EXPRESSION OF *BACILLUS ANTHRACIS* PROTECTIVE ANTIGEN IN VACCINE STRAIN *BRUCELLA ABORTUS* RB51

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Thesis submitted to the Faculty of the Virginia

Polytechnic Institute & State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Veterinary Medical Sciences

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Submitted April, 2000
Blacksburg, VA

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(ABSTRACT)

Bacillus anthracis is a facultative intracellular bacterial pathogen that can cause cutaneous, gastrointestinal or respiratory disease in many vertebrates, including humans. Commercially available anthrax vaccines for immunization of humans are of limited duration and do not protect against the respiratory form of the disease. *Brucella abortus* is a facultative intracellular bacterium that causes chronic infection in animals and humans. As with other intracellular pathogens, cell mediated immune responses (CMI) are crucial in affording protection against brucellosis. *B. abortus* strain RB51 has been shown to be useful in eliciting protective cell mediated immunity and humoral responses against *Brucella* in cattle and other animal species. Since the protective antigen (PA) of *B. anthracis* is known to induce protective antibodies, it was decided that the objective of this research was to test whether the gene encoding PA could be expressed in *Brucella* producing a bivalent vaccine to protect against both brucellosis and anthrax. The *pag* gene was transcriptionally fused to promoters of genes encoding superoxide dismutase or heat shock protein groE, subcloned into a broad host range plasmid (pBBR1MCS) and shown to express in *E. coli* by immunoblotting using antiserum specific for PA. The immunoblot results revealed that *E. coli* produced a PA protein of the expected size. In

addition, the culture medium was shown to contain the same PA protein using immunoblotting. These results show that *E. coli* is capable of expressing *B. anthracis* PA in both the cellular and extracellular forms. The pBB/PA plasmid was used to transform *B. abortus* RB51 and CmR clones screened for the expression of PA by immunoblotting. Twenty clones of strain RB51/pBBSOD were show to express a 30kDa PA protein. Three clones of strain RB51/pBBGroE-PA were shown to express a 63-83kDa protein as detected by antiserum specific for PA. Using the A/J mouse, an immunocompromised vertebrate model, immunization and challenge studies were performed. Preliminary results demonstrate that the bivalent vaccine is capable of producing protection against a live challenge with *B. abortus* and some protection against live non-disease producing spores of *B. anthracis*.

This thesis work is dedicated to

my wonderful parents

Ricky & Lesley Poff

who have stood by my side through every

up and down.

ACKNOWLEGEMENTS

First of all I would like to thank my wonderful advisor, Dr. Stephen Boyle, for always being there for me. You have taught a shy young woman how to find confidence in herself and her abilities. I have grown so much under your leadership. Thank you for listening to my ideas and thoughts and finding ways to tell your stubborn student that, while it is an interesting idea, there is an easier and more practical approach to solving the problem. You have taught me patience, critical thinking skills, and how to look and life and laugh and I will never forget these lessons. I would like to thank Dr. Nammalwar Sriranganathan for his willingness to listen and give advice whether it covered my research or my life. You have also taught me how to laugh at myself and see the good in all situations. I would like to thank Dr. Gerhardt Schurig for his help in designing my experiments and for his patience. Your reassurance in times when I thought my research was falling apart helped more than you could know.

I would like to thank Julie Bard and Dr. Amin Ahmadzadeh for always being there to bounce ideas and theories around. I would like to especially thank Julie for teaching me so many valuable techniques. I would like to thank Dr. Ramesh Vemulapalli for helping me develop my experiments and aiding me with suggestions when I was at a loss as to what to do next.

Thanks to my wonderful labmates, Simge, Nickole, Shella, Virginia, and Michelle, who are more like my siblings. When life gets hard I know I can always turn to you for support, lunch and a good laugh. I also thank Lee Weigt for being such a great guy to work for. Those long, busy hours in the sequencing lab are not as hard with you there.

Thanks to Dr. Harriet Gray for all of the lessons you taught me in my studies as a Hollins undergraduate. Thanks to Dr. Patrick McCarthy, Dr. Bill Whitehurst, and Dr. Debra Clapp for being interested enough in my life to keep tabs on my success.

I would like to thank Dr. Teresa Koehler for supplying the *pag* gene, and Dr. Stephen Leppla for supplying anti-PA sera for my experiments.

Lastly I would like to thank my family for their love and support. Thanks to my cousin Kelley I could always count on a good joke or story to brighten my day. My love and thanks goes to my Grandma and my Nanna whose pride in me inspires me to go on trying. Thanks to my wonderful sister and brother-in-law, Amanda and Buddy, for letting me come to their house for vacations when I needed to get away from it all. I love those soccer games. Most of all I would like to thank my parents for everything they have done and will continue to do for me. Momma, you are my inspiration and my favorite role model. You have no idea how very proud I am of you and your accomplishments. I know that if I work even half as hard as you I will succeed in achieving my dreams. Daddy, you are the only man in my life right now and you hold a place of great respect and love. I can always call on you to help with anything and spending time with you is one of my greatest joys. Thank you both for always pushing me to go for my goals and not to settle. You will never know how much I love and adore you both.

This thesis research was supported by a grant from the United States Department of Agriculture. Financial support for living expenses was provided by a stipend from the Virginia-Maryland Regional College of Veterinary Medicine.

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Amp- ampicillin

AVA- anthrax adsorbed vaccine

BSA- bovine serum albumin

CAMP-

cfu- colony forming unit

Cm- chloramphenicol

CMI- cell-mediated immunity

EF- edema factor

ELISA- enzyme-linked immunosorbent assay

GroE- heat shock protein

HPLC- high profile liquid chromatography

IP- intraperitoneal

kb- kilobase pair

kDa- kiloDalton

LB- Luria-Bertani media

LF- lethal factor

LPS- lipopolysaccharide

MDPH- Michigan Department of Public Health

mL- milliliter

OMP- outer membrane protein

ORF- open reading frame

PA- protective antigen

pag- protective antigen gene

PCR- polymerase chain reaction

R- resistant

RBS- ribosomal binding site

SDS-PAGE- sodium dodecylsulfate polyacrylamide gel electrophoresis

SOD- superoxide dismutase

TSB- trypticase soy broth

U- unit

ul- microliter

USDA- United States Department of Agriculture