Idaho Incubation Fund Program

Final Report Form

Proposal No.	**IF13-006**
Name:	**Juliette Tinker**
Name of Institution:	**Boise State University**
Project Title:	**Characterization of Antigens for a Staphylococcal Bovine Mastitis Vaccine**

Information to be reported in your final report is as follows:

1. Provide a summary of overall project accomplishments to include goals/milestones met, any barriers encountered, and how the barriers were overcome:

During the project period we proposed an extensive characterization of the expression and upregulation of the IsdA surface protein from *Staphylococcus aureus* during clinical and sub-clinical bovine mastitis to better understand the role of this potential vaccine antigen in disease. In addition, we proposed to characterize the expression and immune response to additional *S. aureus* candidate antigens, such as ClfA, FnbpA and IsdH, for incorporation into a multi-subunit bovine mastitis vaccine. Specific goals included 1) to characterize the immune response to IsdA and the expression of *isd*A in healthy cows and cows with clinical and subclinical *S. aureus* mastitis and 2) to assay the immune response to ClfA, IsdH and FnBPA, and gene expression, in healthy cows and cows with clinical and subclinical *S. aureus* mastitis. These goals included a proposed timeline. Table 1 represents the specific goals and those accomplished during the project period. Specific goals that were met during the project period include the sampling and clinical characterization of over 100 cows from both the University of Idaho dairy and a Boise commercial dairy. Samples of milk and serum from cows that were identified as healthy, subclinically infected and clinically infected were taken and characterized for somatic cell count (SCC) and growth of presumptive *Staphylococcus aureus* on manitol salt agar (MSA) (Tables 2 and 3). Samples from the University of Idaho dairy have been further characterized by ELISA assay for IsdA-specific IgA and IgG responses (Figures 1-3), and by PCR for amplification and sequencing of *isd*A, as well as *clf*A, *mec*A and *isd*B surface antigens (Table 2). Commercial sequencing of these antigens from the University of Idaho dairy has been completed and data analysis is underway. Samples from the Boise commercial dairy were received at the end of the project period and further characterization; that will include ELISA analysis for

IsdA-specific responses and PCR analysis, will be completed in the fall of 2013.

Goals that were not met within the proposed time period include the quantitative PCR analysis of *isd*A, *clf*A, *isd*B, and *fnb*P expression (Aims 1 and 2) and ClfA ELISA assays (Aim 2). ELISA assays using the IsdA antigen were completed (Aim 1), however production and expression of additional antigens has been slowed by cloning difficulties. At the end of the project period we successfully cloned and sequenced the *clf*A antigen, and will perform expression studies such that this antigen can be produced for use in ELISAs. Quantitative PCR on milk samples was also not completed due to technical difficulties, however, primers for all proposed antigens have been obtained and tested through the construction of standard curves. All bovine milk samples have been aliquoted and stored in RNA isolation buffer for later RNA extraction to complete these assays. Lastly, proposed opsonization assays were not completed. While these assays will likely enhance current expression data, and may be completed at a later date, they are deemed not necessary for preparation of results and publication.

Current findings indicate that a significant number of subclinically and clinically infected cows from Idaho dairies are infected with *Staphylococcus aureus* or a closely related Staphylococcal species. In addition, a significant number of these isolates contain the *isd*A antigen. From the University of Idaho dairy, 12 out of 50 (24%) cows contained *S. aureus*, as determined by culture, and 10 of these 12 contained *isd*A as determined by PCR (Table 2). From the Boise commercial dairy, 16 of 53 (30%) cows contained *S. aureus*, as determined by culture. Further characterization of *isd*A from the Boise herd is underway. Cows that were subclinically infected also induced a robust humoral response in milk against the IsdA antigen. IsdA-specific IgA, and especially IgG, was significantly higher in animals with subclinical *S. aureus* infections than in healthy cows from the University of Idaho dairy (Figures 1 and 2). IsdA specific serum IgG was not significantly higher in subclinically infected animals, indicating that systemic responses were limited or not induced during this infection (Figure 3). While further analysis of the *clf*A, *isd*B, *fnb*P and *mec*A antigens is underway, current results indicate that the presence of *isd*A is conserved among bovine *S. aureus* isolates and that this antigen is expressed and inducing immune responses during subclinical infection of udder. These results support the use of IsdA as a central protective antigen for use in a vaccine against *S. aureus* bovine mastitis.

