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Research article

Blastomyces dermatitidis Antibody Detection in Dog Serum specimens with Lysate Antigens Prepared from Soil, Dog, Human and Miscellaneous Isolates of the Fungus

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Abstract

The clinical manifestations of blastomycosis range from an asymptomatic infection of the lungs to a life-threatening systemic disease. Because the symptoms of blastomycosis mimic that of various bacterial and viral infections, the development of improved immunodiagnostic tools is imperative. In a previous study done in our lab, 24 lysate antigens obtained from various isolates were tested for immunoreactivity against 12 serum specimens from rabbits previously immunized with *Blastomyces dermatitidis.* The lysates were classified into four different categories based on the isolates from which these antigens were obtained: dog, soil, miscellaneous, and human. From each category, the lysate with the greatest immunoreactivity was ERC-2 (dog, Wisconsin), 85 (soil, Georgia, ATCC 56920), 48938 (bat, India, ATCC 48938), and B5927 (human, Mountain Iron, Minnesota). In the present study, the above four lysates were tested against serum specimens from dogs with known blastomycosis. The mean absorbance values obtained when the four lysates were used to detect antibody in the seven dog sera ranged from 0.763 to 1.150. Although all isolates were able to detect *B. dermatitidis* antibody, the absorbance values varied. The lysate with the greatest absorbance was 48938, followed by 85, B5927, and ERC-2.

Keywords: *Blastomyces dermatitidis*, Blastomycosis, Antibody detection, lysate antigens, ELISA, dog serum

Introduction

Blastomycosis is caused by the inhalation of the thermally dimorphic fungal agent *B. dermatitidis*. This systemic fungal disease is endemic from the Eastern United States to the Great Lakes region, as well as in parts of Africa and India. *B. dermatitidis* is often found in areas rich in moist soil and decaying matter [1,3].

B. dermatitidis is found in a mycelial form in nature. Once inhaled into the lungs, the hyphae convert to broad based yeast cells. This primary pulmonary infection may self-resolve, become chronic, or disseminate. The symptoms of blastomycosis are highly heterogeneous, ranging from an asymptomatic pulmonary infection to a severe, life-threatening systemic disease that can involve the urogenital, skeletal, and the central nervous systems. Cutaneous lesions are common in the disseminated form. Systemic cases of blastomycosis can be fatal if left untreated, particularly if the host is immunocompromised. The symptoms of blastomycosis mimic various viral and bacterial infections; therefore, developing effective diagnostic tools is salient [4,10].

Our lab has focused on using the indirect enzyme-linked immunosorbent assay (ELISA) to test the reactivity of various yeast phase lysate antigens against serum specimens. In a previous experiment, 24 lysates from dog, soil, miscellaneous, and human sources were able to detect *B. dermatitidis* antibody in rabbit sera with varying efficacy. In the present study, the lysate from each category (dog, soil, miscellaneous, and human) with the greatest reactivity was used to detect antibody in dog sera. The goal of these two studies is to determine the efficacy of these antigens in detecting *B. dermatitidis* antibodies in serum specimens. More research is needed to improve the sensitivity and specificity of lysate antigens in detecting *B. dermatitidis* [11-14].

Materials and Method

Lysate antigens

In a previous experiment, 24 yeast lysate antigens were prepared from soil, dog, human and miscellaneous isolates of *B. dermatitidis*. Of the 24 lysates prepared, four were selected to be used in the present study. 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* [15-18] 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

Seven 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 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) 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

Below, Figure 1 shows the mean absorbance in rabbit sera as determined by a previous experiment. The greatest mean absorbance value was 1.961 (ERC-2) for lysates obtained from dog isolates, 1.821 (85) for lysates obtained from soil isolates, 1.595 (48938) for lysates obtained from miscellaneous isolates, and 1.366 (B5927) for antigens obtained from human isolates [15].

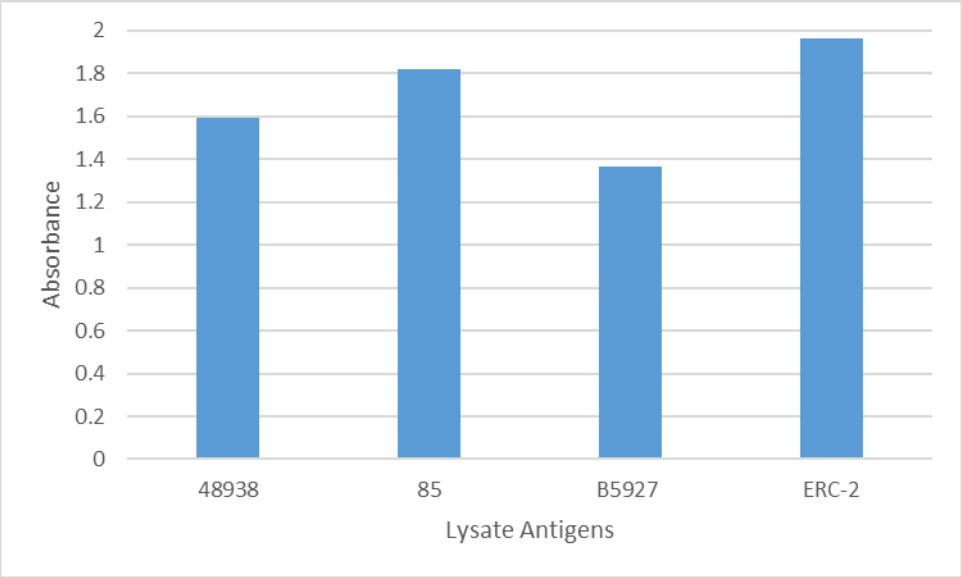

Fig. 1: Mean absorbance of the four lysate antigens in 12 rabbit sera.

