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Research article

Reactivity of Diverse *Blastomyces dermatitidis* Lysate Antigens vs. Serum Specimens from Dogs with Blastomycosis

Jordan T. Hammon, Tyler J. Kennedy and Gene M. Scalarone

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

208-220-0897

E-mail: hammjord@isu.edu

Abstract

Blastomycosis is a disease caused by the parasitic, dimorphic fungus *Blastomyces dermatitidis*. This disease has been a diagnostic challenge due to problems with sensitivity and specificity of the assays. The present study assayed 8 lots of diverse *B. dermatitidis* yeast lysate antigens to determine the reactivity of the reagents. This was determined by comparing antibody detection (enzyme-linked immunosorbent assay; ELISA) with 15 serum specimens from dogs diagnosed with blastomycosis. Seven of the 8 reagents proved to be immunoreactive and were able to detect antibody in each of the 15 different serum specimens. Sensitivity mean absorbance values with the lysates ranged from 0.479 (48089; human isolate from Africa) to 1.464 (T-27; polar bear isolate from Tennessee)

with a mean value for all 8 antigens equal to 1.144. This study generated data making it evident that although the diverse antigenic lysates do display some differences in reactivity, each of them has ability to detect antibody in dog sera in an efficient manner.

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

Introduction

The systemic fungal disease blastomycosis, caused by the etiologic agent *Blastomyces dermatitidis*, is a disease of humans and other animals. Blastomycosis is an endemic disease found in the Southeastern, South-Central and upper Midwestern states of the United States, including areas of Wisconsin, Minnesota and regions of lower Canada. Evidence over the years has indicated that this fungus exists in areas with an abundance of moisture and decaying organic matter [1, 2]. *B. dermatitidis* is thermally dimorphic and acquired by inhalation of the infectious particle (mycelial phase spore) into the lung in which it then converts to a large yeast cell and produces a primary pulmonary acute infection. It may disseminate into other organs of the body including the central nervous system and, as the disease progresses, cutaneous lesions may develop. If a proper diagnosis is not made, or if the disease is misdiagnosed as a bacterial or viral infection, it may be fatal, especially in an immunosuppressed individual [4, 5].

Current laboratory diagnostic methods include culturing or histologic identification, but in many instances these methods may not provide a reliable diagnosis or they may take a considerable amount of time which can delay treatment. During the past several years investigators have made considerable progress with regard to the laboratory diagnosis by developing immunodiagnostic assays for the detection of antibodies or antigens present in patients with blastomycosis [6, 9].

In an effort to contribute to improved immunodiagnostic laboratory assays, our laboratory has been concerned with the preparation and comparative studies of *B. dermatitidis* yeast lysate antigens, prepared from various isolates of the fungus, for the detection of antibodies in sera from immunized and infected animals [10,12]. Encouraging results have been obtained with the *B. dermatitidis* lysate antigens, but other studies are needed to further evaluate the

reagents with regard to sensitivity and specificity of the reagents prepared from diverse isolates of the fungus obtained from human, animal and environmental sources.

Materials and Method

Lysate antigen preparation

Eight yeast lysate antigens were prepared from *B. dermatitidis* isolates (48089: human from Africa; 48938: bat lung from India; 56920: bat liver from India; T-27: polar bear from Tennessee; 449: polar bear from Illinois; 81: seal lion from Tennessee; 104: cat trachea from Tennessee; 103: cat skin from Tennessee). 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* [13-15] and modified in our laboratory for *B. dermatitidis* lysate antigen production [10]. 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 different serum specimens from dogs with diagnosed blastomycosis were provided by Dr. A.M. Legendre (University of Tennessee College of Veterinary Medicine, Knoxville, TN). The dog sera in this study was used to evaluate each yeast lysate antigen's ability to detect antibody.