Table 1. Timeline and specific goals

Goal (Aim)	Month					
	1/2	3/4	5/6	7/8	9/10	11/12
Identification and sampling of cows (1, 2)	X	X	X	X		
Cloning and purification of antigens (1)	X	X				
Cloning and purification of antigens (2)		X	X	X		
ELISA (1)			X	X	X	X
qRT-PCR (1)					X	
ELISA (2)						
qRT-PCR (2)					X	
DNA sequencing (1)				X	X	X
DNA sequencing (2)				X	X	X
Opsonization assays (1,2)						
Data analysis (1,2)					X	X

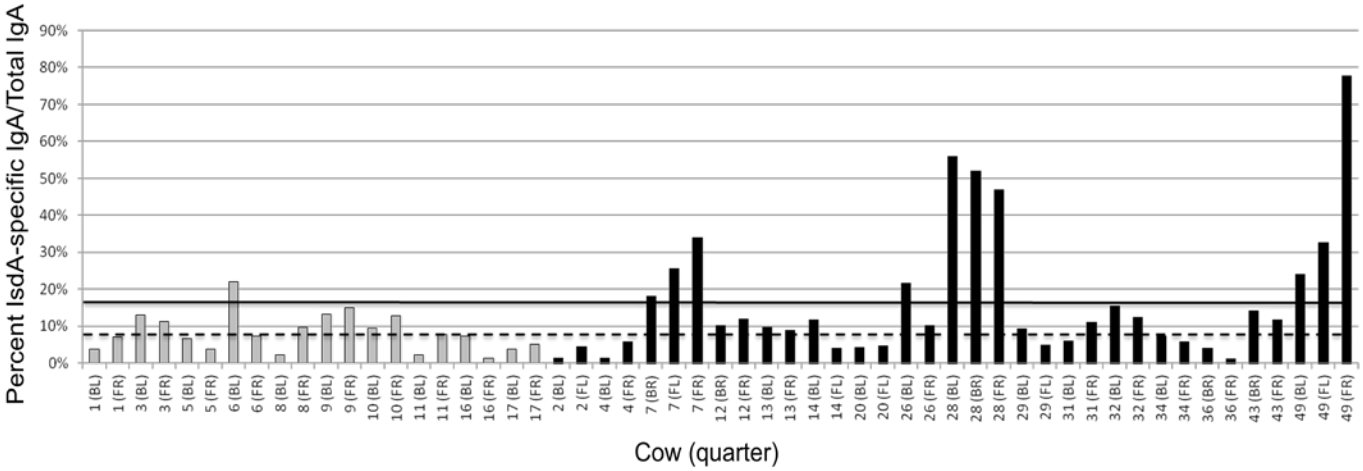

Figure 1. Milk IsdA-specific IgA as a percent of total IgA as determined by ELISA at a 1:8 dilution. Solid bars are cows identified as subclinically infected by culture positive on mannitol salt agar (MSA) and/or high somatic cell count (SCC), and grey bars are cows identified as clinically healthy due to low SCC and absence of culture evidence of *S. aureus*. Solid line represents the average percent IsdA-specific IgA for subclinical cows (17% ± 3% SEM) and dashed line represents the average percent IsdA-specific IgA for healthy cows (8% ± 1.2% SEM).

Table 2. Bovine samples from Univ. Idaho dairy

Cow	Determination U of I	Confirmation MSA	Confirmation MP2	IsdA PCR	IsdB PCR	ClfA PCR	MecA PCR
1	H						
2	S	Y	N	+	+	+	+
3	H						
4	S	Y	N	+	-	-	-
5	H						
6	H						
7	S	Y	Y	+	+	-	+
8	H						
9	H						
10	H						
11	H						
12	S	N	NC	NC	NC	NC	NC
13	S	Y	N	+	+	+	+
14	S	Y	Y	-	-	-	-
15	S*						
16	H						
17	H						
18	S*						
19	H						
20	S	Y	Y	+	+	+	-
21	H						
22	S*						
23	H						
24	S*						
25	H						
26	C*						
27	S*						
28	S	Y	NC	NC	NC	NC	NC
29	S	Y	Y	+	+	-	+
30	S*						
31	S	N	NC	NC	NC	NC	NC
32	S	N	Y	+	+	-	-
33	C*						
34	S	N	NC	NC	NC	NC	NC
35	C*						
36	S	Y	Y	+	-	+	-
37	S*						
38	C*						
39	H						
40	H						
41	C*						
42	H						
43	S	Y	Y	+	+	+	-
44	H						
45	C*						
46	H						
47	S*						
48	H						
49	S	Y	Y	+	+	-	+
50	H						

