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Research article

Comparative Studies on Using Rabbit vs Dog Polyclonal Antibodies for the Detection of *Blastomyces dermatitidis* Antigen in Urine Specimens from Dogs with Blastomycosis

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Abstract

The laboratory diagnosis of blastomycosis, caused by the thermally dimorphic fungus *Blastomyces dermatitidis*, has presented an enigma to medical personnel for many years. Recently many investigators have concentrated on developing antigen detection immunoassays for this disease. The aim of our current project was to evaluate two antibodies from immunized rabbits (anti-B5931; human isolate and anti-T-58; dog isolate) and one pooled antibody preparation from dogs with diagnosed blastomycosis for the detection of *B. dermatitidis* antigen in urine speciemns from dogs with blastomycosis. The competitive enzyme-linked immunossorbent assay (ELISA) was used to compare the rabbit antibodies (Trial 1) with the dog antibody (Trial 2). The T-58 antibody proved to be more sensitive in Trial 1 than the B5931 antibody with sensitivity values for 75% and 59.6% respectively with regard to antigen detection in 52 dog urine specimens. In Trial 2 the pooled dog antibody preparation was able to detect antigen in 44 or 50 (88%) urine specimens which yielded a sensitivity value that was 13% greater than the optimal rabbit antibody in Trial 1. The data obtained in this comparative immunoassay indicates the potential for further development of antigen detection ELISAs.

Keywords: Antigen detection, *Blastomyces dermatitidis*, ELISA, lysate antigens, dog urine specimens

Introduction

Blastomyces dermatitidis is a thermally dimorphic fungus that is pathogenic in humans and other animals. Blastomyces is endemic to North America. It is mainly in the Midwest, around the Great Lakes, the Mississippi and Ohio Rivers. It also exists in a few other areas including regions of Africa and India. The fungus exists in the mycelial phase in the soil or in decaying organic matter. The infectious spore becomes airborne and enters the lungs where the mycelial infectious agent converts into a broad-based budding yeast cell [1-3]. In some instances the immune system may resolve the infection, but in others the disease may progress from the acute to a chronic form in the lungs. It may also disseminate to other internal organs or even to the central nervous system where a fatal meningitis may develop [4-7].

The laboratory diagnosis of blastomycosis in humans and dogs has presented an enigma to clinicians for many years. Culturing or histologic identification of the organism may be performed with success in some instances, but these methods may be time consuming or may not provide an accurate diagnosis in many cases. Therefore, over the past several years, researchers have been concerned with efforts to develop improved immunodiagnostic assays for the detection of *B. dermatitidis* antibody or antigen in serum or urine specimens from humans or dogs with blastomycosis [4,8-17].

Our laboratory has also been concerned with developing improved laboratory diagnostic assays for blastomycosis. We have been performing various enzyme-linked immunosorbent assays (ELISA) with *B. dermatitidis* novel yeast phase lysate antigens, produced in our laboratory, for antibody detection or with antibodies produced with yeast lysates or killed whole yeast cells for antigen detection. The objectives of these studies have been to perform comparative evaluations on antibody detection in serum specimens from immunized rabbits and infected dogs or to detect antigen in urine specimens from dogs with blastomycosis [18-25]. The results of these immunoassays have indicated the potential for further studies on the use of the *B. dermatitidis* antigens or antibodies for the laboratory diagnosis of blastomycosis.

The aim of this current study was to compare *B. dermatitidis* antigen detection in urine from dogs with blastomycosis with rabbit antibodies produced from whole killed *B. dermatitidis* yeast cells of a human or dog isolate (Trial 1) and a pooled antibody preparation from dogs with diagnosed blastomycosis (Trial 2).

Materials and Methods

Antibodies / Urine Specimens

The antibodies were obtained from rabbits immunized with B5931 (Minnesota, human) and T-58 (Tennessee dog) *B. dermatitidis* killed whole yeast cells (Trial 1) or from dogs with diagnosed blastomycosis (Trial 2) and available in our laboratory. Urine specimens (52) were provided by Dr. A.M. Legendre (University of Tennessee College of Veterinary Medicine, Knoxville, TN).

