Quarterly Progress Report for the Period 7/1/00 to 10/30/00

Project Title: Multiple-Locus VNTR Analysis (MLVA) for Bacterial Strain Identification

DOE Project Number: DE-FG03-00NN20102 Northern Arizona University

B&R CODE: **Date:** November 7, 2000

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Progress During the Quarter:

Section 3.6.7 Year 1 Goals and Deliverables

Goal 1. A 25 Marker MLVA System for *Y. pestis*

We currently have 42 VNTR markers working well in our MLVA system. There are a few additional markers that hold great potential for high resolution analysis. We are developing mutational rate experiments to understand these markers in greater detail that will support forensic applications. Mutational rates will allow the development of statistical inferences that may be crucial for prosecution.

Goal 2. MLVA genotype data for 40 *Y. pestis* samples from USAMRIID

Analysis of 12 core samples from USAMRIID has been completed with the full 42 VNTR markers. We have expanded the diverse collection with ~50 diverse isolates from Institut Pasteur. These samples have been previously analyzed by IP using IS elements. Our work will provide a detail comparison of the methods.

Goal 3. MLVA analysis of California, New Mexico and Arizona *Y. pestis* collections.

The MLVA analysis of 96 California samples has been completed. We have completed the wet bench analysis of 35 NM samples and are currently completing the data analysis. We have made a few *Y. pestis* collections from Northern Arizona and have completed the wet bench analysis of these samples. We are now capable of analyzing *Y. pestis* cultures direct from infected fleas.

Goal 4. A 25 marker MLVA system for *B. anthracis*

We have a robust 8 marker MLVA system developed for *B. anthracis*. An additional 28 markers are being applied to a diverse set of 89 strains for further

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phylogenetic analysis. Some of these are multiplexed and capable of being applied more generally.

Goal 5. MLVA genotypic data for ~97 unique *B. anthracis* strains

We have identified a set of 89 unique B. anthracis strains by analysis with the 8 marker MLVA system. These 89 are being analysed with the full set of 36 markers.

Goal 6. Identification of 25 potential VNTR regions for *F. tularensis*

We have identified 6 VNTR regions in the *F. tularensis* genome. These have been use to assay for diversity in a set of ca. 50 isolates from California, Arizona and Oklahoma. Interesting epidemiological ramifications are becoming evident from these data.

Comments

Section 3.6.7 Year 1 Goals and Deliverables

Goal 1. A 25 Marker MLVA System for *Y. pestis*

We are continuing to develop primers, optimizing reactions and testing for primer compatibility in multiplex reactions. A 25+ marker MLVA system will be developed by the end of the contract period.

Goal 2. MLVA genotype data for 40 *Y. pestis* samples from USAMRIID

The core set of *Y. pestis* samples from USAMRIID represent a very diverse collection and its analysis is intended as a broad assay of diversity. A full analysis of 40 samples may not be necessary in order to accomplish this goal and we have selected a representative core set of 12, instead. Additional diversity strains are being obtained and analyzed. Include in our recent additions are a set of 50+ strains from Institut Pasteur. This collection has been analyzed using IS elements and will represent an excellent comparison of methods.

Goal 3. MLVA analysis of California, New Mexico and Arizona *Y. pestis* collections

MLVA analysis of the California samples will be accomplished with this contract period. The wet bench portion of the MLVA has been accomplished on 35 New Mexico samples. We are performing the computer analysis at this time. AZ flea are being directly analyzed as well as strains are being isolated.

Goal 4. A 25 marker MLVA system for *B. anthracis*

With identification of the additional ~30 potential VNTR regions, the prospects for a 25 marker MLVA system appear very good. We will be screening these regions for variation over the next few months. In addition, we will be attempting to combine these markers into multiplex reaction cocktails.

Goal 5. MLVA genotypic data for ~97 unique *B. anthracis* strains

The new markers identified, as a part of goal number 4 will be used to analyze the unique set of 89 strains. This will be accomplished over the next few months.

Goal 6. Identification of 25 potential VNTR regions for *F. tularensis*

We have screened all available *F. tularensis* genomic sequence and are waiting for additional sequence data from the consortium or the new LLNL sequencing effort.

Funding Status	**Operations**	**Capital**
Uncosted from previous FY.	0	0
Current FY	$ 270,000	$ 30,000
Total Funding Available	$ 270,000	$ 30,000
$ Spent this quarter:	$ -	$ -
$ Spent year-to-date	$ 186,551	$ 3,540
$ Remaining for this FY	$ 57,169	$ 26,460
Anticipated uncosted current FY funds	0	0

Technical Reports/Presentation

Journal Article: Schupp, J.M., A. M. Klevytska, L.B. Price, and P. Keim. 2000. *VrrB*, A Hypervariable Open Reading Frame in *Bacillus anthracis*. *J. Bacteriology*. 182:3989-97.

Journal Article: Smith, K.L., V. DeVos, H. Bryden, L.B. Price, M.E. Hugh-Jones, P. Keim. 2000. Genetic Diversity and Ecology of *Bacillus anthracis* in the Kruger National Park. *Journal of Clinical Microbiology*. 38:3780-4.

