isolates showed the least reactivity to the dog sera, ranging from 3.0- 0.329.

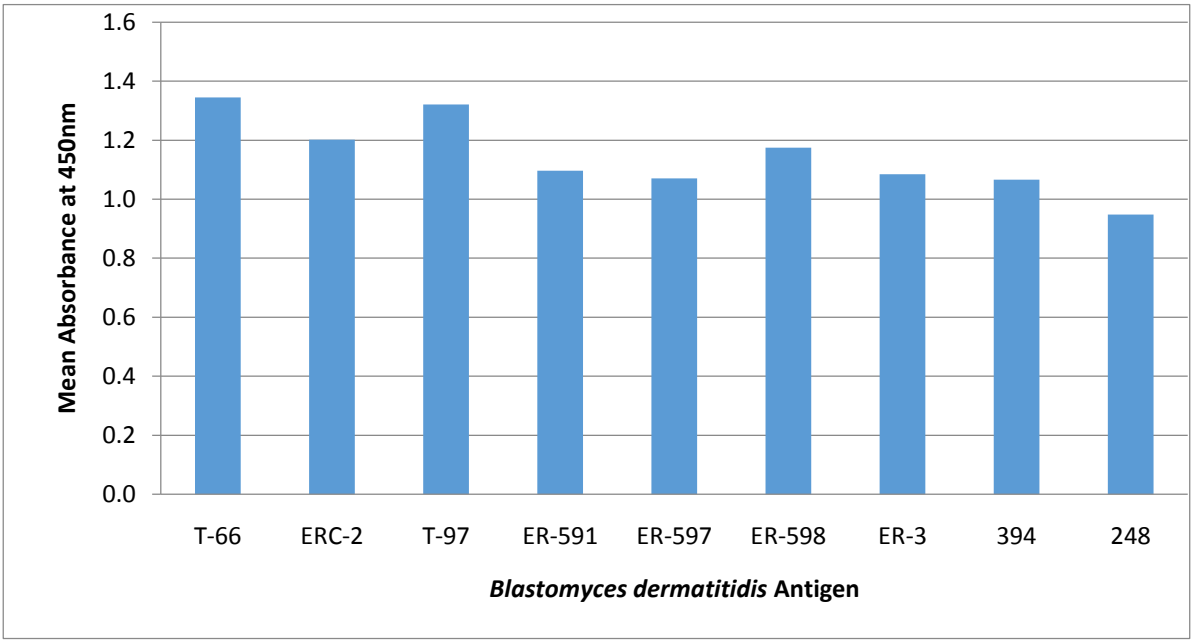

Figure 1: *Blastomyces dermatitidis* antibodies produced in dogs were used to detect 9 lysate antigens with ELISAs. (Organism sources: T-66, ERC-2 and T-97 from dog; ER-591, ER-597 and ER-598 from human; ER-3, 394 and 248 from soil.) Thirty individual antibodies were used to detect antigen in triplicate.

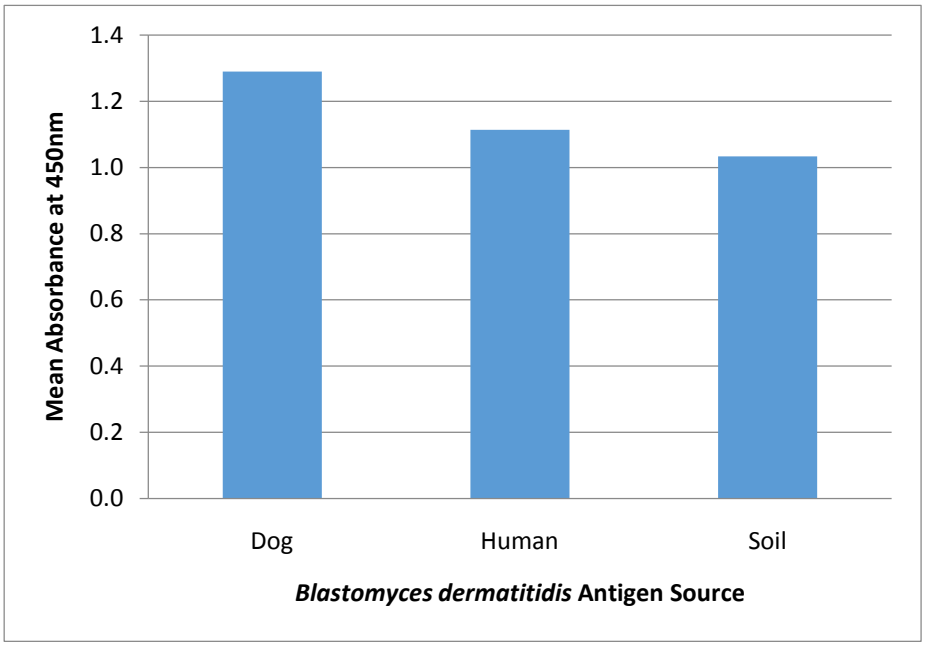

Figure 2: Antibody detection with the three dog, human and soil isolates

Conclusion

The focus of this study was to compare the antibody detection capability of nine different yeast lysate antigens of *B. dermatitidis* from various isolates. This was accomplished by reacting the lysate antigens with sera acquired from dogs that had been infected with *B. dermatitidis*. Two lysates prepared from the human isolates and two of the soil isolates showed similar levels of reactivity. As illustrated in Figure 1, lysate antigens T-66 (dog: Tennessee) and T-97 (dog: Tennessee) had the highest absorbance values of 1.345 and 1.321 were thus slightly more efficient at detecting antibody in the dog serum specimens. Lysate antigen 248 (soil: Eagle River Wisconsin) was the least reactive at detecting antibody with a mean absorbance value slightly lower than the others, a value equal to 0.948. The differences in the overall reactivity of each antigen was due to the amount of antibody present in the sera from the dogs. 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 three sources had the ability to detect *B. dermatitidis* antibodies in the dog sera. The ultimate aim of this experiment and ones to follow is to increase sensitivity and specificity of assays using B. dermatitidis lysate antigens.

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