

**Diagnosis of and Protection Against Infectious *Brucella* Aerosols**

DHS Priority Areas Addressed	<input checked="" type="checkbox"/> Prevention <input checked="" type="checkbox"/> Detection <input checked="" type="checkbox"/> Response <input type="checkbox"/> Recovery <input type="checkbox"/> Education/Risk Communication			
Proposal Section Addressed	Sections 5.1.1, 5.2.1, 5.3.1, 5.3.4, and 5.3.5			
Investigators	TAMU: Thomas Ficht, Renee Tsois, and Garry Adams			
Objectives	Deliverables	Progress Toward Deliverables	Percent Complete	
To evaluate molecular approaches for the ability to rapidly identify and distinguish infected animals	Complete <i>Brucella</i> specific host gene expression biomarker analysis in cattle.	Based on preliminary data showing biomarker candidates using the Ciphergen SELDI-TOF apparatus with banked sera from experimentally infected and control animals, experimental animals were bred and exposed to <i>B. abortus</i> . Sera, plasma and lymphocytes were collected and exposure was confirmed using serological tests. Plasma, sera and lymphocytic proteins will be analyzed in collaboration with the GI lab (TAMU), PNNL and UTSW. Different chip surfaces (hydrophobic, anionic, cationic, and metal binding) were evaluated to determine which chemistry provided the best serum profiles in terms of number and resolution. The IMAC-30 chip (metal binding) chip was found to give the best profile for bovine serum. In addition, RNA extracted from the lymphocytes will be analyzed using bovine microarrays. Host invasion of Peyer’s patches by <i>B. melitensis</i> and the presence of <i>Brucella</i> in systemic blood within 30 minutes was confirmed, and the <i>in vivo</i> transcriptional profiles of both <i>B. melitensis</i> and bovine host were characterized at multiple times during the first 4 h post-infection, providing evidence for diagnostic host biosignatures. The <i>virB</i> operon was down-regulated providing evidence that it may not be essential for <i>in vivo B. melitensis</i> invasion. Also pro-apoptotic and anti-inflammatory expression profiles were identified in the host during the first 4 h PI, and genes encoding proteins related with interruption of pregnancy and abortion were differentially expressed. Furthermore, these results demonstrate that <i>Brucella</i> are able to invade the host via intestinal Peyer’s patches and are present in systemic blood by 30 min post-infection. A common set of genes was	70%	

		identified as differentially expressed in <i>Brucella</i> , while two different transcriptional profiles were observed in the bovine host early in the infection.	
	Confirm the VirB12 protein encoded within the T4SS locus as a biomarker of field strain infection	An ELISA based protocol has been developed to utilize the immunogenic protein VirB12 as a diagnostic antigen. Detection of anti-VirB12 antibody has proven to be highly specific in distinguishing exposed cattle, goats and mice. Vaccine candidates identified (products 4 & 5 below) are under development. It is expected that VirB12 may be used as a biomarker for exposure to field strain infection while vaccinated animals will remain negative for VirB12 antibody. Double knockout mutants will be constructed using one of the vaccine strains described below to take advantage of this marker.	100%
	Finalize real-time PCR probes selected by proteomic analysis to distinguish between <i>Brucella</i> species	Proteomic analysis of <i>Brucella</i> is underway at PNNL. It is expected that this will provide markers for infection and detection. Taqman probes have been designed to take advantage of the sequence variation (SNPs) at the <i>omp2</i> (porin) locus. Since there is not a unique probe for each species, a combination of probes (n=4) is required to identify all six species. Additional genetic variation may be necessary when distinguishing field strains. Candidates include genes that are expressed specifically in the infected host. Recent work in another lab points to the use of housekeeping genes in accomplishing this task. However, the sensitivity of this method for direct detection does not appear to be diagnostically useful.	80%
To evaluate vaccine candidates that are safe and efficacious for use against aerosol challenge and identify correlates or protective immunity	Vaccine Candidates - Combine deletion mutations to enhance safety & diagnostic potential of efficacious <i>Brucella</i> vaccine	Deletion of luxR produces a vaccine strain that fails to induce splenomegaly. Perhaps even more important vaccination with this mutant protects against splenomegaly and colonization by virulent challenge. Attenuated mutants carrying combinations of gene mutations are under construction and will be evaluated using the mouse model and will be funded by DOD. Such combinations are expected to enhance safety, although they may limit immune protection due to rapid clearance. Combinations of genetic defects will be based on a $\Delta$ luxR genetic background.	100%
	Evaluate oral microencapsulated brucella vaccine delivery to enhance mucosal immunity	Microencapsulation of highly attenuated mutants was shown to enhance the level of immune protection using IP vaccination and IP and aerosol challenges. Mice have been vaccinated intranasally and are awaiting challenge in studies supported by DOD.	100%
	<i>Brucella</i> Vaccine product develop & technology transition commercialization will continue with NIH support	Application for vaccine product development in nonhuman primates will proceed through NIH U01 mechanism along with microencapsulation of other vaccines (DOD).	100%