Figure 2 shows the mean absorbance values obtained in the present experiment in which the four lysate antigens were used to detect antibody in the seven dog sera. Although all the reagents were able to detect antibody in the dog sera, the immunoreactivity varied. The lysate with the greatest absorbance was 48938 with a mean of 1.150, followed by 85 with a mean of 1.059, B5927 with a mean of 0.943, and ERC-2 with a mean of 0.763.

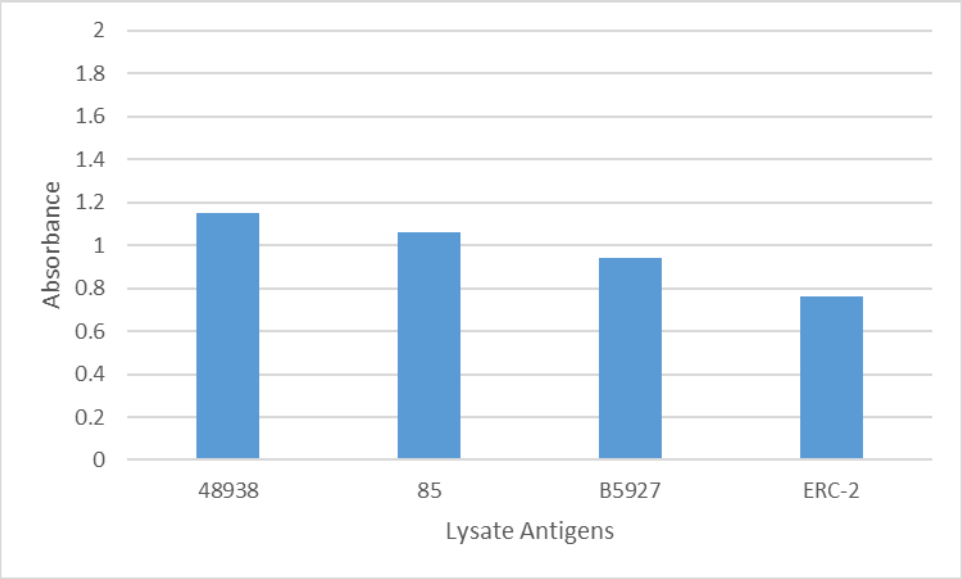

Fig. 2: Mean absorbance of the four lysate antigens in seven dog sera.

Figure 3 shows the overall mean of the antigens in detecting antibody in the rabbit and dog sera. Overall, 85 was the most effective at detecting antibody in the dog and rabbit sera with a mean absorbance of 1.440, followed by 48938 with a mean absorbance of 1.372, ERC-2 with a mean absorbance of 1.362, and B5927 with a mean absorbance of 1.154.

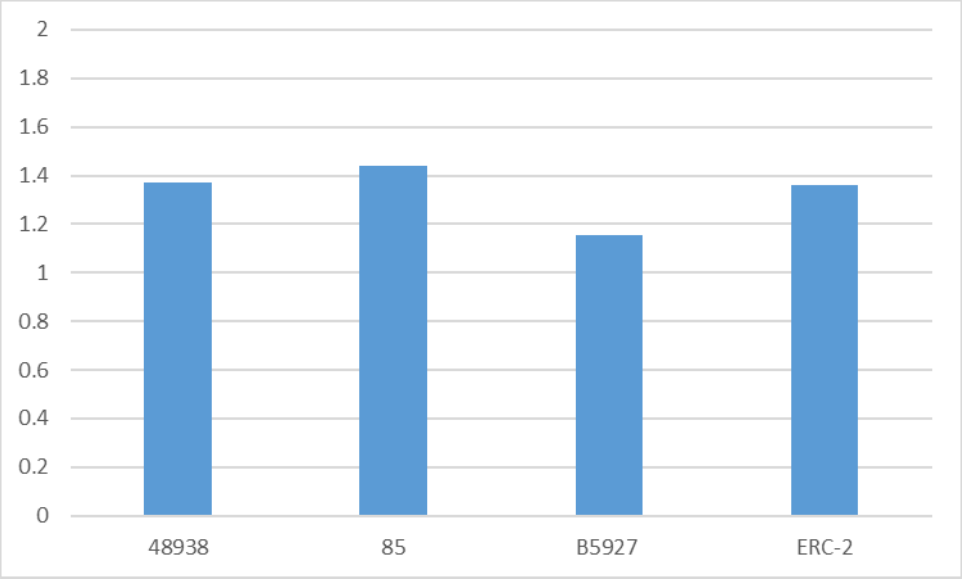

Fig. 3: Mean absorbance of the 4 lysate antigens in the 12 rabbit sera and 7 dog sera.

Conclusion

A previous experiment found that the lysate antigens ERC-2, 85, 48938, and B5927 were able to detect antibody in rabbit sera with values ranging from 1.366 (B5927) to 1.961 (ERC-2). The present study found that these four lysates were able to detect antibody in the dog sera with mean absorbance values ranging from 0.763 (ERC-2) to 1.150 (48938). All the lysates were more reactive with the dog sera than the rabbit sera. For the rabbit sera, ERC-2 was the most reactive and B5927 was the least. For the dog sera, 48938 was the most reactive and ERC-2 was the least. Overall, 85 was the most effective at detecting antibody in dog and rabbit sera, followed by 48938, ERC-2, and B5927. The previous study quantified the immunoreactivity of the above lysate reagents in rabbit sera. The present study found that these lysates were also effective immunodiagnostic reagents for detecting antibody in dog sera.

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