Journal Article: Turnbull, P., P. Jackson, K. Hill, A-B, Koltso, Keim, P. 2000. Systematic Relationships within *Bacillus anthracis*. "In: Applications and Systematics for Bacillus and Relatives. 2001. (Berkeley,RCW, Heyndrickx M, Logan NA, de Vos P, eds). Blackwell Science, Oxford" In press.

Journal Article: Keim, P, and KL Smith. 2000. Genome analysis for strain discrimination. In: "Anthrax Toxins." ed. T. Koehler. Current Topics in Microbiology and Immunology. Springer Verlag. In review.

Talk: "The rapid evolution of VNTR sequences in slowly evolving *B. anthracis*"
Evolution of Pathogens Conference – Institut Pasteur Oct. 2000

Talk: "Bioterrorism Attribution Using DNA Fingerprinting"
Banbury Conference on "The Challenge of Infectious Disease"
Cold Spring Harbor Symposium, Oct. 2000

Talk: MLVA analysis of toward an evolutionary understanding of *B. anthracis*. The 54th International Conference on Zoonotic Diseases. Ft. Collins Colorado August 2000.

Talk: A High Resolution Typing system for *Y.pesits*: Ecology and Evolution of Plague. The 54th International Conference on Zoonotic Diseases. Ft. Collins Colorado August 2000.

Talk: MLVA analysis of *F. tularensis* in California, Arizona and Oklahoma. The 54th International Conference on Zoonotic Diseases. Ft. Collins Colorado August 2000.

Talk: Molecular Epidemiology of Anthrax. The 54th International Conference on Zoonotic Diseases. Ft. Collins Colorado August 2000.

Talk: Crystal City VA - CBNP BioFoundations Annual report meeting July 2000.

Talk: July 3, MLVA for Epidemiological Analysis of Bacterial Pathogens, Gene Eskew and Bill Barnum- hosts, Oklahoma Deptartment of Agriculture, Animal Industry Division, State of Oklahoma, Oklahoma City, Oklahoma.

Talk: July 5, MLVA for Epidemiological Analysis of Bacterial Pathogens, Oklahoma Veterinary Medical Diagnostic Laboratory and Becky Morton, School of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma.

Talk: July 6, MLVA for Epidemiological Analysis of Bacterial Pathogens,, Kristy Bradley, Michael Lytle and Epidemiology Division staff- hosts, Oklahoma Department of Health, Department of Health and Human Services, State of Oklahoma, Oklahoma City, Oklahoma.

Talk: July 10, MLVA in general, Virginia Shehee- hosts, Chair Emeritus, Shreveport Medical Center and Shreveport Biomedical Research Foundation, Shreveport, Louisiana.

Talk: July 11, Yp MLVA Epi, Suzanne Barth and staff, Jane Barlow and Pam Willson- hosts, Microbiology Division and Zoonosis Division, Texas Department of Health, State of Texas, Austin, Texas.

Talk: July 13, Yp MLVA Epi, James Alexander and Bob Gilliland- hosts, Zoonosis Division and Wildlife Division, Texas Department of Health, State of Texas, Canyon, Texas.

Talk: July 15, Yp MLVA Epi, Jan Buck, Regional Zoonosis Division, Texas Department of Health, State of Texas, Fort Worth, Texas.

Talk: August 2, Epidemiological Applications of the *Bacillus anthracis* Multi-locus VNTR Analysis, International Northwestern Conference on Diseases in Nature Transmissible to Man, Fort Collins, Colorado.

Talk: September 14/15, Keim Genetics Laboratory BSL-2 Facility and NAU Emergency Response Personnel, Northern Arizona University Police Department, Flagstaff, Arizona.

Talk: September 13, Bacterial Virulence Mechanisms of *Bacillus anthracis*, Bacterial Virulence Mechanisms Seminar, Department of Biology, Northern Arizona University, Flagstaff, Arizona.

Talk: September 19, Yp MLVA Epi, Landscape Ecology of Plague, Madison Wildlife Disease Center and the Vector Borne Disease Unit, CDC, Fort Collins, Colorado.

Talk: September 26, Research Opportunities for Veterinarians, Pre-medical Proffesionals class, Department of Biology, Northern Arizona University, Flagstaff, Arizona.

Talk: October 18, High Resolution Fingerprinting of *Yersinia pestis*, Interstate Working Groups for the Recovery of the Black-tailed Prairie Dog, Rapid City, South Dakota.

Talk: October 23, *Mycobacterium tuberculosis* MLVA Epi, Tom Kraunbull, Hansen's Disease Center, Louisiana State University, Baton Rouge, Louisiana.

Talk: October 24, Ba and Yp MLVA Epi, Gus Kosulous, Department of Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana.

Talk: November 1, Ft MLVA Epi, Kristi Bradley and Michael Lytle, Oklahoma Department of Health, Department of Health and Human Services, State of Oklahoma, Oklahoma City, Oklahoma.

Talk: November 3, Ft MLVA Epi, Becky Morton, Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater, Oklahoma.