

Research article

Antibody Detection in Dogs with Blastomycosis: Comparison of *Blastomyces dermatitidis* Lysate Antigens Prepared from Dog and Soil Isolates

Jordan T. Hammon, Austin C. Boyd and Gene M. Scalarone

Department of Biological Sciences, Idaho State University, Pocatello, Idaho, USA
E-mail: hammjord@isu.edu



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

Abstract

Blastomyces dermatitidis, the causative agent of blastomycosis, has presented diagnostic dilemmas to clinicians for many years due to problems with sensitivity and specificity. This study compared six *B. dermatitidis* yeast lysate antigens, three prepared from soil isolates and three from dog isolates to assess antibody detection using the enzyme-linked immunosorbent assay [ELISA] in 20 serum specimens from dogs with diagnoses blastomycosis. All six reagents proved to be immunoreactive and were able to detect antibody in each of the sera with only slight variations in the mean absorbance values evidenced. Sensitivity mean absorbance values ranged from 0.658 (47; dog from Minnesota) to 0.733 (98 and 42913; dog from Minnesota and dog from Tennessee respectively) with a mean value for all six antigens equal to 0.710. This study generated data making it apparent that although the antigenic lysates do display some differences in reactivity, each of them has ability to detect antibody in dog sera in an efficient manner. This study is part of ongoing evaluations in which we have been performing comparative studies on a large number of *B. dermatitidis* yeast phase lysate antigens prepared from human, animal and environmental isolates of this dimorphic fungus.

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

Introduction

Blastomycosis, a pulmonary and potentially systemic fungal disease caused by *Blastomyces dermatitidis*, is an infection of humans and other animals. This disease has been associated with regions of the United States where there is an abundance of water and decaying vegetation including states that border the Mississippi and Ohio Rivers and also states like Minnesota, Wisconsin, areas of lower Canada, and even in certain regions of Africa and India [1,2].

The disease state originates when an individual inhales mycelial spores into the lung which then have the ability to convert to broad-based budding yeast cells. This primary acute infection may progress to a chronic state or even disseminate to other organs including the production of cutaneous lesions or infection of the central nervous system which may be fatal depending on the immunological status of the patient. Often times the disease is not diagnosed in the desired time frame or a misdiagnosis is made as a bacterial or viral infection which may certainly lead to problems regarding antimicrobial treatment [3,4].

Various techniques have been used in the clinical laboratory for the diagnosis of blastomycosis including microscopy, culturing and histopathologic methods. In some instances these methods have provided a reliable diagnosis, but in other instances a diagnosis may not be achieved or the time interval required for the diagnosis of the infection may be quite lengthy. Therefore investigators have spent much effort during the past several years in attempting to develop improved immunodiagnostic assays for antibody and antigen detection in blastomycosis [5-9]. For many years our laboratory has developed and evaluated yeast phase lysate antigens prepared from a variety of *B. dermatitidis* isolates and the utilization of such reagents for the detection of antibodies in serum specimens from immunized and infected animals [10-14]. In many instances we have shown the utility of some of these novel lysate antigens with regard to antibody detection, but these comparative studies have also indicated that additional evaluations with some of the more recently prepared lysates are necessary. This study compares six new lysate preparations (three each from dog isolates and soil isolates) for antibody detection in serum specimens from dogs with diagnosed blastomycosis.

Materials and Methods

Lysate antigen preparation

Six yeast lysate antigens were prepared from *B. dermatitidis* isolates (397: soil from Georgia; 98: dog from Minnesota; 47: dog from Tennessee; 248: soil from Eagle River Minnesota; 42913: dog from Tennessee; A2: soil from Canada). 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* [15-17] 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. 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

Twenty 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 assess 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 [11-14]. 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

Figure 1 demonstrates that each of the six *B. dermatitidis* yeast lysate antigens from these isolates were reactive with the dog serum specimens. Mean absorbance values ranged from 0.658 (dog; Tennessee) to 0.733 (dog; Minnesota and dog; Tennessee) with a mean value for all six antigens equal to 0.710. The mean absorbance value difference between the lysate showing the greatest value and the lysate with the lowest value was 0.075. Figure 2 shows the range of these yeast phase lysate antigens based on their reactivity with a total of 20 different dog sera. Lysate 98 (dog; Minnesota) showed the greatest range of reactivity among the dog sera with a range of 0.440-1.468 while lysate 47 (dog; Tennessee) presented the lowest range of reactivity between dog serum specimens with a range of 0.412-1.003. The average range between yeast lysate antigens with respect to their reactivity with 20 different dog sera was 0.812.

