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Research article

A Comparative Study Using 28 *Blastomyces dermatitidis* Yeast Lysate Antigens for Antibody Detection in Serum Specimens from Immunized Rabbits and Dogs with Blastomycosis

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Abstract

Blastomycosis is the systemic fungal infection of animals and humans. It has presented a diagnostic challenge to clinicians and laboratory personnel over the years. Our laboratory has been concentrating on the development of antigenic reagents from the yeast phase of *Blastomyces dermatiditis* in various isolates in order to evaluate these lysate antigens with regards to antibody detection in blastomycosis. This study evaluated 12 serum specimens (6 from dogs with blastomycosis and 6 rabbits with blastomycosis) to determine which lysate detected antibody with greater efficacy. Twenty out of the 28 lysates had better detection values with the rabbit serum, while the remaining 8 lysate samples had greater absorbance values (detection) with the dog serum. When tested on the human antigen (B5934) the combined absorbance values between rabbit and dog sera was 0.655, the lowest absorbance value observed (Figure 1). Soil antigen (248) displayed the highest combined absorbance value of 1.689. The overall mean absorbance value of all 28 lysates for both dog and rabbit serum was 1.138.

Keywords: Blastomycosis, *Blastomyces dermatitidis*, ELISA, Antibody detection, Lysate antigens

Introduction

Blastomycosis is a pulmonary disease produced in humans and animals by the thermally dimorphic fungal pathogen *Blastomyces dermatitidis*. Environmental temperature is the determining factor for transformation in morphology of *B. dermatitidis* from the mycelial form to the yeast form. The mycelial form of the fungus is the vegetative part of the plant that grows in soils and other locations close to 25°C. This form produces microscopic airborne reproductive cells called spores. These spores are infectious particles that use the respiratory system of humans and canines as a portal of entry. Once in the body at a temperature of 37°C, the spores transform into the second morphological phase of *B. dermatitidis* – yeast cells [1-4].

The proliferation of the yeast cells in the lungs leads to the blastomycosis disease that results in symptoms characteristic to tuberculosis and influenza: fever, chills, chest pain, and productive cough. These symptoms are usually followed by cutaneous skin lesions containing the fungus. However, dissemination of blastomycosis can result in infection of epithelial cell linings and any other organ in the body. Other areas of manifestation can include larynx, adrenals, heart, kidneys, prostate, uterus, and meningeal layers of the brain. The fungus can produce extensive disease and possibly death in patients with AIDS or other diseases that compromise the immune system [4-9].

In recent years researchers and physicians have stimulated considerable interest with their attempts to develop improved methods to combat the dramatic increase in the systemic fungal diseases. One major problem is that the disease is either not diagnosed or misdiagnosed as some other type of microbial disease. Ongoing research activities have been centered on developing novel ways of diagnosing, preventing and treating these potentially devastating infections. With regard to diagnosis of the disease various methods have been employed including culturing and histopathology, but in many instances these procedures are time-consuming or may fail to yield the desired results. The use of immunological assays in the clinical laboratory generally provide for a more rapid diagnosis, but most of the previous tests for the detection of antibodies have been of limited value due to problems with sensitivity and specificity [6, 7, 10-12].

For the past several years our laboratory has been involved in the development of antigenic reagents from various isolates of *B. dermatitidis* [13-20] and the evaluation of these novel yeast phase lysate antigens for the detection of antibodies or antigens in blastomycosis. These studies have contributed greatly to our knowledge concerning potential avenues of approach with regard to future investigations, but they have also indicated a need for more studies on antigen purification and antigenic components from various isolates of the organism in order to better understand the immunobiology of *B. dermatitidis*. This type of study will certainly aid us and other researchers involved in trying to discover more about the importance of the antigenic composition of new reagents and how this knowledge may allow for the development of improved immunodiagnostic reagents and new approaches to immunization for this fungal disease.

Materials and Methods

Lysate Antigens

Mycelial phase cultures of 28 different human, animal or environmental *B. dermatitidis* isolates (bat: 2; soil: 7; sea lion: 2; cat: 2; polar bear: 1; dog: 6; human: 8) were converted to yeast cells by culturing at 37 C on brain heart infusion agar. Yeast phase lysate reagents were prepared by a method similar to one that was previously used for the production of antigen from *Histoplasma capsulatum* [21-23] and modified in our laboratory for *B. dermatitidis* lysate antigen production [13]. 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, 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 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 protein concentration.