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 [10-12]. 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

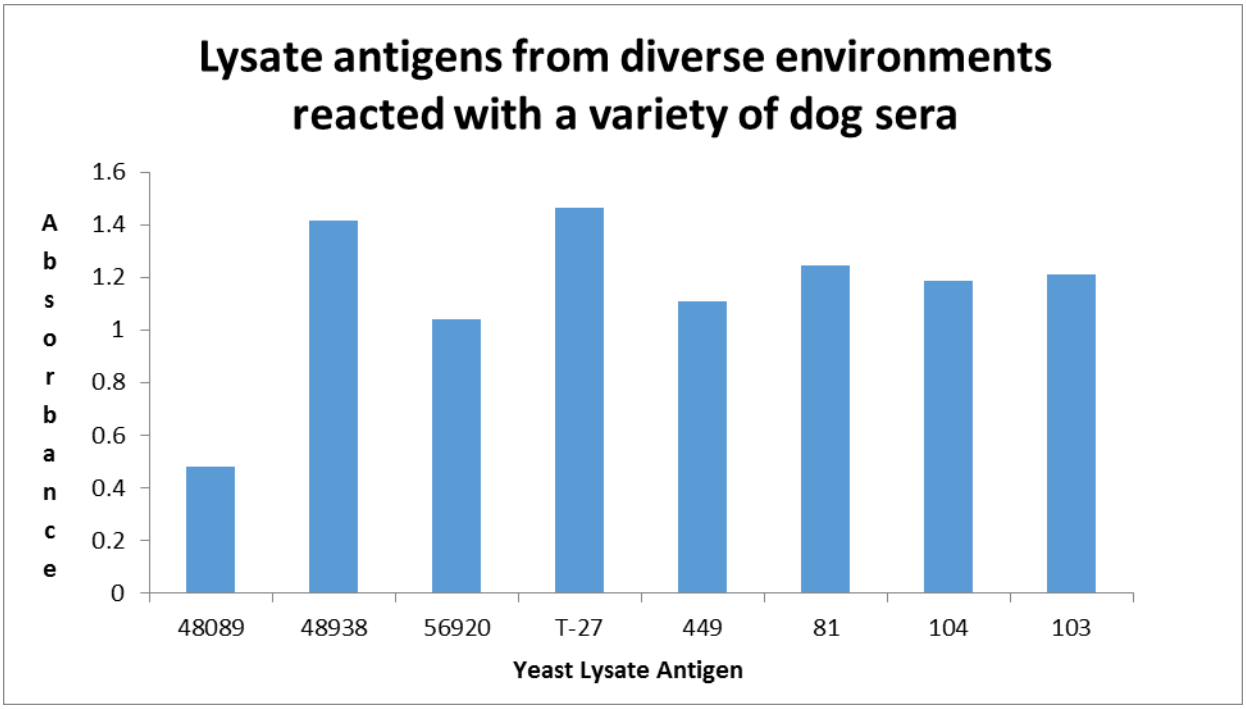

Figure 1. Mean reactivity of 8 *Blastomyces dermatitidis* yeast lysate antigens (48089: human from Africa; 48938: bat from India; 56920: bat from India; T-27: polar bear from Tennessee; 449: polar bear from Illinois; 81: sea lion from Tennessee; 104: cat from Tennessee; 103: cat from Tennessee) reacted with 15 different dog serum specimens.

Figure 1 illustrates that all of *B. dermatitidis* yeast lysate antigens from these diverse environments except 48089 (human from Africa) were reactive with dog serum specimens. Mean absorbance values ranged from 0.479 (human

from Africa) to 1.464 (polar bear from Tennessee) with a mean value for all 8 antigens equal to 1.144. The mean absorbance value difference between the lysate showing the greatest value and the lysate with the lowest value was 0.985.