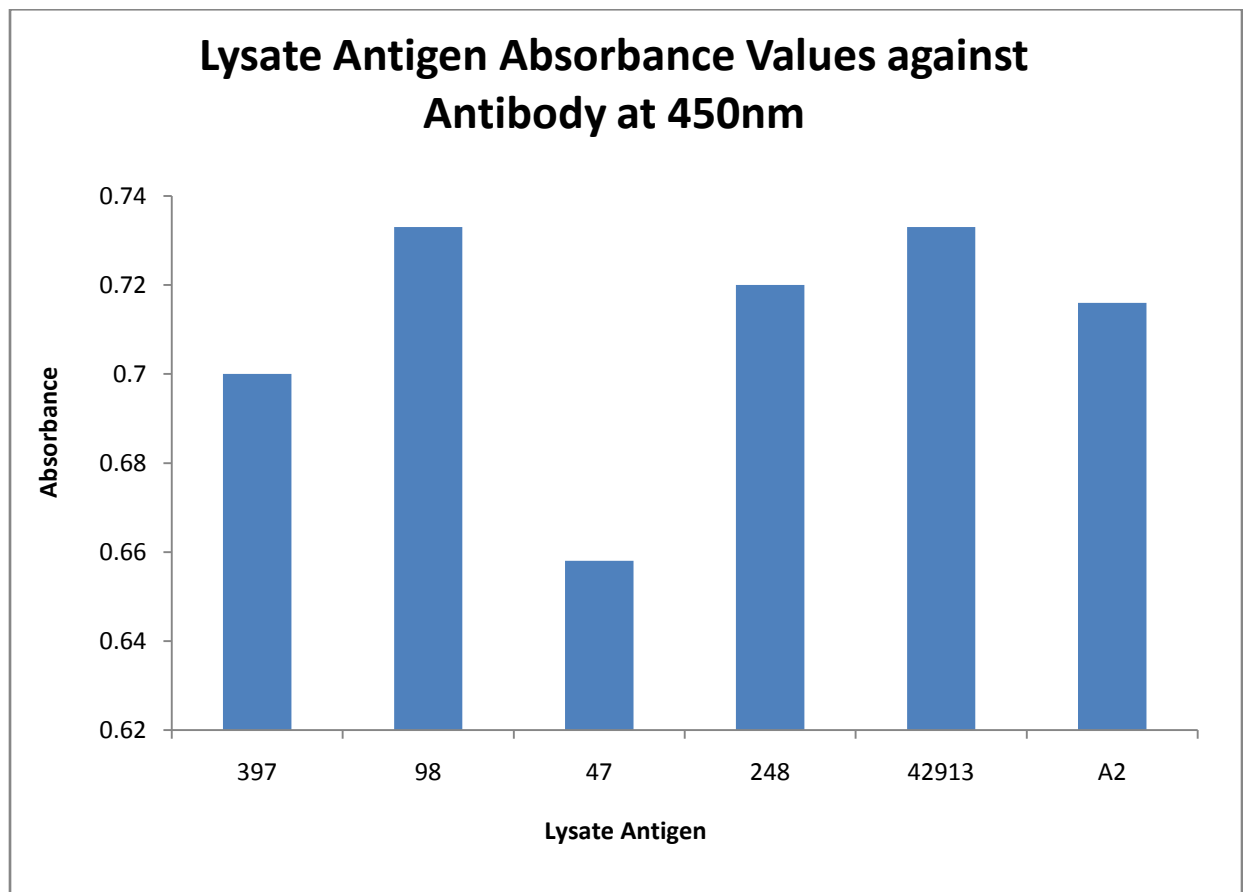


Figure 1. Mean reactivity of 6 *Blastomyces dermatitidis* yeast lysate antigens (397: soil from Georgia; 98: dog from Minnesota; 47: dog from Tennessee; 248: soil from Eagle River Minnesota; 42913: dog from Tennessee; A2: soil from Canada; reacted with 20 different dog serum specimens.