## Highlight for Research Briefs

- Vaccine strains for livestock superior to those currently marketed have been produced using specific genetically derived *Brucella* mutants.
- Immune protection against aerosol challenge has also been demonstrated and additional improvements in performance have been achieved through use of delivery in a controlled release format.
- Aerosol challenge reveals differences in the timing of systemic infection and the need to provide improved protection against aerosol infection in the lung via stimulation of mucosal immunity.
- Although characterization of the cytokine response was unrevealing when comparing vaccine strains, an enhanced response was characteristic of encapsulated vaccine.
- Improved diagnostics for the purposes of surveillance are under development using novel biomarkers including the deletion of the *Brucella* virB12 and identification of host responses using SELDI-TOF technology.
- Pathomics Discovery Platform has been implemented for discovery of improved vaccine design, host biosignature based diagnostic platforms, and influencing discovery of chemotherapeutics.

## Interpretive Summary

Improved surveillance of livestock and vaccination of agricultural species against *Brucella abortus* infection is the focus of this study. In the area of surveillance, candidate biomarkers for infection have been identified from retrospective samples using novel protein analysis and Pathomics Discovery Platform technologies, and is currently being validated using an experimental herd infected in Fall of 2006.

In support of vaccine development, a mouse model has been used to demonstrate that protection provided by genetically derived *Brucella* mutants, depending on the genes deleted, can be superior to currently available vaccine strains. Mutants that persist longer provide improved protection while mutants that are cleared more rapidly are not as effective. In order to improve efficacy of highly attenuated vaccine candidates, immunity was enhanced using microencapsulated oral vaccine. Use of microencapsulation is expected to provide controlled release over time, targeted tissue specificity and enhanced mucosal immune stimulation and will be used as cross-cutting platform for other vaccines of importance to biodefense.

Aerosol challenge has been evaluated as a likely source of bioterrorist exposure and lung infection has revealed prolonged persistence of the organism systemically. Oral and intranasal inoculation using microencapsulation is expected to provide improved immune protection against this route of infection.

The Pathomics Discovery Platform approach yields revealing information for cross-cutting applications to biodefense issues.

## Results and Interpretations

1. Significant levels of protection against intraperitoneal and aerosol challenge were demonstrated for candidate vaccines developed via genetic engineering and delivered via injection. Prolonged survival of vaccine strains corresponded with enhanced protection. Promising candidates have been identified and trials in small ruminants confirm protection in multiple hosts.
2. Oral and intranasal routes of vaccination are under investigation. Oral inoculation has proven to provide protection equivalent to parenteral inoculation.
3. Microencapsulation of candidate strains has revealed enhanced efficacy in parenteral and oral inoculations, and is expected to improve delivery by stabilizing and reducing the vaccine dose necessary for protection. Although characterization of the cytokine response was unrevealing when comparing vaccine strains an enhanced response was characteristic of encapsulated vaccine.
4. Candidate biomarkers of infection have been detected in retrospective samples derived from exposed and naïve animals. These will be evaluated in the

experimentally exposed animals described above. These same samples will also be evaluated for additional biomarkers by our collaborators at PNNL using highly sophisticated separation and analysis approaches.

