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Research article

Blastomyces dermatitidis Yeast Lysate Antigens: Antibody Detection in Dogs with Blastomycosis

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Abstract

For the past several years our laboratory has been involved with the production and evaluation of Blastomyces dermatitidis yeast phase lysate antigens for the detection of blastomycosis in serum specimens using the enzyme-linked immunosorbent assay (ELISA). The aim of this comparative evaluation was to utilize 6 lysate antigens prepared from dog isolates (T-58, T-66 and T-2 from Tennessee and ERC-2 and WI-R from Wisconsin and M97 from Minnesota) to detect antibodies in serum specimens from dogs with blastomycosis. All six of the lysates were able to detect antibodies in the 20 dog sera with mean absorbance values ranging from 1.043 (WI-R) to 1.586 (T-66). It was interesting to note that the three southern isolates were slightly more reactive with the dog sera (all from dogs from southern states) than the three northern isolates of the fungus with mean absorbance values of 1.321 and 1.188 respectively. Additional studies are in progress to further evaluate the lysate reagents for antibody detection in a variety of serum specimens.

Keywords: Blastomyces, Blastomycosis, ELISA, Antibody Detection, Dogs, Lysate Antigens

Introduction

The disease blastomycosis, produced by Blastomyces dermatitidis is a systemic fungal infection of animals and humans. It is a dimorphic organism that exists in

soil or in decomposing organic matter as a mycelial spore. Endemic areas for blastomycosis have been in southeastern and south-central states that border the Ohio and Mississippi Rivers and upper Midwestern states including areas in Wisconsin and Minnesota. The fungus exists in environments with an abundance of water (lakes, rivers, etc.) which facilitates the growth and potential spread of the infectious mycelial spores [1, 2].

B. dermatitidis is present in the mycelial form in nature or in the laboratory at approximately 25 C and has the ability to convert to the yeast phase at 37 C in the lungs of the infected host or at the higher temperature in the laboratory. The disease may be self-resolving or it may exist as an acute or chronic state in the lungs, where it may be misdiagnosed as tuberculosis or some other microbial pulmonary disease. If the disease is not diagnosed or untreated while in the lungs it may disseminate to other organs including the skin, eyes (ocular involvement in dogs), bones and possibly to the central nervous system where fatal meningitis may develop [3-7].

The diagnosis of blastomycosis has presented clinicians with varying degrees of difficulty over the years. In some instances culturing or histopathological examination, which have been the gold standard for diagnosis, may be beneficial, but in some patients these methods may not yield positive results due to the time required for culture growth or procedures involved in invasive testing. This has led to investigators concentrating on research designed to improve immunological assays, which tend to provide a more rapid diagnosis, but problems still exist with regard to the sensitivity and specificity of immunoassays [8-11]. A recent study [12] on the use of B. dermatitidis surface protein in an enzyme immunoassay for antibody detection in blastomycosis indicated a sensitivity value of 88% in patients with blastomycosis. Thus progress is being made and investigations continue on improved antibody or antigen det3ection immunoassays.

Over the past several years our laboratory has developed novel yeast phase lysate antigens and utilized these in various immunoassays for antibody detection in serum specimens from infected dogs and immunized rabbits [13-19], but these studies have only opened up new avenues of approach with regard to how we might improve immunodiagnostic assays in the future. Therefore this present study was designed to determine the reactivity of 6 B. dermatitidis lysate antigens in the indirect ELISA to detect antibodies in serum specimens from dogs with diagnosed blastomycosis.

Materials and Method

B. dermatitidis yeast phase lysate reagents from dog isolates (T-58, T-66, T-2 : Tennessee isolates, and ERC-2 and WI-R: Wisconsin isolates, M-97: Minnesota isolate) were prepared following a protocol similar to the one used for the production of antigen from Histoplasma capsulatum [20--22] and modified in our laboratory for B. dermatitidis lysate antigen production [23]. The yeast phase cells were grown for 7 days at 37°C in a chemically defined medium (glucose, 10.0 g; potassium phosphate monobasic, 1.5 g; calcium chloride dehydrate 0.15 g; magnesium sulfate, 0.5 g; ammonium sulfate, 2.0 g; L-asparagine, 2.0 g; L-cysteine, 0.2g; and pH adjusted to 6.2 with 5 N sodium hydroxide) in an incubator shaker, harvested by centrifugation (700 x g; 5 min) followed by washing with distilled water, re-suspended in distilled water, and then 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. Protein determinations were performed on the lysates using the BCA protein assay kit (Pierce Chemical Company,

Rockford, IL, USA), and dilutions of the antigenic reagents used in the ELISA assays were based on protein concentration.