Serum specimens

Six serum specimens from dogs with diagnosed blastomycosis were provided by Dr. A.M. Legendre (University of Tennessee College of Veterinary Medicine, Knoxville, TN). In addition 6 serum specimens from rabbits immunized with various yeast lysate antigens prepared from *B. dermatitidis* were also assayed to determine and compare the efficacy of the lysates as immunodiagnostic reagents.

Enzyme-linked immunosorbent assay (ELISA)

The ability of each of the 28 yeast lysate reagents to detect antibodies in the above serum specimens was determined using the indirect enzyme-linked immunosorbent assay (ELISA). 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 (Thermo-Fisher). 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 (1:2500 dilution; 100 ul) were added to the microplate wells 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-species (either dog or rabbit) IgG (H & L) peroxidase conjugate (Kirkegaard and Perry) was added to each well and incubated for 30 min at 37 C. The plates were again washed as above and 100 ul of Ultra TMB peroxidase substrate (Pierce/ThermoFisher) was be added to each well and incubated for approximately 2 min at room temperature. The reaction will be stopped by the addition of sulfuric acid and the absorbance read at 450 nm using a BIO-RAD 2550 EIA reader.

Results

The mean absorbance values of the 28 *B. dermatitidis* lysate antigens, when evaluated using the ELISA method to detect the presence of antibodies for Dog and Rabbit sera are shown in Figure 1.When evaluating the dog and rabbit sera individually, lysates 48938, 56920, 86, 394, Canada soil, 397, 85, 248, ER-3, 449, 103, ERC-2, 42913, 02, 643, 598, 48089, B5927, B5929, B5931 showed greater absorbance values with the rabbit serum compared to the dog serum. Lysates 81, 104, T-27, T-58, 42847, 97, B5926, B5934 seemed to have a higher absorbance value when tested against the dog serum than rabbit serum.