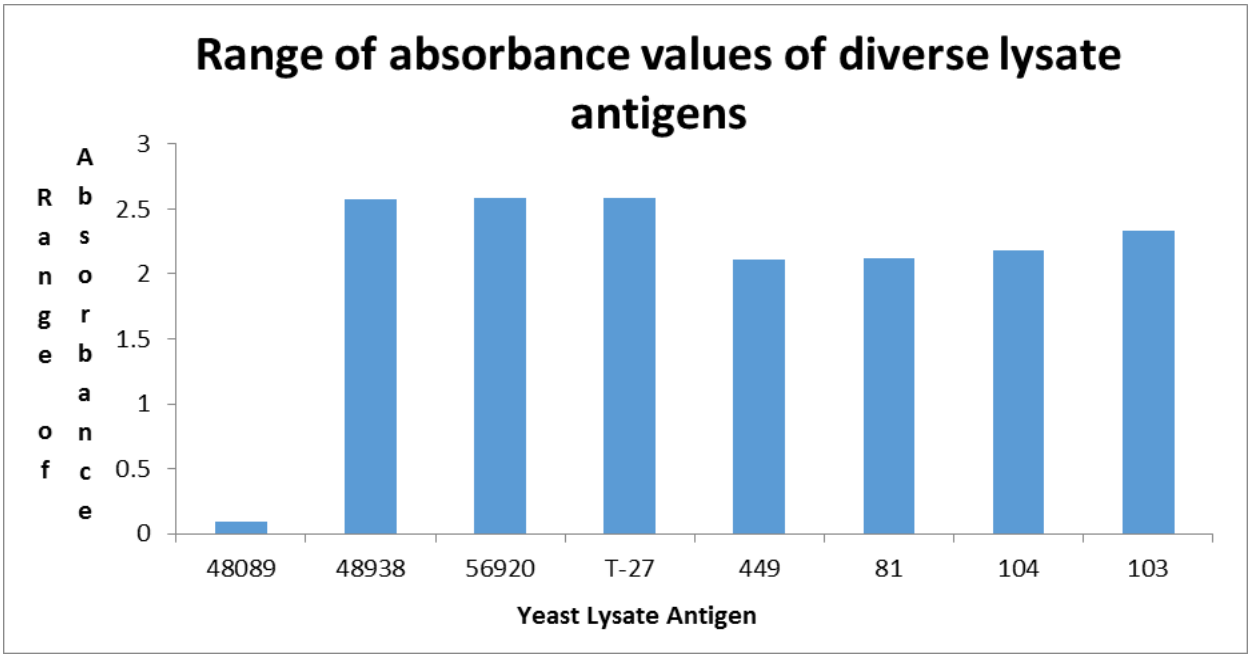

Figure 2. Range of reactivity between *Blastomyces dermatitidis* yeast lysate antigens and dog sera.

Figure 2 shows the range of these exotic yeast phase lysate antigens in their reactivity with a total of 15 different dog sera. The T-27 (polar bear from Tennessee) lysate antigen showed the greatest range of reactivity among the dog sera with a range of 0.395-2.504 while the 48089 (human from Africa) lysate antigen showed the lowest range of reactivity between dog serum specimens with a range of 0.323-0.410. The average range between yeast lysate antigens with respect to their reactivity with 15 different dog sera was 2.071. The aim of this current research was to compare the antibody detection capability of diverse yeast lysate antigens of *B. dermatitidis* from a variety of environments. This was achieved by reacting those lysate antigens with a variety of dog sera acquired from dogs infected with *B. dermatitidis*. The lysates were prepared and then stored at 4°C. Seven of the 8 yeast lysate antigens exhibited a great deal of reactivity and were able to detect antibody in each of the 15 *B. dermatitidis* dog sera. The variations in reactivity that were observed with the 8 different lysates were likely associated with antigenic differences and variations in 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 situations. This is certainly an important consideration in the production and use of such preparations for the laboratory diagnosis of fungal diseases. This present study demonstrates that the yeast lysate antigens from diverse environments had the ability to detect *B. dermatitidis* antibodies in a sensitive manner and to a similar degree. The continuation of studies in this respect will further examine yeast lysate antigens for their ability to detect antibody in specimens from humans and other animals with blastomycosis.

Acknowledgement

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