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Research article

Blastomyces dermatitidis: Antibody Detection on Canine Serum Specimens with Lysate Antigens Prepared from Human, Animal, and Environmental Isolates

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Abstract

The diagnosis of blastomycosis is a concern to veterinarians and other health professions in the southern states and along the Mississippi River. Recent research in our laboratory has been associated with the production of *Blastomyces dermatitidis* yeast lysate antigens and the evaluation of these antigens for antibody detection in serum specimens from various sources. This study evaluated *B. dermatitidis* lysate antigens from a variety of sources against canine antibody to determine reactivity of the lysates using the indirect ELISA. Of the five lysate antigens tested (397-Soil-Georgia, 46-Canine-TN, 41-Feline-TN, 20-Canine-TN, and KY-Human) 46-Canine-TN had the greatest mean absorbance value of 1.864. The range of all mean absorbance values were between 1.039 – 1.864.

Keywords: *Blastomyces dermatitdis,* Antibody Detection, ELISA, Lysate Antigens

Introduction

Blastomycosis is a respiratory fungal disease transmitted by inhalation to the hosts, which are predominately human and canine. The infecting agent of interest, *B. dermatitidis*, is a thermally dimorphic fungus living as a mold in damp or humid conditions. *B. dermatitidis* is primarily located in the southeastern part of the United States along the Mississippi River. Wisconsin, Minnesota, and southern parts of Canada are other endemic regions where the fungus may flourish [1-3].

The mycelial phase of the fungal organism grows at a temperature of 25 °C and produces microscopic spores known as conidia. These infectious agents enter the host by way of the respiratory system. Once inhaled, the spores have a unique ability to convert into budding yeast cells at a temperature of 37 °C [1-5]. The yeast cells may be contained in the lungs or disseminate and become an invasive disease to immunocompromised individuals or individuals in the debilitating stages of AIDS, causing a serious or fatal infection. Misdiagnosis of *B. dermatitidis* is common due to related symptoms of tuberculosis and if mistreated or left untreated may become invasive and disseminate to the immune system, bones, central nervous system, and skin involvement [4-9].

It has become a growing concern among researchers with respect to developing improved methods on diagnosing this fungal disease. A combination of clinical history, visualization of the yeast cells and/or by immunodiagnostic methods to detect antibody or antigen in clinical specimens is required for a proper laboratory diagnosis of blastomycosis. Reliable methods of diagnosing blastomycosis is paramount to reducing cross-reactivity with *Histoplasma capsulatum*. Inaccurate laboratory conclusions, may lead to an increased probability of the physician prescribing an un-effective plan of treatment. In addition, many physicians may misdiagnose a blastomycosis infection and assume the infecting agent is a bacterial or viral pathogen. The misdiagnosis of blastomycosis is the driving force for why researchers continue to improve and develop new methods of proper diagnosis that provide fast and reliable results [8-10].

The immunodiagnostic analysis of blastomycosis has been a continuing focus in our research facility [11-14]. The aim of this study was to perform a comparative evaluation of *B. dermatitidis* lysate antigens prepared from human, animal, and environmental strains of the organism for the detection of antibodies in canine serum specimens.

Materials and Method

Lysate Antigens

The lysate antigens were prepared from mycelial phase culture of five different *B. dermatitidis* isolates: 397-Soil-Georgia, 46-Canine-TN, 41-Feline-TN, 20-Canine-TN, and KY-Human. These isolates were converted to yeast cells by culturing at 37 ºC on brain heart infusion agar. The yeast phase and mycelial phase lysate reagents in this study were prepared through a similar method used in the production of antigen from *H. capsulatum* [14-17] and this method was modified in our laboratory for *B. dermatitidis* lysate antigen production [11]. The yeast cells were grown for seven days at 37 ºC, in a chemically defined medium, in an incubator shaker, and subsequently harvested by centrifugation (700x g; 5 min). The cells were washed with distilled water, re-suspended in distilled water and allowed to lyse for seven days at 37 ºC in water with shaking. The preparations were centrifuged, filter sterilized, merthiolate added (1:10000) and stored at 4 ºC for further use. Protein determinations were performed on the lysates using the BCA Protein Assay Kit (Thermo-Fisher Pierce) and dilutions of the antigenic reagents used in the assays were based on the protein concentrations.