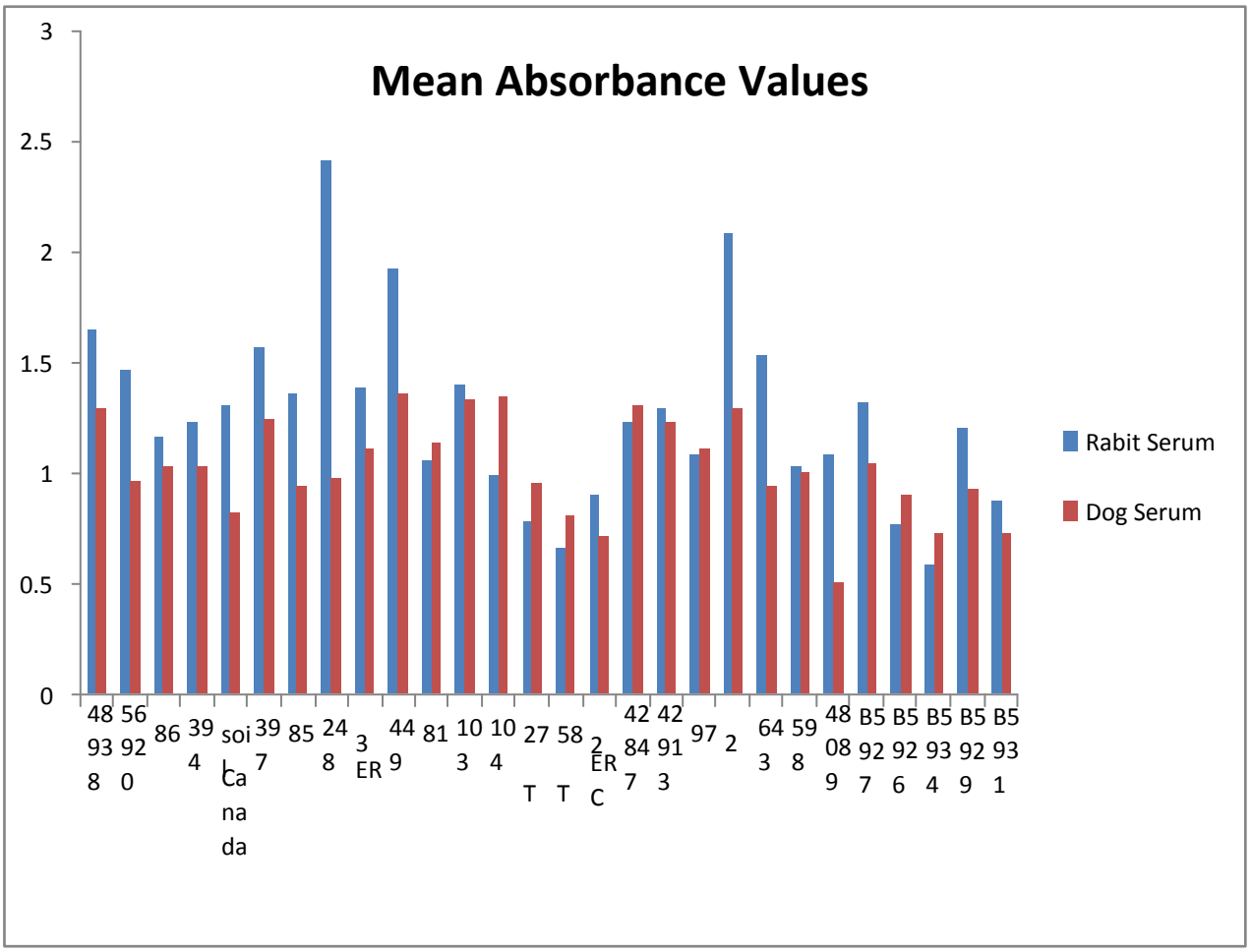

Figure 1: Overall mean absorbance values obtained from 28 yeast lysate antigens using ELISA to detect antibodies in dog and rabbit Serum.

When tested on the human antigen (B5934) the combined absorbance values between rabbit and dog sera was 0.655, the lowest absorbance value observed (Figure 2). Soil antigen (248) exhibited the highest combined absorbance value of 1.689. The overall mean absorbance value of all 28 lysates for both dog and rabbit serum was 1.138.

Out of the two bat lysate samples 48938 showed a higher mean absorbance value of 1.464 than lysate 56920 absorbance value 1.211. The highest of the seven soil samples was lysate 248 with absorbance value of 1.689, also the overall highest absorbance value within the 28 lysates. The lowest of the soil samples was lysate Canada soil sample at 1.058. Both sea lion lysates tested high with 449 (1.636) being higher than 81 (1.176).

Of the two cat lysates, 103 had a higher absorbance value than 104 with 1.361 over 1.164. There was only one polar bear lysate (T27) with its absorbance value being 0.863. Six dog antigens were tested with lysate 02 with a mean absorbance value of 1.682. The low was dog antigen ERC-2 with an absorbance value of 0.803. Eight human antigens were examined with lysate 643, with mean absorbance of 1.236 being the high. The low, which was the overall lowest was lysate B5934 with a mean absorbance of 0.655.

