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Research article

Antigen Detection in Urine Specimens from Dogs with Blastomycosis: Comparative ELISA Determinations with Serum from Rabbits Immunized with *Blastomyces dermatitidis* Dog Isolates

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Abstract

The systemic fungal infection blastomycosis, caused by *Blastomyces dermatitidis*, infects mammals including humans and dogs. Developmental improvements have drastically increased in the detection and diagnosis of the disease. This study was designed to detect the presence antigen in urine from dogs diagnosed with blastomycosis using the competitive enzyme-linked immunosorbent assay (ELISA) method with known antibodies T-66 (Tennessee dog) and WI-J (Wisconsin dog). A combination of the two antibodies was also used to determine if such a preparation might be efficacious in detecting antigen. Based on the results, each of the known antibodies detected the presence of antigen in the urine specimens from pre-treatment to 30, and 60 days post- treatment. Although each known antibody detected the presence of antigen, the combination of T-66+WI-J showed a higher degree of sensitivity with each of the urine specimens than the individual antibodies with mean absorbance values with the pre-treatment specimens 1.248 to 1.627 as compared to an absorbance value of 1.790 with the positive control. This study provides evidence that the combination of antibodies is more efficient in detection of antigens than single antibodies and our laboratory will continue to evaluate the combinations of antibodies when detecting antigens

Keywords: Blastomycosis, Competitive ELISA, Antibody detection, Lysate Antigens

1. Introduction

Blastomycosis is a systemic fungal infection caused by the thermally dimorphic organism that infects mammals, especially dogs and humans [1]. The infection is initiated when spores that have been produced by the filamentous phase of the fungus, is inhaled. The organism exists in this stage in nature or in the laboratory at 25° C and has the ability to convert to the yeast phase at 37° C in the lungs of the infected host. The disease may be self-resolving, or it may exist as an acute infection that can be characterized by cough, chills, headaches, malaise, fever, chest pain, night sweats and weight loss. It may also progress to a more chronic state and has a greater possibility to be misdiagnosed as tuberculosis or other diseases [1-7]. Early symptoms can easily go unnoticed in dogs and therefore detection may not occur until *B. dermatitidis* has disseminated. In dogs the infection may disseminate to the eyes, bones, skin or lymph nodes and produce an immune response directed against the yeast phase antigens.

The amount of interest among researchers with respect to increasing the development and improving diagnostic assays has increased dramatically due to a rise of the systemic fungal diseases [8-11]. Blastomycosis, as well as the other systemic mycoses have been important diseases to physicians since they can not only infect individuals with normal immune systems, but also are a cause for concern in hosts with deficiency diseases that compromise the immune system. Human diagnosis can be especially difficult with individuals that are immuno-compromised because the current immunodiagnostic techniques require the production of an adequate antibody response. Therefore a number of researchers have concentrated on developing or improving antigen detection assays for blastomycosis or other fungal diseases [12-16].

The emphasis of study in our laboratory over the past several years has been on the development of novel yeast phase lysate antigens from various strains of *B. dermatitidis* and the utilization of these reagents in immunodiagnostic assays for not only the detection of antibody, but also for antigen detection [17-25]. The focus of this current research was to evaluate the efficacy of different known antibodies and a combination of those same antibodies in antigen detection in urine specimens from dogs with diagnosed blastomycosis using a competitive ELISA.

2. Materials and Method

2.1 *Antibodies*

The antibodies were obtained from rabbits immunized with T-66 (Tennessee dog) and WI-J (Wisconsin dog) *B. dermatitidis* yeast phase lysate antigens and available in our laboratory. The rabbits were housed in accordance to the NIH guide for Care and Use of Laboratory Animals with approval from the Idaho State University IACUC.

2.2 *Urine Specimens*

The urine specimens were provided by Dr. A.M. Legendre (University of Tennessee College of Veterinary Medicine, Knoxville, TN). Of the 67 urine specimens 64 were obtained from dogs previously diagnosed with blastomycosis ranging from: pre-treatment, 30, and 60 days of treatment from several locations within the endemic area of North America. Three of the urine specimens were obtained from normal uninfected dogs with the disease and used as a negative control. No competitive binding was observed in urine specimens from uninfected dogs.

2.3 *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 T-66 (Tennessee dog 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 1:900 antibody obtained from rabbits immunized with T-66 (Tennessee dog), WI-J (Wisconsin dog), and an antibody combination of T-66 and WI-J were added to microcentrifuge tubes (200 µl plus 200 ul of each urine specimen) and incubated for 30 min at 37° C. Following this incubation step 100 ul of the antibody-urine mixture from the microdilution tubes was added to the above plates containing the T-66 antigen and incubated for 30 min at 37 C. The plates were again washed as above and 100 ul of goat anti-rabbit 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 TMB peroxidase substrate (Pierce: Thermo-Fisher) was added to each well and incubated for approximately 3 min at room temperature. The reaction was stopped with 2 N H_2SO_4 and the absorbance was read using a BIO-RAD 2550 EIA reader at 450 nm. Positive controls containing known T-66 antigen coated on the plate and the sera from the immunized rabbits were run to determine the baseline value in which all of the urine were compared.