Serum Specimens

Twenty serum specimens from canines diagnosed with 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 the five lysate reagents to detect antibodies in the above serum specimens was determined using the indirect enzyme-linked immunosorbent assay (ELISA). Each of the five lysate antigens was diluted (2000ng/mL of protein) in a carbonate-bicarbonate coating buffer (pH 9.6) and added to triplicate wells (100µL) of a NUNC 96-well micro plate (Thermo-Fisher). The plates were then incubated overnight at 4 ºC in a humid chamber followed by washing three times with phosphate buffered saline solution containing 0.15% Tween 20 (PBS-T). The serum specimens (1:2000 dilution; 100µL) were added to the micro plate wells and incubated for 30 minutes at 37 ºC in a humid chamber. Following this incubation the wells were washed as previously mentioned and 100µL of goat anti-canine IgG (H & L) peroxidase conjugate (Kirkegaard and Perry; KPL) was added to each well and incubated for 30 minutes at 37 ºC. The plates were washed again as aforementioned and 100µL of Sure Blue Reserve TMB peroxidase substrate (KPL) was added to each well and incubated for approximately two minutes at room temperature. The reaction was stopped by the addition of Stop Solution (KPL) and the absorbance read at 450nm using a BIO-RAD 2550 EIA reader.

Results and Discussion

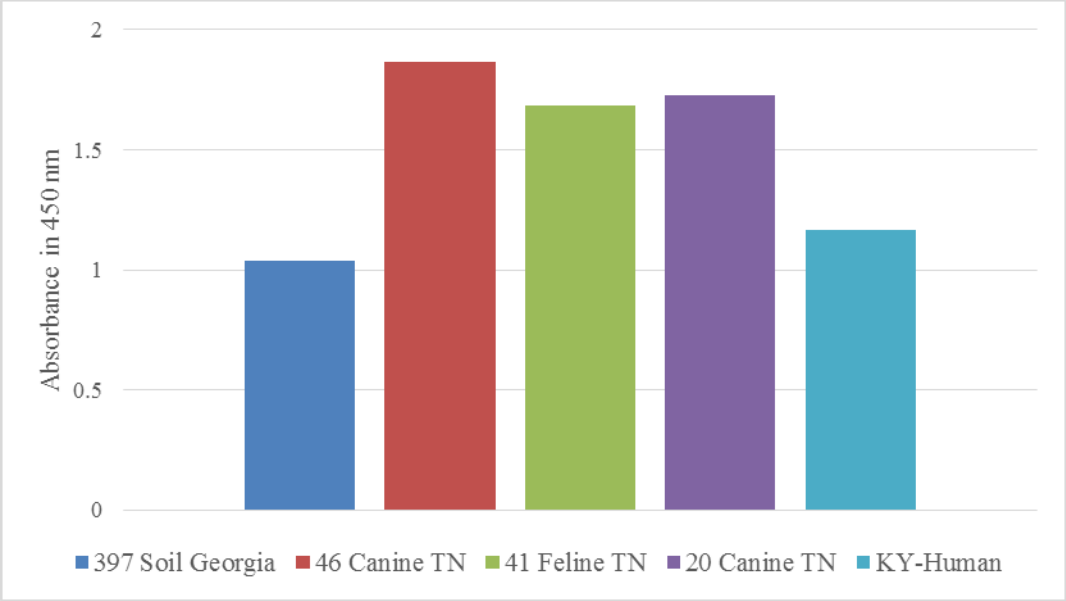

Figure 1. Mean absorbance values of antibodies detected with five *B. dermatitidis* lysate antigens.

Figure 1 illustrates the mean absorbance values obtained for the five *B. dermatitidis* lysate antigen reagents when used to detect antibodies present in canine serum specimens. All five of the lysate antigens used in the study were able to detect antibodies from the twenty canine serum samples. The three antigen samples from Tennessee, two being canine and one feline, had a mean absorbance value greater than the soil sample or sample from a human host. Both canine antigens outperformed the other antigens with, 46-Canine-TN exhibiting the optimal reactivity with a mean absorbance value of 1.864, and 20-Canine-TN had the second greatest mean absorbance value of 1.727. The feline lysate detected antibody presence with a mean absorbance value of 1.684, which is comparable to the canine

antigens', yet considerably outperformed the human and soil samples. The 397-Soil-Georgia antigen was least likely to produce positive verification of antibody presence in a canine serum with a mean absorbance value of 1.039. The KY-Human antigen produced a stronger detection of antibody presence with a mean absorbance value of 1.163, but was non-comparable to the canine or feline yeast lysates.

The research data provided by this study is pertinent for the ability to detect antibodies in hosts that have been infected with blastomycosis. The current study found that lysate antigen 46-Canine-TN outperformed four other lysate antigens in regards to detection of antibodies in twenty canine serum specimens. The antigen isolate may be used in future laboratory diagnostic tests of *B. dermatitidis* infections. Continual research is required for the ability to use antigen 46-Canine-TN as the standard for *B. dermatitidis* diagnostic testing. One concern is whether the antigen cross reacts with *H. capsulatum*, a similar fungal organism. Future studies will be conducted to evaluate the cross reactivity with Histoplasmosis, along with the continuation of analyzing antibodies produced by various hosts of *B. dermatitidis.*

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