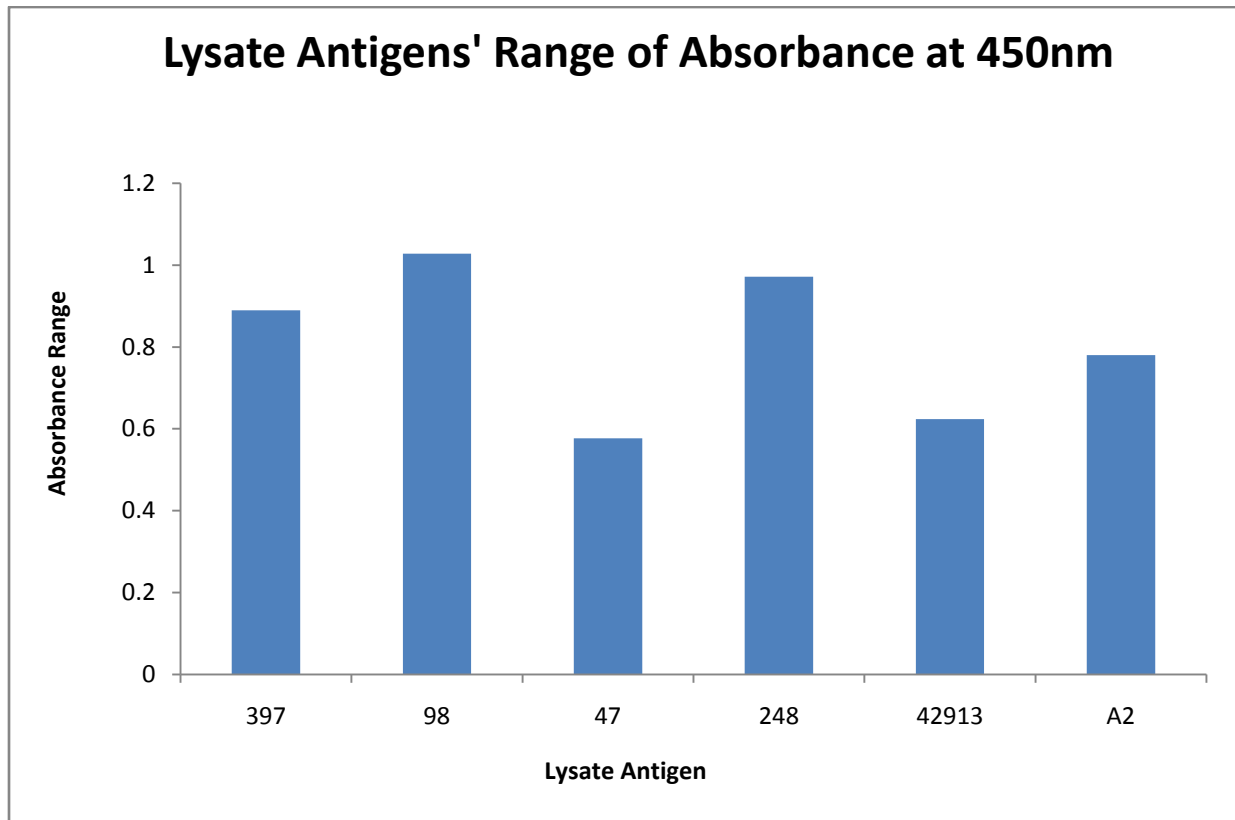


Figure 2. Range of reactivity between *Blastomyces dermatitidis* yeast lysate antigens against dog sera.

Discussion/Conclusion

The focus of this study was to compare the antibody detection capability of six different yeast lysate antigens of *B. dermatitidis* from various locations. This was accomplished by reacting the lysate antigens with an assortment of dog sera acquired from dogs that had been infected with *B. dermatitidis*. Five of the six lysate antigens proved to have similar ability to detect antibody in the dog sera. As illustrated in Figure 1, lysate antigens 98 (dog; Minnesota) and 42913 (dog; Tennessee) had the highest absorbance values of 0.733 and were thus slightly more efficient at detecting antibody in the dog serum specimens. Lysate antigen 47 (dog; Tennessee) indicated it was the least reactive at detecting antibody by displaying a mean absorbance value slightly lower than the others, a value equal to 0.658. The slight differences in reactivity of the lysates were likely due to 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 study makes evident that the yeast lysate antigens from these environments had the ability to detect *B. dermatitidis* antibodies to a similar degree. The continuation of studies in this respect will further examine yeast lysate antigens for their ability to detect antibody in serum specimens from humans and other animals. The ultimate aim of this experiment and ones to follow is to decrease the number of misdiagnosed cases of blastomycosis.

Acknowledgements

This research was supported through the Department of Biological Sciences at Idaho State University, Pocatello, Idaho, USA.