Results and Discussion

The mean absorbance values obtained when the 6 B. dermatitidis yeast lysate antigens, prepared from dog isolates, were utilized in the ELISA to detect antibodies in 20 serum specimens from dogs with diagnosed blastomycosis, are shown in Figure 1. One lysate antigen, T-66, prepared from a dog isolate from Tennessee, was optimal with regard to immune reactivity with the dog sera from Tennessee. Another lysate preparation (ERC-2, prepared from a dog isolate from Wisconsin was second with regard to reactivity in the dog sera. All six of the lysates were able to detect antibody in the 20 dog sera with mean absorbance values ranging from 1.043 (WI-R) to 1.586 (T-66). It was interesting to note that the three southern isolates were slightly more reactive with the dog sera (all from dogs from southern states) than the three northern isolates with mean absorbance values of 1.321 and 1.188 respectively. Additional studies are required in order to further evaluate the lysate preparations and to determine if the geographical origin of the B. dermatitidis cultures may play a role with regard to the utilization of the lysates prepared from the isolates as immunodiagnostic reagents in the clinical laboratory.

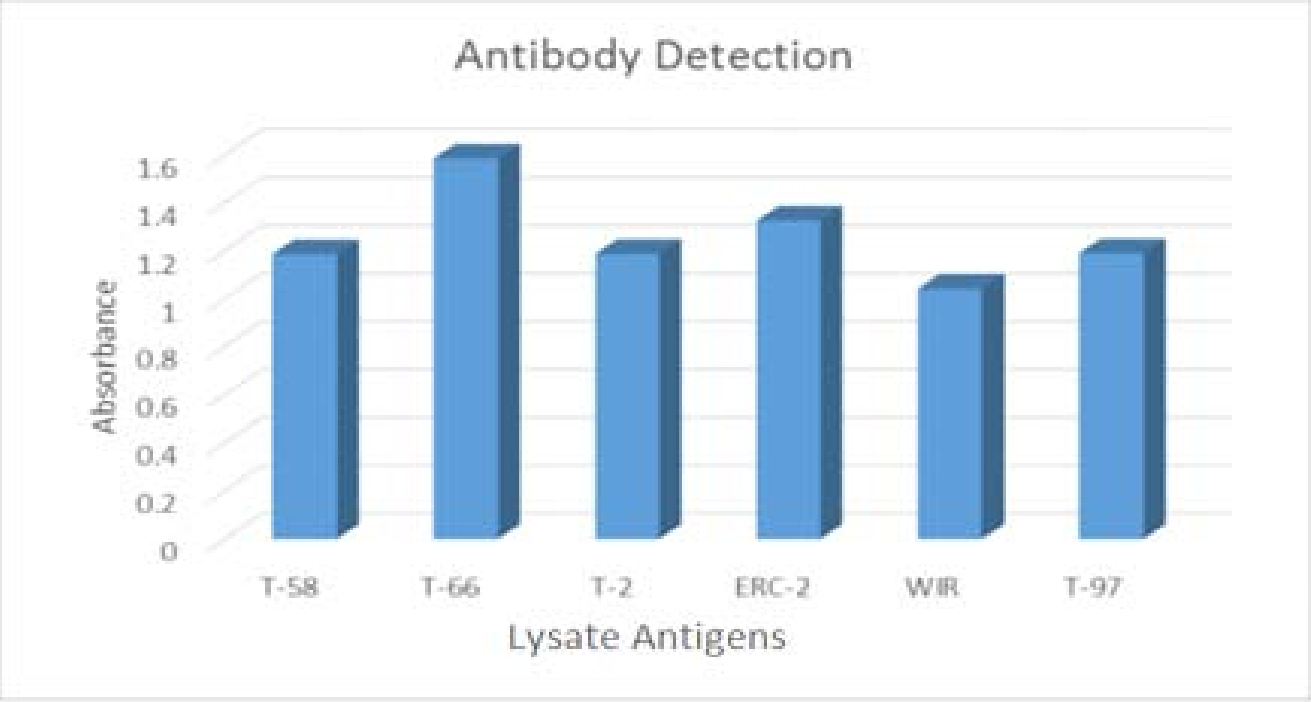

Figure 1: Mean absorbance values at 450nm for each of the six yeast lysate antigens

Conclusion

This study illustrates that while effective ELISA data may be achieved by using B. dermatitidis antigen isolates from several different yeast lysates, the most effective antibody antigen combinations resulted from using those lysates obtained from the same geographic region. This data is important in a laboratory or in clinical diagnostic settings to

ensure the greatest sensitivity in sera analysis. Further studies will be needed to determine what specific role the source environment plays in its effects on the antigen antibody complex formation.

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