Lysate antigen preparation

The yeast lysate antigen used to coat the microdilution plates was prepared as follows: *B. dermatitidis* lysate (B5896, human isolate, Minnesota) was prepared by a method similar to one that was previously used for the production of yeast lysate antigen from *Histoplasma capsulatum* [28-30] and modified in our laboratory for *B. dermatitidis* lysate antigen production [20]. The yeast phase cells were grown for 7 days at 37°C in a chemically defined medium in an incubator shaker, harvested by centrifugation (700 x g; 5 min), followed by washing with distilled water, resuspended in distilled water and then allowed to lyse for 7 days at 37°C in water with shaking. The preparation was centrifuged, filter sterilized, merthiolate added (1:10,000) and stored at 4 C. A protein determination was performed on the lysate using the BCA Protein Assay Kit (Pierce Chemical Company, Rockford,

IL) and the dilution used in the ELISA was based on protein concentration.

Competitive ELISA method

The horseradish peroxidase competitive binding inhibition ELISA was used for the detection of *B. dermatitidis* antigens in the urine specimens. Microdilution plates (96 well NUNC, Thermo-Fisher) were coated with 100 µl of B5896 (Minnesota human isolate) lysate antigen that was diluted (2000 ng ml^{-1}) in a carbonate-bicarbonate coating buffer (pH 9.6).The plates were 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). Dog urine and 1:2200 antibody obtained from rabbits immunized with either B5931 (Minnesota human) or T-58 (Tennessee dog) (Trial 1) or pooled antibody from infected dogs (Trial 2) were added to microcentrifuge tubes (200 µl plus 200 µl of each urine specimen) and incubated for 30 min at 37° C. Following this incubation step 100 µl of the antibody-urine mixture from the microdilution tubes was added to the above plates containing the B5896 antigen and incubated for 30 min at 37º C. The plates were again washed as above and 100 µl of goat anti-rabbit IgG or anti-dog IgG horseradish peroxidase conjugate (Kirkegaard and Perry Laboratories, KPL) was added to each well and incubated for 30 min at 37° C and were washed as above. Then 100 µl of Sure BlueTMB peroxidase substrate (KPL) was added to each well and incubated for approximately 2 min at room temperature. Stop Solution (KPL) was added to each well and the absorbance was read using a BIO-RAD 2550 EIA reader at 450 nm. Positive controls containing known B5896 antigen coated on the plate and the above sera from the immunized rabbits or infected dogs were used to determine the baseline value to which all of the urine specimens were compared.

Results

Trial 1

Trial 1 consisted of two competitive antigen detection assays using urine specimens from dogs with diagnosed blastomycosis by comparing two rabbit serum derived antibodies (Figures 1 and 2). Antibody B5931detected antigen in 31 of the 52 urine samples (59.6% sensitivity) (Figure 1). In contrast, antibody T-58 detected antigen in 39 out of the 52 urine samples (75% sensitivity) (Figure 2). The sensitivity achieved with the T-58 antibody was 15.4% greater than that of the B5931 antibody.