3. Results and Discussion

The mean absorbance values for the detection of antigens in urine from pre-treated dogs with the known antibodies T-66, WI-J, and the combination T-66+WI-J are found in **Figure 1**. The detection of antigen is compared to the controls of each known antibody with the T-66 antigen. Each of the mean absorbance for the antigens was below the values of the controls, ranging from 0.755 to 1.456,while the control values ranged from 1.038 to 1.791.

The mean absorbance values obtained for the detection of antigens in urine from dogs treated for 30 days are shown in **Figure 2**. Each of the mean absorbance values for the antigens was below that of the controls, ranging from 0.473 to 1.065 with the control values ranging from 0.914 to 1.334.

The mean absorbance values of the detection of antigens in urine from dogs treated for 60 days are shown in **Figure 3**. Each of the mean absorbance values for the antigens is below that of the controls ranging from 0.584 to 1.154 with the control values ranging from 1.073 to 1.524.

The mean absorbance value of the detection of antigen in normal urine from dogs with the known combination antibodies T-66+WI-J is shown in **Figure 4.** The detection of the antigens in the normal urine was compared to the control of T-66+WI-J with the antigen T-66. Unlike the other assays the mean absorbance for the normal dog urine is above the control value. The mean absorbance for the normal urine is 1.439 and the value of the control is 1.352.

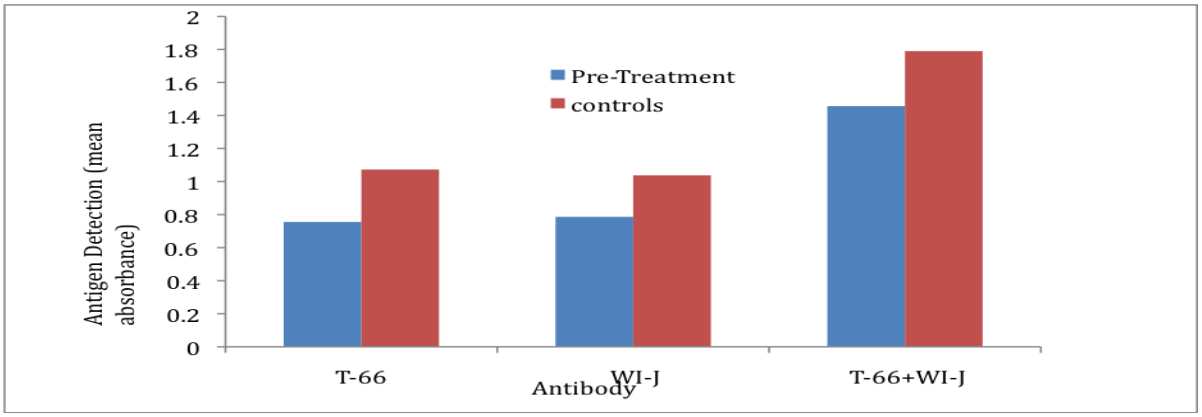

Figure 1: Comparison of the three known antibodies for the detection of antigen in urine from pre-treatment dogs diagnosed with blastomycosis.

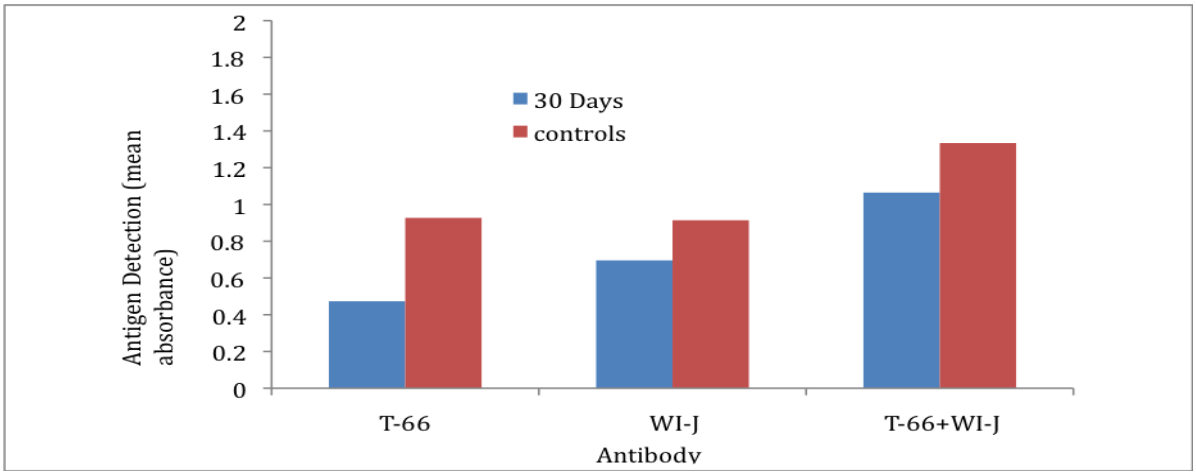

Figure 2: Comparison of the three known antibodies for the detection of antigen in urine from dogs that have been treated for 30 days for blastomycosis.