5. Deletion of virB12 has been combined with attenuating mutations to produce a vaccine with enhanced capacity to distinguish infected from vaccinated animals (DIVA).

## Technology Transition

Microencapsulation technology used to enhance aerosol protection through oral and intranasal delivery includes a proprietary additive that further enhances immune stimulation owned by Texas A&M System and licensed by NanoRelease Technologies, College Station, TX.

The concept of using *Brucella* spp. Locus *omp2* as a diagnostic target is patented U.S. Patent No. 5,310,649: Method for Detecting Species and Biovars of *Brucella*. Thomas A. Ficht, Blair A. Sowa and L. Garry Adams. 1994.

Vaccine challenges scheduled in the final year were designed to evaluate the safety and efficacy of double mutants and those lacking virB12 as a potential diagnostic marker.

## Status of Funding

Funding is frozen pending CDC report concerning a failure to report a human exposure during aerosol experimentation. Experiments in progress have been suspended, although vaccinated animals are still maintained by CMP (LARR).

## Publications

- Arenas-Gamboa, A.M., T.A. Ficht, M.M. Kahl-McDonagh, G. Gomez, A.C. Rice-Ficht. Deletion of *luxR* enhances Safety in S19 and Confers Protection against Wild-type Challenge in BALB/c Mice when Delivered in a Sustained Release Vehicle. Manuscript in preparation.
- Arenas-Gamboa, A.M., T.A. Ficht, M.M. Kahl-McDonagh, A.C. Rice-Ficht. Immunization with a Single Dose of a Microencapsulated *Brucella melitensis* Mutant Enhances Protection Against Wild-type Challenge. Manuscript submitted to *Infect. Immun.*, 2007.
- Kahl-McDonagh, M.M., A. M. Arenas-Gamboa, and T.A. Ficht. Aerosol Infection of BALB/c Mice with *Brucella melitensis* and *Brucella abortus* and Protective Efficacy Against Aerosol Challenge. *Infect. Immun.* Accepted with revision.
- Kahl, M., Tsohis, R. M., and Ficht, T.A. 2006. Evaluation of Protection Afforded by *Brucella abortus* and *Brucella melitensis* unmarked deletion mutants exhibiting different rates of clearance in BALB/c mice. *Infect. Immun.* 74:4048.
- Kahl, M, Davis, D.S., Elzer, P.H., Hagius, S., Adams, L.G., and Ficht, T.A. 2006. A Novel Unmarked Deletion Mutant of *B. melitensis* does not Colonize Fetal Tissues and Affords Significant Protection Against Infection in the Pregnant Goat Model. *Vaccine.* 24:5169.
- den Hartigh, A. B., Y. H. Sun, D. Sondervan, N. Heuvelmans, M. O. Reinders, T. A. Ficht, and R. M. Tsohis. 2004. Differential requirements for VirB1 and VirB2 during *Brucella abortus* infection. *Infect Immun* 72:5143-9.
- Rossetti, C. A., Drake, K. L., Galindo, C. L., Ficht, T. A., Garner, H. R. and Adams, L. G. Host and *Brucella melitensis* Temporal Gene Expression Profiles in an *in vitro* Model of Infection. Manuscript in preparation.
- Rossetti, C. A., Galindo, C. L., Ficht, T. A., Johnston, S. A., Garner, H. R. and Adams, L. G. Identification of *Brucella melitensis* Invasive Candidate Genes in Non-Professional Phagocytic Cells by Microarray Analysis. Manuscript submitted.
- Rossetti, C. A., Galindo, C. L., Drake, K. L., Ficht, T. A., Johnston, S. A., Garner, H. R. and Adams, L. G. Temporal Global Gene Expression Analysis of the *in*

*vivo* Initial Interactions of both *Brucella melitensis* and the Bovine Host. Manuscript in preparation.