* not *S. aureus*
NC = not completed

H = healthy, S = subclinical, C= clinical

Table 3. Bovine samples from Boise dairy

Cow	Determination U of I	Confirmation MSA
1	H	
2	H	
3	H	
4	H	
5	S*	N
6	H	
7	S	Y
8	S	Y
9	S*	N
10	H	
11	S	Y
12	H	
13	S*	N
14	H	
15	H	
16	H	
17	C*	N
18	S*	N
19	S	N
20	S	Y
21	H	
22	H	
23	H	
24	S	Y
25	C	Y
26	H	
27	H	
28	S	Y
29	H	
30	C*	N
31	C*	N
32	C*	N
33	S	Y
34	C	Y
35	C*	N
36	C*	N
37	C	Y
38	C	Y
39	C	Y
40	C*	N
41	S	Y
42	H	
43	S	Y
44	C	Y
45	S*	N
46	H	
47	C	Y
48	S*	N
49	S*	N
50	S*	N
51	C*	N
52	S*	N
53	C*	N

* not *S. aureus*
NC = not completed

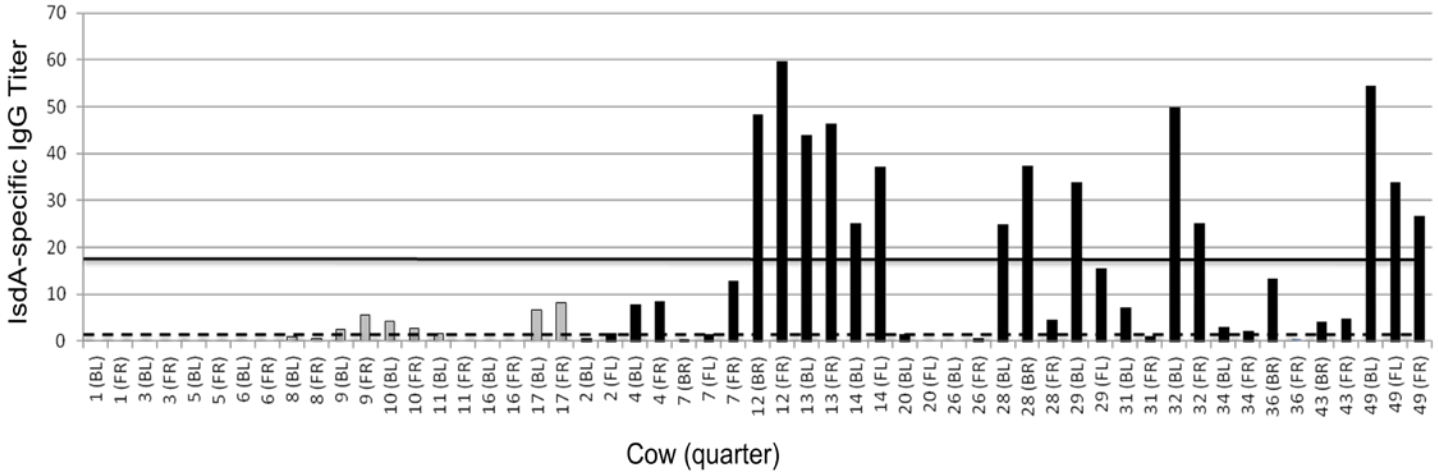

Figure 2. Milk IsdA-specific IgG titer as determined by ELISA. Solid bars are cows identified as subclinically infected by culture positive on mannitol salt agar (MSA) and/or high somatic cell count (SCC), and grey bars are cows identified as clinically healthy due to low SCC and absence of culture evidence of *S. aureus*. Solid line represents the average IsdA-specific IgG titer for subclinical cows (18.3 ± 3.2 SEM) and dashed line represents the average IsdA-specific IgG titer for healthy cows (1.7 ± 0.6 SEM). Endpoint titers were defined as the reciprocal of the last sample dilution giving an absorbance of 0.2 *A*370 units above background.