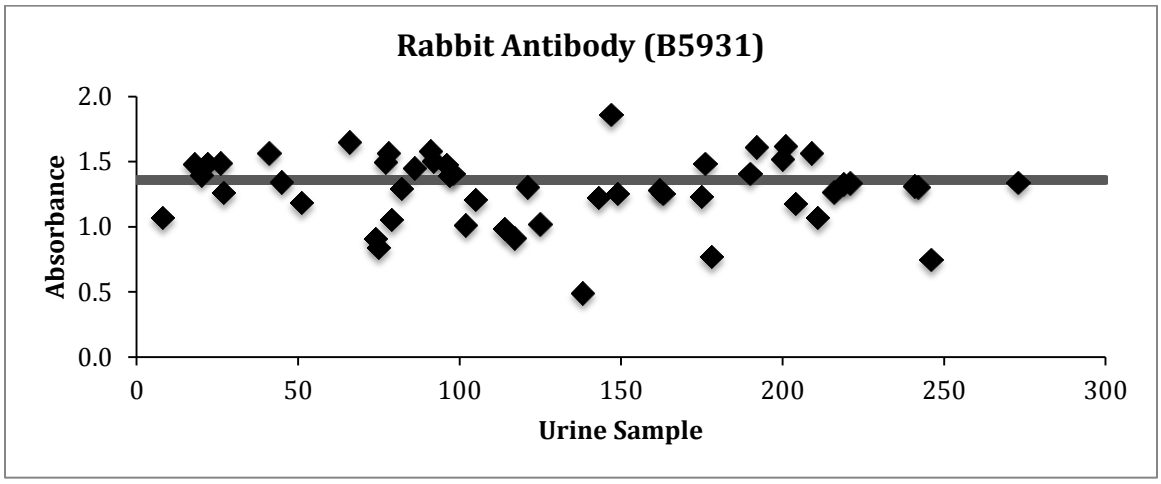

Figure 1: Antigen detection with Rabbit antibody B5931 with a final dilution of 1:4400 when admixed with the urine samples when used to detect *B. dermatitidis* antigen in 52 dog urine samples having been diagnosed with blastomycosis. The rabbit serum control (1:4400), as identified by the horizontal line, had a mean absorbance value of 1.359.

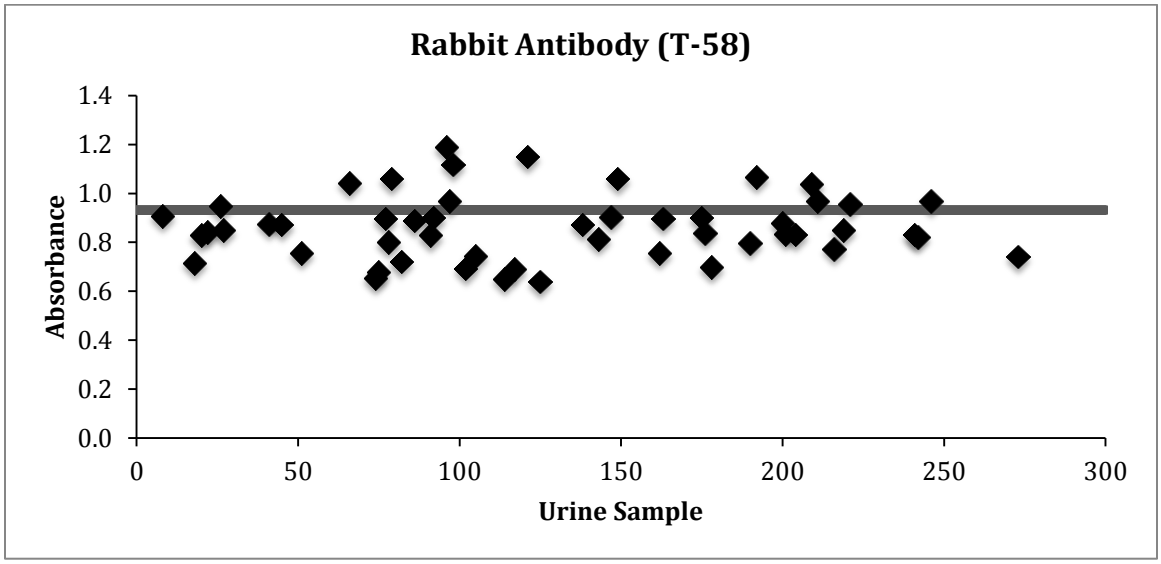

Figure 2: Antigen detection with Rabbit antibody T-58 with a final dilution of 1:4400 when admixed with the urine samples when used to detect *B. dermatitidis* antigen in 52 dog urine samples having been diagnosed with blastomycosis. The rabbit serum control (1:4400), as identified by the horizontal line, had a mean absorbance value of 0.932.

Trial 2

In Trial 2 T-58 antibody, which was the most sensitive in Trial 1, was compared with a pooled antibody preparation obtained from two dogs with diagnosed blastomycosis for antigen detection in the same dog urine specimens (Figures 2 and 3). The results revealed that the pooled dog antibody preparation detected antigen in 44 of the 50 urine samples with a sensitivity value of 88%, which was 13% higher than the T-58 Trial 1 antibody (Figure 3).