References

- [1] M.A. Pfaller and D.J. Diekema, "Epidemiology of invasive mycoses in North America. *Critical Reviews in Microbiology*, vol. 36, no.1, pp. 1-53, 2010.
- [2] A.F. DiSalvo, "Blastomycosis", in *Topley and Wilson's Microbiology and Microbial Infections*," L. Collier,ed., pp. 337-355, Arnold Publishers, London, UK, 9th edition,1998.
- [3]R.W., Bradsher, S.W. Chapman and P.G. Pappas. 2003. "Blastomycosis." *Infectious Disease Clinics of North America*, Vol.17, pp. 21-40, 2003.
- [4] J.R. Bariola and K.S. Vyas, "Pulmonary Blastomycosis," *Seminars in Respiratory Critical Care Medicine*, Vo. 32, No. 6, pp. 745-753, 2011.
- [5] M. Saccente and G.L. Woods, "Clinical and Laboratory Update on Blastomycosis," *Clinical Microbiology Reviews*, Vol. 23, No. 2, pp. 367-381, 2010.
- [6] J.A. McKinnell and P.G. Pappas, "Blastomycosis: New Insights into Diagnosis, Prevention, and Treatment," *Clinical Chest Medicine*, Vol. 30, pp. 227-239, 2009.
- [7] D. Spector, A.M. Legendre, J. Wheat, D. Bemis, B. Rohrbach, J. Taboada and M. Durkin, "Antigen and Antibody Testing for the Diagnosis of Blastomycosis in Dogs," *Journal of Veterinary Internal Medicine*, Vol. 22, pp. 839-843, 2008.

- [8] B.S. Klein, R.A. Squires, J.K. Lloyd, D.R. Ruge and A.M. Legendre, "Canine Antibody Response to *Blastomyces dermatitidis* WI-1 Antigen," *American Review of Veterinary Research*, Vol. 61, No. 5, pp. 554-558, 2000.
- [9] K.S. Vyas, J.R. Bariola and R.W. Bradsher, "Advances in the Serodiagnosis of Blastomycosis," *Current Fungal Infection Reports*, Vol. 2, pp. 227-231, 2008.
- [10] S.M. Johnson and G.M. Scalarone, "Preparation and ELISA Evaluation of *Blastomyces dermatitidis* Yeast Phase Lysate Antigens," *Diagnostic Microbiology and Infectious Diseases*, Vol. 11, pp. 81-86, 1989.
- [11] J.P. Poole, R.T. Cox, E.R. Cobb, A.R. Boyd and G.M. Scalarone., "Comparison of antibody detection with lysate antigens prepared from human and dog isolates of *Blastomyces dermatitidis*." *US Open Infectious Diseases & Immunity Journal*, Vol. 1, No. 1, pp. 1-5, 2014.
- [12] T.R. Allison, J.C. Wright and G.M. Scalarone, "*Blastomyces dermatitidis*: Stability Studies on Different Yeast Lysate Antigens," *Open Journal of Immunology*, Vol. 3, pp. 98-102, 2013.
- [13] C. Rayner, T. Blackwell, T. Hine, J. Court and G.M. Scalarone, "A comparative study using 28 *Blastomyces dermatitidis* yeast lysate antigens for antibody detection in serum specimens from immunized rabbits and dogs with blastomycosis." *US Open Infectious Diseases & Immunity Journal*, Vol. 1, No. 2, pp. 1-8, 2014.
- [14] K. Mondada, J. Fullmer, E. Hungerford, K. Novack, K. Vickers and G.M. Scalarone, "*Blastomyces dermatitidis*: Antibody detection in sera from dogs with blastomycosis with yeast lysate antigens produced from human and dog isolates," *Veterinary Medicine International*, ID 376725, 2014
- [15] H.B. Levine, G.M. Scalarone and S.D. Chaparas, "Preparation of Fungal Antigens and Vaccines: Studies on *Coccidioides immitis* and *Histoplasma capsulatum*," *Contributions to Microbiology and Immunology*, Vol. 3, pp. 106-125, 1977.
- [16] H.B. Levine, G.M. Scalarone, G.D. Campbell, R.C. Graybill and S.D. Chaparas, "Histoplasmin-CYL, A Yeast Phase Reagent in Skin Test Studies in Humans," *American Review of Respiratory Diseases*, Vol. 119, pp. 629-636, 1979.
- [17] G.M. Scalarone, H.B. Levine and S.D. Chaparas, "Delayed Hypersensitivity Responses of Experimental Animals to Histoplasmin from the Yeast and Mycelial Phases of *Histoplasma capsulatum*," *Infection and Immunity*, Vol. 21, pp. 705-713, 1978