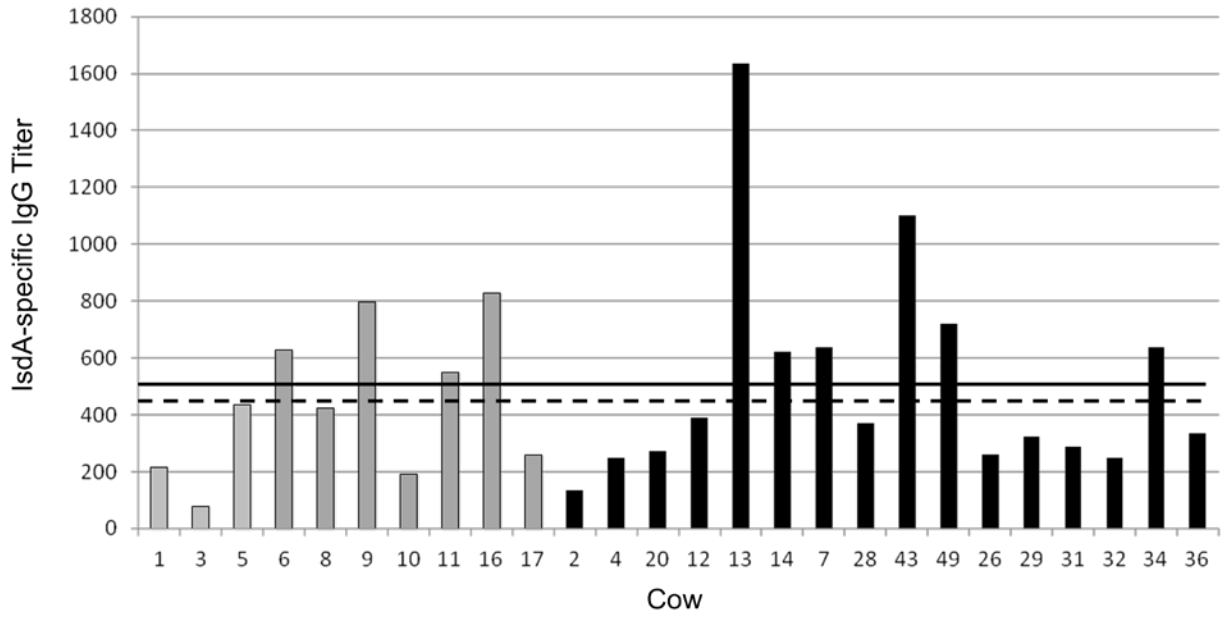

Figure 3. Serum IsdA-specific IgG titer as determined by ELISA. Solid bars are cows identified as subclinically infected by culture positive on mannitol salt agar (MSA) and/or high somatic cell count (SCC), and grey bars are cows identified as clinically healthy due to low SCC and absence of culture evidence of *S. aureus*. Solid line represents the average IsdA-specific IgG serum titer for subclinical cows (514.57 ± 97 SEM) and dashed line represents the average IsdA-specific IgG titer for healthy cows (440.2 ± 82 SEM). Endpoint titers were defined as the reciprocal of the last sample dilution giving an absorbance of 0.2 *A*370 units above background.

2. Describe the current state of the technology and related product/service:

"Cholera toxin chimera and its use as a staph vaccine". U.S. Patent pending. Submitted 12/16/11. Application # 13328686 (Amendment submitted 5-17-13).

A USDA AFRI Foundational Grant (# 2013-01189- Novel vaccines to prevent bovine mastitis caused by *Staphylococcus aureus* and *Streptococcus uberis* $500,000/ 5yrs) was recommended for funding in July of 2013, start date of Dec. 1, 2013. This grant will provide resources to perform additional immunological testing in a large sample herd as well as a small contained Staphylococcal challenge study. Resources will also be used to promote pre-licensure approval of this investigational new animal drug.

3. List the number of faculty and student participants as a result of funding:

2 faculty (Dr. Tinker and Dr. McGuire), 1 post-doctoral fellow (Katherine Hunt, Univ. of Idaho), 1 Master's graduate student (Tyler Wines, Boise State) and 6 undergraduate students (Univ. of Idaho and Boise State) were involved in bovine sampling and characterization. Shandra Jeffries (B.S. '13 Boise State) was specifically very involved in IsdA expression, *clf*A cloning and antigen-specific ELISA analysis.

4. What are the potential economic benefits:

According to a study in 2008 from Boise State's College of Business and Economics, Idaho is ranked third behind California and Wisconsin for the most milk and cheese produced in the nation. In 2008, Idaho dairy farms produced over 12 million pounds of milk at an estimated $2.1 billion. This industry also directly employed an estimated 13,180 people, and indirectly another 18,357 people. An effective *S. aureus* vaccine to prevent mastitis will have a significant impact on the dairy industry. In addition, the ease of administration and reduction of cost as a result of novel delivery of this proposed vaccine will promote its use over other potential mastitis vaccine candidates. Due to multiple agents of bovine mastitis as well as

