Detection of Antibodies in Dogs with Blastomycosis: Comparative Studies Using Yeast Lysate Antigens Prepared from Isolates of *Blastomyces dermatitidis* from a Minnesota Outbreak

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Abstract

Blastomyces dermatitidis, a thermally dimorphic systemic fungal organism, has been the etiologic agent associated with several outbreaks of blastomycosis in the United States. In the present study antibody detection comparative evaluations were performed using seven yeast lysate antigens prepared from *B. dermatitidis* isolates from human isolates collected during an outbreak of blastomycosis in Mountain Iron, Minnesota to determine the sensitivity of each reagent . The enzyme linked immunosorbent assay (ELISA) was used to detect antibodies in serum specimens, collected at pre-treatment and post-treatment intervals, from 42 dogs with blastomycosis. Mean ELISA absorbance values ranged from 0.352 (B5934) to 0.701 (B5896) when the lysate antigens were tested against a panel of 42 sera. Reactivity determinations at the six time intervals (pre-treatment and days 30,60,90,120 and 180 post-treatment) indicated that the absorbance values obtained with the optimal B5896 yeast lysate ranged from 0.819 (pre-treatment) to 0.281 (180 days post-treatment). All seven *B. dermatitidis* yeast lysate antigens detected antibodies in the dog sera, but one reagent (B5896) exhibited exceptional reactivity and may prove to be useful for the clinical detection of blastomycosis in animals and humans.

Introduction

In September 1999, an outbreak of blastomycosis was reported in the Mountain Iron, Minnesota [2]. Eighteen human cases of blastomycosis were diagnosed by culture or observation of the yeast cells in sputum or bronchial washings. There seemed to be a relationship of disease to disruption of soil at a new home excavation site. We were able to obtain several cultures from this outbreak.

For the past several years our laboratory has been interested in the preparation and evaluation of antigens prepared from *B. dermatitidis* isolates from various geographical regions endemic for blastomycosis [1, 3, 4, 8]. In the present study assays were performed using yeast phase lysates prepared from *B dermatitidis* isolates obtained from the outbreak to determine the reactivity profile of each antigenic reagent.

Materials & Methods

Antigens**:** Yeast phase lysate antigens were prepared from seven *B. dermatitidis* isolates (B5898, B5926, B5931, B5896, B5927, B5895, B5934) using a method similar to that previously used for the production of antigen from *Histoplasma capsulatum* [6, 7] and was modified for *B. dermatitidis* lysate antigen production [5]. Briefly, yeast phase cells were grown for seven days at 37 C in a chemically defined medium (5) with shaking, harvested by centrifugation (700 x g), followed by washing with distilled water and then allowed to lyse for seven days at 37 C in water with shaking. The reagent was centrifuged (700 x g),filter sterilized and stored at 4 C. Protein determinations were performed using the BCA Protein Assay Kit (Pierce Chemical Company, Rockford, IL). Dilutions of the antigenic reagents were based on protein concentration.

Serum specimens: Serum specimens from 42 dogs with blastomycosis that were previously collected and available in our laboratory were used to assess the reactivity of seven *B. dermatitidis* lysate antigens. Various serum specimens were tested ranging from pre-treatment to180 days post-treatment.

***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; peroxidase system) as follows: Each lysate antigen was diluted (100 ng of protein/mL) in a carbonate-bicarbonate coating buffer and then added to wells (100 ul) of Costar plates (Fisher Scientific, Pittsburgh, PA). The plates were incubated overnight at 4 C in a humid chamber followed by washing three times with phosphate buffered saline containing Tween 20 (PBS-T). The serum specimens (1:5000 dilution) were added to triplicate microplate wells and incubated for 30 min at 37 C in a humid chamber.
The wells were washed as above and goat anti-dog IgG (H & L) peroxidase conjugate (Kirkegaard and Perry, KPL, Gaithersburg, MD) was added to each well and incubated for 30 min at 37 C. The plates were washed as above and 100 ul of peroxidase substrate (Ultra TMB; Pierce) was added to each well and incubated for approximately 2 min at room temperature . The reaction was stopped by the addition of 100 ul of 2N sulfuric acid and the absorbance read at 450 nm using a BIO-RAD 2550 EIA reader. Wells containing only substrate and stop solution were used as blanks to zero the EIA reader.