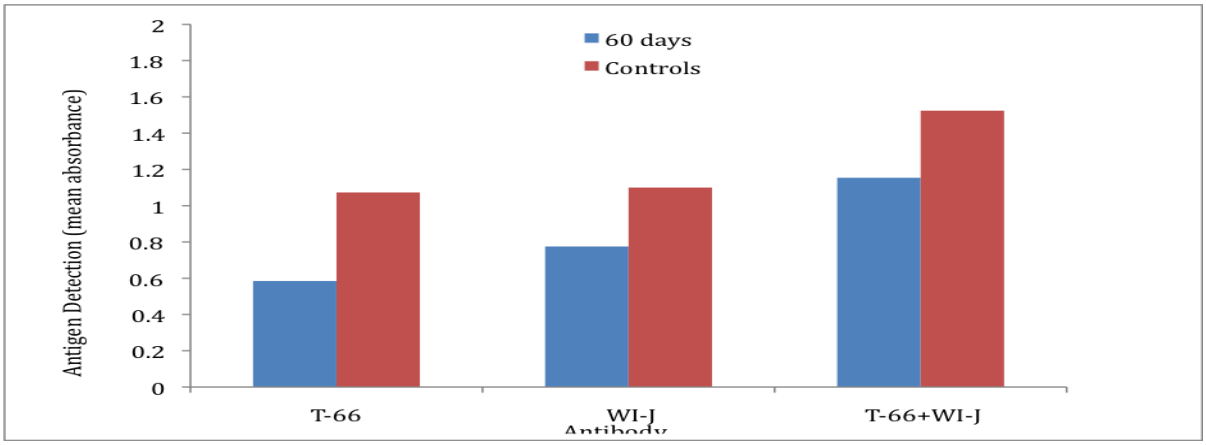

Figure 3: Comparison of the three known antibodies for the detection of antigen in urine from dogs that have been treated for 60 days for blastomycosis.

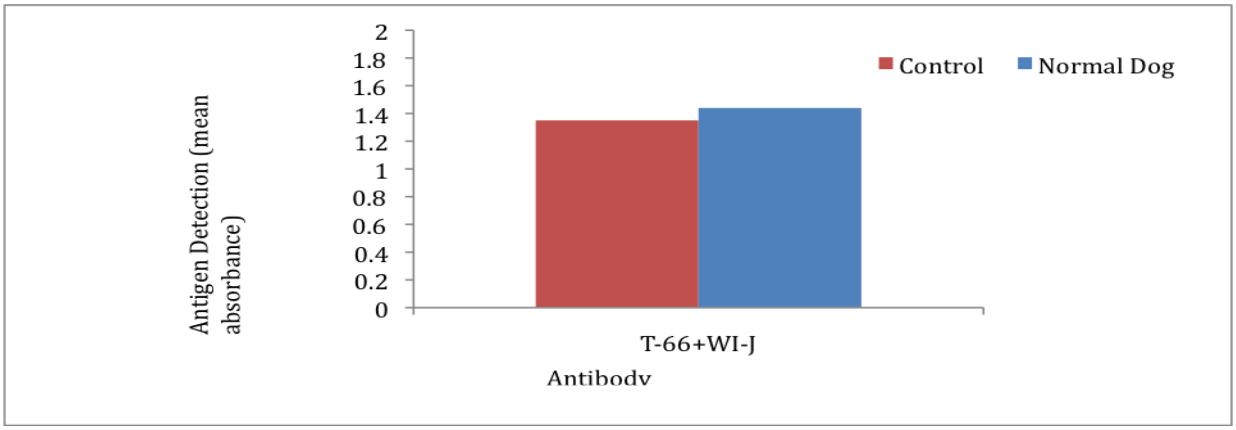

Figure 4: Comparison between the combinations of known antibodies (T-66+WI-J) with antigen detection in normal dog urine.

4. Conclusion

This study indicates that the combination of antibodies has proved more effective in detection of antigens in urine of dogs that have been diagnosed with blastomycosis. The combination of T-66+WI-J antibodies was optimal with respect to antigen detection in each of the urine specimens ranging from pre-treatment, 30 days, and 60 days of treatment. The urine specimens from uninfected dogs provided evidence that non-specific reactions were not contributing to the results obtained in this competitive ELISA for antigen detection. This study provides evidence of the potential of such an assay and we continue the study of such combinations of known antibodies for detection of *B. dermatitidis* antigen.

5. Acknowledgement

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