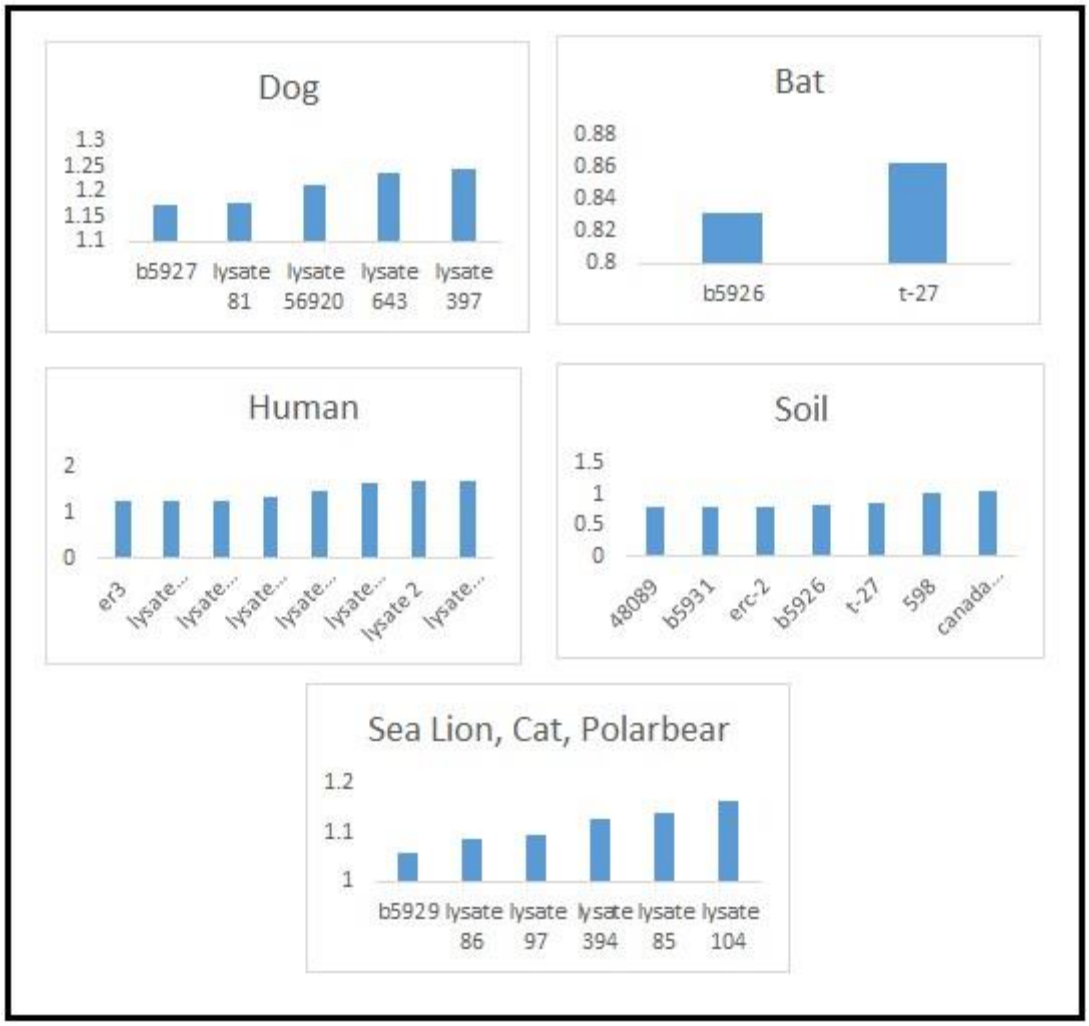

Figure 2: Mean absorbance values of lysates grouped by their donor organism. Due to the limited number of samples from the sea lion, cat and polar bear, the absorbance values were combined on one graph.

Discussion/Conclusion

When developing antigenic reagents for the immunodiagnosis of blastomycosis, it is desirable that the antigens are able to detect antibody in a variety of organisms from a wide range of geographical regions. For the past several years our lab has been focused on evaluating and developing *B. dermatitidis* yeast lysates. These lysates are prepared from isolates of the fungus taken from human, animal, and environmental sources. The use of such lysate antigens can help in the detection of antibodies being produced from the serum samples (most commonly dog or rabbit).

Recent studies have used lysate antigens from dogs, humans, and other sources and compared them using dog sera, but recently we have found an interest in comparing lysate antigens from multiple sources and comparing them with dog and rabbit sera. The study was designed to test 28 lysates prepared from various sources to detect antibody in both dog sera and rabbit sera. All 28 reagents were able to detect antibody in both the dog and rabbit sera. The most reactive agents were found to be in combination with the rabbit sera.

The results from the trials indicated that antigens prepared from *B. dermatitidis* isolates were overall more efficient as immunodiagnostic reagents in the sera from the rabbits. The most efficient immunodiagnostic reagent for detection of antibody in the rabbits sera was soil sample
248 with an absorbance value of 2.404. The least efficient immunodiagnostic reagent was human lysate B5934. The most efficient immunodiagnostic reagent for detecting antibody in the sera for dogs was sea lion lysate 449 with an absorbance value of 1.352. The least efficient immunodiagnostic reagent was human lysate 48089. This study provides the need for further investigation associated with the most reactive lysates for both dog and rabbit sera in order to develop a future test that will be simple and accurate for the detection of blastomycosis in animals and humans.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] 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.

