Idaho Incubation Fund Program

Progress 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 progress report is as follows:

1. Provide a summary of project goals/milestones for the period just completed, accomplishments for the period just completed, and plans and goals for the coming quarter:

   We have previously constructed and purified chimeric *S. aureus* proteins as potential vaccines against bovine mastitis in dairy cattle. Preliminary data indicates that the vaccine containing the *S. aureus* IsdA antigen is immunogenic when delivered intranasally. The proposed project will permit a better understanding of the role of IsdA and additional vaccine candidates in bovine mastitis. The goals of the proposed project were two-fold: 1) to characterize the immune response to the IsdA antigen in healthy dairy cows, and cows with subclinical and clinical *S. aureus* mastitis and 2) to characterize the immune response to additional *S. aureus* antigens, including ClfA, IsdH and FnBP.

   During the previous project period Dr. McGuire (co-P.I. University of Idaho) obtained IACUC approval, contacted and arranged for the involvement of a commercial dairy, as well as the University dairy. The IsdA protein was expressed in large quantities in preparation for ELISA assays at Boise State. In addition the ClfA and IsdB antigens were cloned into vectors for the expression of novel chimeric vaccines and ClfA was cloned into a vector for affinity purification.

   For the project period just completed (second four months Jan-April 2013) the proposed timeline includes ELISA and qPCR studies on dairy cow samples for Aim 1, and cloning and purification of antigens for Aim 2. Three University of Idaho students and one Post-doctoral fellow (Katherine Hunt) are, and will continue to be, involved in culturing, sampling and shipping samples to Boise State. During this period, 50 cows from the University of Idaho dairy were identified and categorized as healthy, subclinically infected or clinically infected based upon somatic cell count (SCC) and colony forming unit determination (CFU) (Table 1). Data on CFU, colony color and SCC was recorded for each quarter from all 50 cows. 2 ml of blood and milk samples from each quarter were frozen and shipped to

Boise State for additional culture and storage of *S. aureus* isolates, ELISA, PCR analysis and sequencing. 100 ul of milk samples from at least two quarters of each subclinical cow was plated and confirmed for growth on MSA and MP2 agar. From these samples, a total of 20 presumptive *S. aureus* isolates from 12 cows were stocked for further analysis. Continued analysis of these samples has included characterization of cultures on blood agar, coagulase reactivity and agglutination reaction. DNA was extracted from each *S. aureus* sample and primers for 16S rRNA, *isd*A, *isd*B, *clf*A, and *mec*A have been used in traditional PCR as summarized in Table 1. PCR reactions have been purified and 16S rRNA, as well as isdA PCR will be sequenced. *isd*A, *isd*B and *clf*A encode adhesins and important *S. aureus* virulence factors. The presence of *mec*A is indicative of methicillin-resistant or MRSA isolates, and was identified in 5 of 12 subclinical cows (Table 1). 20 μl of each milk sample was also removed and frozen in RNA buffer for future RNA isolation and qPCR. Milk samples from clinically or subclinically infected cows, as well as a select group of presumptively healthy cows, were analyzed by ELISA assay for IsdA-specific IgA (Figure 1). Standard curves for quantitative PCR using *isd*A, *mec*A and *isd*B specific primers have been constructed using control *S. aureus* genomic DNA. ELISA assays on anti-IsdA IgG in milk and serum are currently underway. Attempts to purify ClfA, IsdH or IsdB alone for ELISA analysis have thus far not been successful, and are ongoing. At Boise State, a master's-level graduate student, Tyler Wines, and two undergraduates, Shandra Jeffries and Emily Price are involved in culturing, ELISA and PCR analysis. Efforts are also underway at the U of I for sampling of another 50 cows from a commercial dairy.

Plans for the upcoming final quarter include the completion of screening, sampling and shipping milk and blood from the commercial dairy, and stocking of *S. aureus* samples at Boise State. Anti-IsdA ELISA analysis on all clinical and subclinical cows will be completed. In addition, genomic isolation and PCR will be completed, and all 16S rRNA and *isd*A PCR samples will be sequenced. qRT-PCR will be performed on the samples from the U of I dairy.