Results

The ability of each antigenic reagent to detect antibodies in serum specimens from dogs with blastomycosis is shown in Figure 1 (sera collected at various time intervals) and Figure 2 (sera collected prior to treatment and 30, 60, 90, 120 and 180 days post-

treatment) respectively. One of the yeast phase lysate antigens (B5896) prepared from the *B. dermatitidis* isolates exhibited the greatest reactivity when tested against the serum specimens from 42 dogs with blastomycosis and the same pattern was evident when reactivity at the various time intervals was determined. The other yeast lysate reagents were less reactive with mean absorbance values ranging from 0.655 (B5895) to the lowest absorbance value of 0.352 with the B5934 antigen.

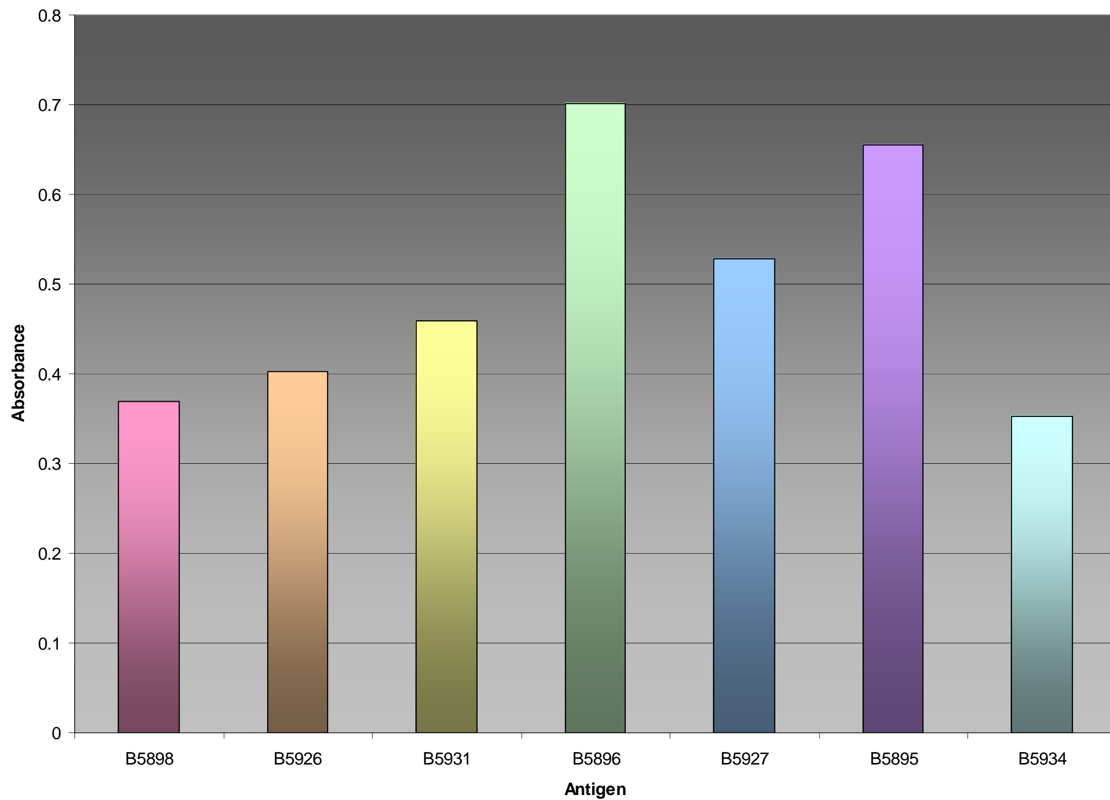

Figure 1: The ability of each antigenic reagent to detect antibodies (mean absorbance values) in serum specimens from all dogs with blastomycosis (sera collected at various time intervals).