varying clinical and subclinical outcomes of disease, it is notoriously difficult to estimate industry losses as a result of *S. aureus* infection. However, a report from the National Mastitis Council in 1996 estimated the economic loss from bovine mastitis to be $184 per cow, and $1.7 billion annually in the U.S. These calculations include: reduced and lost milk production, replacement costs, extra labor, treatment and veterinary services for infected cows. The cost and limited efficacy of treatment has resulted in increased demand for preventative measures within the dairy industry, and this demand has coincided with an overall global demand for more veterinary vaccines to promote agriculture. According to a 2012 GBI Research report, the global market for veterinary vaccines has grown steadily at a rate of 1% annually since 2002. The largest market within this sector is the bovine vaccines market, which is estimated to be $1.6 billion in the U.S.

5. Description future plans for project continuation or expansion:

In the immediate future, samples from the Boise dairy will be characterized by PCR, sequencing, qPCR and ELISA. Data will be analyzed and prepared for publication (Tyler Wines, MA candidate, Boise State).

USDA funding (# 2013-01189, above) will provide essential resources to continue to obtain pilot data for this potential vaccine over the next five years. Pilot data will be used to continue to promote FDA licensure and marketing. The USDA grant was made possible due to the collaborations and resources maintained from this Idaho SBOE HERC funding. Additional collaborations with industry will continue to be explored through consultation (Dr. Brian Mitchell, Dairy TEAM) and collaboration (Dr. Mark McGuire, Univ. of Idaho).

6. Please provide a final expenditure report (attached) and include any comments here:

7. List invention disclosures, patent, copyright and PVP applications filed, technology licenses/options signed, start-up businesses created, and industry

involvement:

“Cholera toxin chimera and its use as a staph vaccine”. U.S. Patent pending. Submitted 12/16/11. Application # 13328686 (Amendment submitted 5-17-13).

Technology Brokerage Agreement for collaborator/consultant during project period: Dr. Brian Mitchell, DairyTeam Nutrition and Veterinary Consulting, Eagle, ID. Dr. Mitchell has expertise in veterinary vaccines and numerous ties to the dairy industry in Idaho. Dr. Mitchell is developing connections to national vaccine companies.

8. Any other pertinent information:

FINAL EXPENDITURE REPORT

A. FACULTY AND STAFF		
Name/Title	$ Amount Requested	Actual $ Spent
Juliette K. Tinker	**6002.00**	**6002.00**
B. VISITING PROFESSORS		
Name/Title	$ Amount Requested	Actual $ Spent
C. POST DOCTORAL ASSOCIATES/OTHER PROFESSIONALS		
Name/Title	$ Amount Requested	Actual $ Spent
D. GRADUATE/UNDERGRADUATE STUDENTS		
Name/Title	$ Amount Requested	Actual $ Spent
Shandra Jeffries	**5500.00**	**5500.00**
Emily Price	**2500.00**	**2500.00**
E. FRINGE BENEFITS		
Rate of Fringe (%)	$ Amount Requested	Actual $ Spent
Juliette Tinker (35%)	**2100.70**	**2100.70**
Shandra Jeffries/ Emily Price (9%)	**560.3**	**560.3**
PERSONNEL SUBTOTAL:	**16663.00**	**16663.00**
F. EQUIPMENT: (List each item with a cost in excess of $1000)		
Item/Description	$ Amount Requested	Actual $ Spent
1. **Pipettmen – Fisher Scientific**	**2000.00**	**1042.08**
2.		
3.		
4.		
EQUIPMENT SUBTOTAL:	**2000.00**	**1042.08**
G. TRAVEL		
Description	$ Amount Requested	Actual $ Spent
1.		
2.		
3		
TRAVEL SUBTOTAL:		

H. PARTICIPANT SUPPORT COSTS:		
Description	$ Amount Requested	Actual $ Spent
1.**University of Idaho Subcontract**	**20,000.00**	**19,709.00**
2.		
3		
PARTICIPANT SUPPORT COSTS SUBTOTAL:	**20,000.00**	**19,709.00**

I. OTHER DIRECT COSTS:			
Description		$ Amount Requested	Actual $ Spent
1.**Materials and Supplies**		**10,837.00**	**11,044.93**
2.**Publication costs**		**500.00**	**0.00**
3.			
OTHER DIRECT COSTS SUBTOTAL:		**11337.00**	**11.044.93**
TOTAL COSTS (Add Subtotals):		**50,000.00**	**48459.01**
TOTAL AMOUNT REQUESTED:			**50,000.00**
TOTAL AMOUNT SPENT:			**48,459.01**