2. Provide a summary of budget expenditures for the period just completed:

Budget Expenditures (The University of Idaho):

	REQUESTED	USED
Total salaries and fringe benefits	**$10,265**	**$1,470**
Total supplies	**$7,360**	**$7,128**
Total other expenses	**$ 1,500**	**$ 46**
Total travel	**$ 875**	**$1,120**
Total direct costs	**$20,000**	**$9,764**

Budget Expenditures (Boise State):

Total salaries and fringe benefits	**$16,663**	**$2005.00**
Total supplies	**$13,337**	**$4553.65**
Total direct costs	**$30,000**	**$6558.65**

3. List patents, copyrights, plant variety protection certificates received or pending:

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)

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

Completed 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.

5. Include funding burn rate:

$2393.83/mth

6. Any other pertinent information:

(see attached Table and Figure)

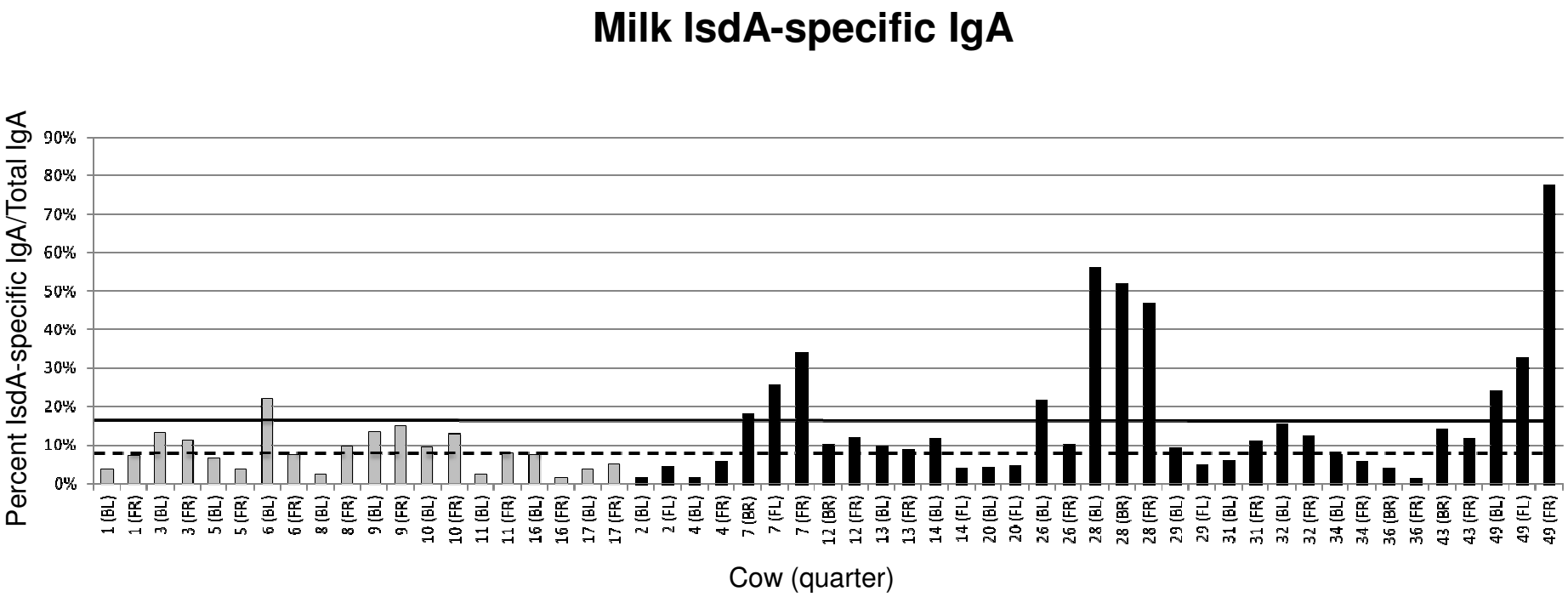

Figure 1. Milk IsdA-specific IgA as a percent of total IgA as determined by ELISA at a 1:8 dilution. Solid bars are cows identified as subclinically infected by culture positive on mannitol salt agar (MSA) and/or high somatic cell count (SCC), and grey bars are cows identified as clinically healthy due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

Table 1. Characterization of *S. aureus* isolates

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