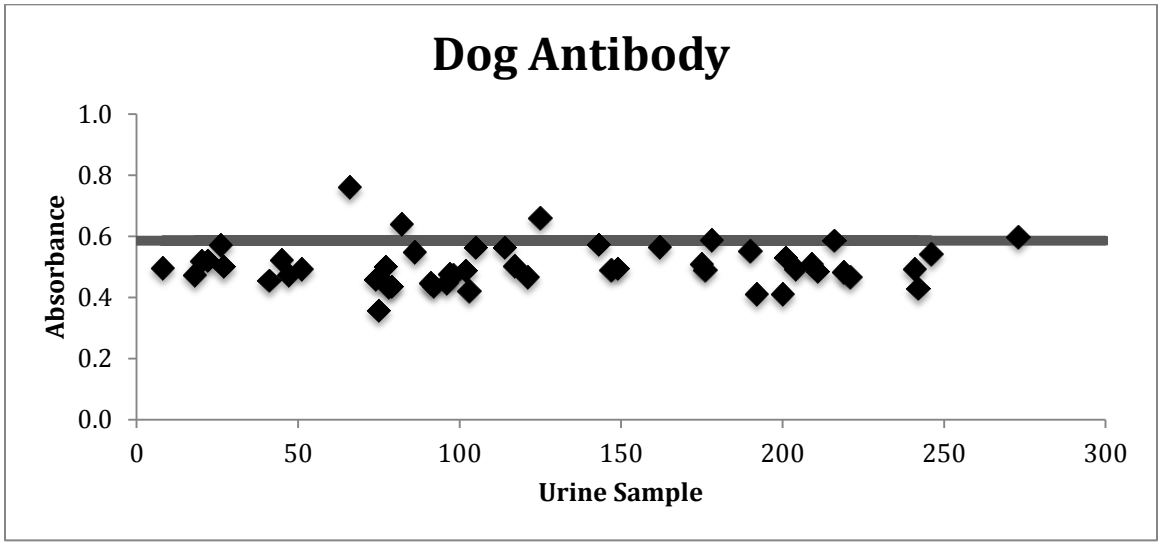

Figure 3: Antigen detection with pooled dog antibody with a final dilution of 1:2000 when admixed with the urine samples when used to detect *B. dermatitidis* antigen in 50 dog urine samples having been diagnosed with blastomycosis. The dog serum control (1:2000), as identified by the horizontal line, had a mean absorbance value of 0.586.

Discussion/Conclusion

Endemic to North America, the thermally dimorphic fungus *B. dermatitidis* has an airborne mode of infection and is pathogenic to humans and other animals including dogs [1-3]. If not naturally resolved, a lack of early detection could result in a systemic progression with possible fatal health implications. Often misdiagnosed as a bacterial pulmonary infection, the proper course of treatment may be delayed leading to dissemination of the yeast form of *B. dermatitidis* to other organs including the central nervous system [4-7]. Antigen detection of *B. dermatitidis*, as is represented in the study, may provide for early detection and the progression to a fatal prognosis may be avoided. The research advances by investigators involved in the development of improved and reliable immunodiagnostic assays is certainly needed for the efficient and accurate diagnosis of blastomycosis [4, 8-17].

The use of antigen detection immunoassays may lead to a faster detection rate compared to the time frame involved for adequate antibody production by the patient needed for successful detection of the infection to be achieved. This concept is especially important in immunocompromised patients with blastomycosis.

When the most sensitive of the two rabbit antibodies in Trial 1, anti-T-58, was compared to the pooled antibody preparation from two dogs with diagnosed blastomycosis (Trial 2), it was determined that the dog antibody was able to detect *B. dermatitidis* antigen in a higher percentage (88% sensitivity) of the dog urine specimens versus a sensitivity value of 75% with the optimal rabbit antibody.

This research work has provided encouraging data on the potential use of various antibody preparations for the detection of *B. dermatitidis* antigen in urine from dogs with blastomycosis. The results also indicate that additional studies with different antibody preparations produced in rabbits immunized with *B. dermatitidis* yeast phase lysate antigens and killed whole cell antigens or antibodies obtained from dogs with blastomycosis are certainly required in an effort to produce an antigen capture antibody that is specific and reliable for early detection of blastomycosis in animals and humans.

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