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Research article

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

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Abstract

Acquired by the inhalation of the dimorphic fungus *Blastomyces dermatitidis*, blastomycosis is a systematic disease that can prove fatal when misdiagnosed, particularly among the immunosuppressed. Although microbiological culturing or histologic identification can be performed successfully, the development of improved immunodiagnostic assays in patients with blastomycosis is essential to preventing fatalities. A prior study done by our lab showed that lysate antigens prepared from 9 *B. dermatitidis* isolates from a human outbreak of blastomycosis were reactive with serum specimens from dogs with diagnosed blastomycosis. The goal of the present study was to test the reactivity of the 9 *B. dermatitidis* isolates against serum specimens from rabbits immunized with blastomycosis. Results using the indirect ELISA showed that the mean absorbance ranged from to 1.363 to 0.718. All of the 9 reagents were able to detect antibodies in the rabbit sera, but 6 of the reagents (B5926, B5927, B5929, B5896, B5931, B5934) were moderately reactive (mean absorbance value: 1.270) and 3 of the antigens (B5895, B5898, B5894) were less reactive (mean absorbance value: 0.858). The data obtained from this comparative evaluation illustrates the efficacy of yeast lysate antigens prepared from human isolates in the detection of *B. dermatitidis* antibodies in immunized rabbits

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

Introduction

Blastomycosis is caused by the inhalation of the thermally dimorphic fungus *Blastomyces dermatitidis*. This disease is endemic in areas of the Southeastern and upper Midwestern United States and in regions of lower Canada. Cases are also found in regions of India and Africa. Prevalent in moist environments that are rich in decaying matter, *B. dermatitidis* is found in a mycelial state in nature [1,2]. When *B. dermatitidis* becomes airborne and is inhaled into the lungs, the infectious agent converts to a broad-based yeast cell. What starts as an acute pulmonary infection frequently disseminates into other organs of the body, including the skin, resulting in cutaneous lesions. The disease can also progress to the central nervous system, causing meningitis.

Blastomycosis is often misdiagnosed as a viral or bacterial infection, such as tuberculosis. In some cases, microbiological culturing and histologic identification have been successful. However these methods can lead to a misdiagnosis and are often time consuming. Depending on the immunological state of the individual, blastomycosis can be fatal. An accurate diagnosis is essential for the proper treatment to be administered in a timely fashion. Therefore, recent researchers have focused on improving immunodiagnostic assays for the detection of blastomycosis [3-7].

Our lab has been involved with the use of lysate antigens from various *B. dermatitidis* isolates by performing comparative evaluations for antibody detection in the sera of various infected and immunized animals. Additional studies have been performed on as the detection of antigens in urine samples of infected dogs [8-15]. In a prior study, lysate antigens prepared from isolates from a human outbreak of blastomycosis were used in detecting antibodies in dog sera using the indirect ELISA. This present study used the indirect ELISA method to test these same antigens against rabbit sera. Collectively these studies provide evidence that human lysate antigens can be used to detect *B. dermatitidis* antibodies. More evaluations are needed to determine the sensitivity and specificity of human lysate antigens in detecting 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 came from rabbits immunized with Blastomyces dermatidis lysate antigens.

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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-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

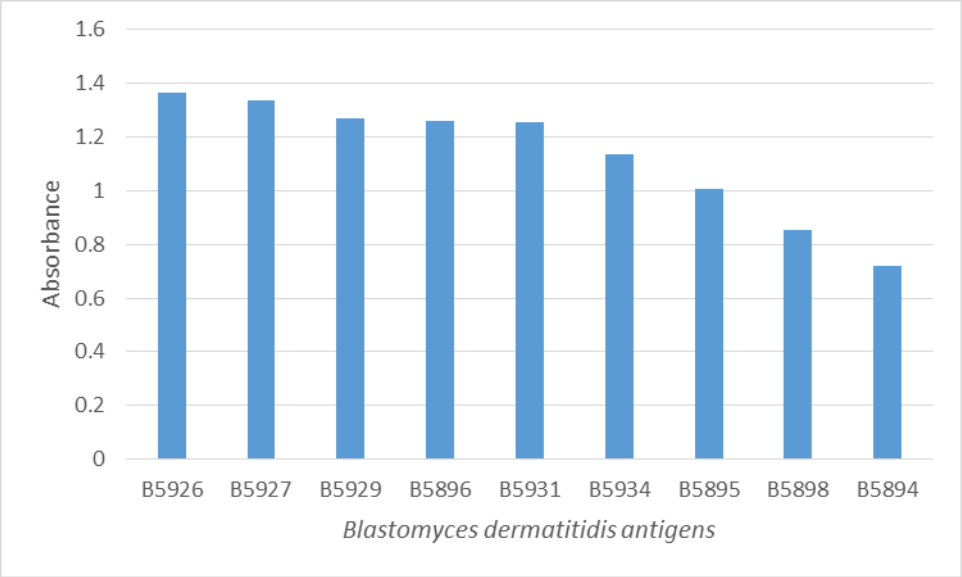

Figure 1: Mean absorbance as determined with 9 *B. dermatitidis* lysate antigens

As seen in Figure 1, the mean absorbance of the 9 lysate antigens when used to detect antibodies in the 15 rabbit sera ranged from 1.368 to 0.718. All of the antigens were able to detect *B. dermatitidis* antibodies in the rabbit sera, but 6 were moderately reactive (B5926, B5927, B5929, B5896, B5931, B5934) with a mean absorbance value of 1.270. In contrast, 3 of the antigens (B5895, B5898, B5894) were less reactive (mean absorbance value: 0.858).

In a previous study the 9 lysate antigens from isolates from the outbreak in Minnesota were successful in detecting *B. dermatitidis* antibodies in dog sera with an absorbance value ranging from 1.860 to 1.036. The efficacy of the antigens in detecting blastomycosis in the dog serum was greater, but ultimately the antigens were reliable at detecting blastomycosis in both types of serum.

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The results from this study show that the 9 lysate antigens from isolates of a human outbreak in Minnesota can be used to detect *B. dermatitidis* in rabbit sera. This study provides further evidence that lysate antigens prepared from human isolates can be used to diagnose blastomycosis in serum using an indirect ELISA. Additional studies are needed to further test the reactivity and sensitivity of human yeast phase antigens in order to further develop immunodiagnostic assays.

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