[2] 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

[3] B.S. Klein, J.M.Vergeront, R.J. Weeks, Kumar, U.N., Mathai, G., Varkey, B., Kaufman, L., Bradsher, R.W., Stoebig, J.R., and Davis, J.P., "Isolation of Blastomyces dermatitidis in soil associated with a larger outbreak of blastomycosis in Wisconsin," Journal of Infectious Diseases, Vol. 155, pp. 262-268, 1986.

[4] R.W., Bradsher, S.W. Chapman and P.G. Pappas. 2003. "Blastomycosis." Infectious Disease Clinics of North America, Vol.17, pp. 21-40, 2003.

[5] J.R. Bariola and K.S. Vyas, "Pulmonary Blastomycosis," Seminars in Respiratory Critical Care Medicine, Vo. 32, No. 6, pp. 745-753, 2011.

[6] M. Saccente and G.L. Woods, "Clinical and Laboratory Update on Blastomycosis," Clinical Microbiology Reviews, Vol. 23, No. 2, pp. 367-381, 2010.

[7] J.A. McKinnell and P.G. Pappas, "Blastomycosis: New Insights into Diagnosis, Prevention, and Treatment," Clinical Chest Medicine, Vol. 30, pp. 227-239, 2009.

[8] J.A. Smith and C.A. Kauffman, "Pulmonary Fungal Infections,"Respirology, Vol. 17, pp. 913-926, 2012.

[9] J.A. Smith and C.A. Kauffman, "Blastomycosis," Proceedings of the American Thoracic Society, Vol. 7, No. 3, pp. 173-180, 2010.

[10] 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.

[11] 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.

[12] 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.

[13] S.M. Johnson and G.M. Scalarone, "Preparation and ELISA Evaluation ofBlastomyces dermatitidis Yeast Phase Lysate Antigens," Diagostic Microbiology and Infectious Diseases, Vol. 11, pp. 81-86, 1989.

[14] C.M. Sestero and G.M. Scalarone, "Detection of IgG and IgM in Sera from Canines with Blastomycosis Using Eight Blastomyces dermatitidis Yeast Phase Lysate Antigens," Mycopathologia, Vol. 162, pp. 33-37, 2006.

[15] W.O. Hatch and G.M. Scalarone, "Comparison of Colorimetric and Chemiluminescent ELISAs for the Detection of Antibodies to Blastomyces dermatitidis," Journal of Medical and Biological Sciences, Vol. 3, No 1, pp. 1-6, 2009.

[16] W. Christenson, R. Horton, K. Campbell, K. Meacham, A. Schroeder and G.M. Scalarone, "Comparison of antibody detection with Blastomyces dermatitidis yeast lysate antigens in serum specimens from immunized rabbits and infected dogs," Open Journal of Immunology, vol. 1, pp. 74-79, 2011.

[17] J.L. VanDyke, A.R. Boyd, J. Sorensen, T. Hine, C. Rayner, A. Zamora and G.M. Scalarone, "Detection of Antibodies in Serum Specimens from Dogs with Blastomycosis with Lysate Antigens Prepared from Four Blastomyces dermatitidis Dog Isolates: Individual Antigens VS Antigen Combinations," Open Journal of Veterinary Medicine, Vol.3, pp. 237-241, 2013.

[18] 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.

[19] J.C. Wright, T.E. Harrild and G.M. Scalarone, "The Use of Isoelectric Focusing Fractions of Blastomyces dermatitidis for Antibody Detection in Serum Specimens from Rabbits Immunized with Yeast Lysate Antigens,"Open Journal of Veterinary Medicine, Vol. 2, pp. 237-241, 2012.

[20] Alex R. Boyd, Jamie L. VanDyke and Gene M. Scalarone 2013. Blastomyces dermatitidis yeast lysate antigen combinations: Antibody detection in dogs with blastomycosis. Veterinary Medicine International 2013: ID 940126, 4 pages. http://dx.doi.org/10.1155/2013/940126.

[21] 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.

[22] 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.

[23] 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