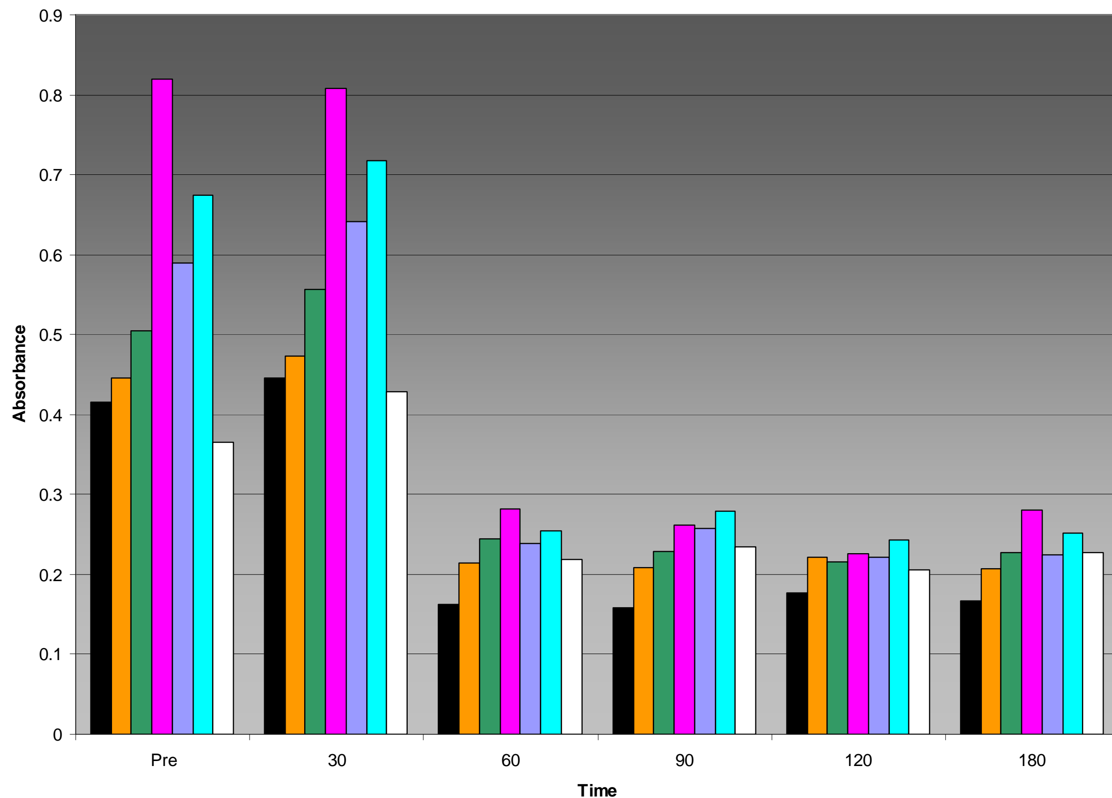

Figure 2: The ability of each antigenic reagent to detect antibodies (mean absorbance values) in serum specimens from dogs with blastomycosis. Specimens collected prior to treatment and at 30, 60, 90, 120 and 180 days post-treatment. The seven bars at each time interval are the antigens in order from left to right as follows: B5898, B5926, B5931, B5896, B5927, B5895 and B5934.

Discussion

Evidence from the assays indicated that one *B. dermatitidis* yeast phase lysate antigenic preparation (B5896) was the most efficacious with respect to antibody detection in the serum specimens from dogs with blastomycosis. The amount of antibody detected with all of the lysate reagents was greatest at the pre-treatment and 30-day time intervals and decreased markedly in the 60 to 180 days serum specimens. It was interesting to note

that all seven antigens reacted in a similar manner with each of the 42 serum specimens. For example, the optimal antigen (B5896) presented a maximum absorbance value of 1.978 with one dog serum specimen while the other lysate antigens also exhibited high absorbance values ranging from 1.420 (B5895) to 0.660 (B5934). The amount of antibody in each individual dog specimen varied based on the disease status of the animal as well as the response to treatment, but the reactivity pattern was similar with each antigen. Thus it was determined that there were differences in reactivity even though all of the yeast phase lysate antigens were prepared from human isolates from the outbreak of blastomycosis in the town of Mountain Iron, Minnesota. Research is continuing to further evaluate these lysate antigens by performing additional isolation and purification procedures in an attempt to further elucidate sensitivity and specificity characteristics of each. The ultimate aim of our research is to determine if one or a combination of *B. dermatitidis* yeast phase lysate antigens can be utilized in immunodiagnostic assays for the definitive diagnosis of blastomycosis.

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Acknowledgements

This research was supported by the Department of Biological Sciences, Idaho State University. *Blastomyces dermatitidis* isolates provided by the Centers for Disease Control (CDC) Atlanta, GA and serum specimens provided by A. M. Legendre (University of Tennessee College of Veterinary Medicine, Knoxville, TN).