9th ASM Biodefense and Emerging Diseases Research Meeting

Omni Shoreham Hotel  Washington, DC

February 6 – 9, 2011 (Sunday–Wednesday)

www.asmbiodefense.org
SAVE THE DATE!

10th ASM Biodefense and Emerging Diseases Research Meeting

February 26-29, 2012 | Omni Shoreham Hotel | Washington, DC

www.asmbiodefense.org
Dear Colleagues,

Welcome to the American Society for Microbiology’s 9th Annual Biodefense and Emerging Diseases Research Meeting in Washington, DC. The ASM Biodefense Research Program Committee has assembled a program that includes experts in multiple relevant fields presenting in several different session formats. The 2011 program is intended to provide each attendee with the opportunity to participate in educational programming that has the most value and relevance to their individual professional needs. For this year’s meeting we have brought together individuals who are carrying out research to defend against the growing threats of bioterrorism and emerging infectious diseases and decision makers shaping the future of the biodefense research agenda.

The meeting opens Sunday at noon with four in-depth Focus Sessions followed by the Keynote Session Sunday afternoon. This year’s Keynote Speaker, Donald A. Henderson, M.D., from the Center for Biosecurity of UPMC in Baltimore, Maryland, will present “The Eradication of Smallpox: A Continuing Saga.” We cordially invite all meeting attendees to the Opening Reception in the Exhibit Hall immediately following the conclusion of Dr. Henderson’s talk.

Beginning Monday morning, daily Plenary Sessions include “Smallpox Vaccines,” “Jamming Nanomachines,” and “New Interventions and the Challenges of Emerging Infections.” Diverse concurrent symposia are also scheduled for Monday and Tuesday afternoon. Finally, a Discussion Roundtable Session on “Medical Countermeasure Development in the U.S. Government” will enhance your meeting experience. Please check the details in this Final Program/Abstracts book to identify the presentations that most appeal to you.

From over 180 accepted abstract submissions, the Program Committee has organized 2 Poster Sessions and 6 Highlighted Oral Abstract Sessions. Poster presentations will be presented in the Exhibit Hall, Monday and Tuesday afternoons. Posters will be on display from 12:00 noon – 3:30 PM and presenters will be available from 1:00 – 3:00 PM to answer your questions. The Exhibit Hall also features 40 exhibitors with the latest information on new products. The Highlighted Oral Abstract Sessions occur Monday and Tuesday from 3:00 – 4:00 PM. Please check the details in this Final Program/Abstracts book.

Again, the Program Committee welcomes you and looks forward to meeting you as we continue to work together in addressing the challenges that lie ahead.

Sincerely,

Connie S. Schmaljohn, Ph.D.
ASM Biodefense Research Meeting Co-Chair

Paul Keim, Ph.D.
ASM Biodefense Research Meeting Co-Chair
9th ASM Biodefense and Emerging Diseases Research Meeting

Program-at-a-Glance

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<tr>
<td>Registration</td>
<td>10:00 AM – 7:00 PM</td>
<td>7:30 AM – 5:00 PM</td>
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<tr>
<td>Keynote Session</td>
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<td>8:30 AM – 12:00 PM</td>
<td>8:30 AM – 12:00 PM</td>
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<tr>
<td>Exhibits</td>
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<td>Poster Presentations</td>
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<td>1:00 PM – 3:00 PM</td>
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<tr>
<td>Highlighted Oral Abstract Sessions</td>
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<tr>
<td>Concurrent Symposia</td>
<td></td>
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<td>4:15 PM – 6:15 PM</td>
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<td>Discussion Roundtable</td>
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<tr>
<td>Opening Reception</td>
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Complimentary lunch for all attendees will be available in the Exhibit Hall from 12:00 PM – 1:00 PM, Monday and Tuesday, February 7th and 8th.
DONALD A. HENDERSON, MD; Center for Biosecurity of UPMC, Baltimore, MD.

Donald Ainslie Henderson, known as D.A., was born in Lakewood, Ohio, in 1928, to David and Grace Henderson. He received his bachelor of arts degree from Oberlin College in 1950. In 1951, he married Nana Irene Bragg. The couple have three children. Henderson received his medical degree from the University of Rochester in 1954 and was an intern and resident at the Mary Imogene Bassett Hospital in Cooperstown, New York. From 1955 to 1966 he also worked for the Centers for Disease Control.

Henderson ran the World Health Organization’s smallpox eradication program from 1966 to 1977. During the 20th century, at least 300 million people died of smallpox. As late as 1960, about five million people a year died of the disease. The eradication of smallpox has been called “perhaps the greatest medical feat of all time.” “An ancient, contagious and particularly hideous disease, smallpox kills a third of those infected with it, and Dr. Henderson is one of the few doctors in this country today to have actually seen a case,” noted Sheryl Gay Stolberg in an article in the New York Times, dated November 18, 2001. “The World Health Organization’s smallpox eradication program, which Dr. Henderson ran from 1966 to 1977, was, he said, the effort of countless public health workers who toiled under grueling conditions, often living in villages without electricity and running water, in nations torn apart by war. They operated under the principle of ‘ring vaccination,’ containing outbreaks by vaccinating every patient infected, and everyone around those patients, moving outward in concentric circles until the virus stopped spreading.”

Smallpox experts believe the eradication effort, carried out with the former Soviet Union during the Cold War, succeeded because of Henderson’s determination. When the health minister of Ethiopia would not cooperate with him, Henderson made his way into the country and got to know the personal physician of the country’s emperor, Haile Selassie. When Henderson suspected the Russians were giving him an inferior smallpox vaccine, he traveled to Moscow — violating orders from his superiors, who were worried about a diplomatic nightmare — and demanded a better vaccine.

After finishing his work with the World Health Organization, Henderson served as dean of the Johns Hopkins School of Public Health from 1977 to 1990. He then worked for the administration of President George Bush, serving as science adviser to the White House from 1991 to 1993, as associate director of the Office of Science and Technology Policy. Henderson worked for the Department of Health and Human Services (HHS) from 1993 to 1995 as deputy assistant secretary of HHS for health and science. He left in 1995 because he felt he was being underutilized and returned to the Johns Hopkins School of Public Health as a professor. Because of his fears about bioterrorism, in 1997, Henderson founded and became director of the Johns Hopkins University Center for Civilian Biodefense Studies, a research institution.
Symposia

Symposia consist of 4-5 faculty, invited by the Biodefense Research Program Committee, who present lectures on a specific topic followed by a moderated Q&A period.

Poster Sessions

All accepted abstracts are scheduled for poster presentation based on the December 2010 review of all abstract submissions by the Biodefense Research Program Committee. Posters are on display in the Exhibit Hall from 1:00 PM – 3:30 PM, Monday, February 7th, and Tuesday, February 8th. Presenting authors have been instructed to remove their posters by 4:30 PM on Tuesday. Each presenter will be available at his or her poster to answer questions from 1:00 PM – 3:00 PM either Monday or Tuesday as scheduled in this program.

Focus Sessions

Focus sessions are in-depth educational activities that highlight a specific topic area. These sessions precede the Keynote session and are an opportunity for attendees to explore new and emerging issues with a group of faculty experts.

Plenary Sessions

Daily Plenary Sessions will be held Monday, Tuesday, and Wednesday morning. These sessions provide all attendees an opportunity to collaborate on presentations of science, policy, and public health issues. The speakers were invited by the Biodefense Research Program Committee based on their professional expertise and achievement. Plenary Sessions may have a moderated Q&A period.

Highlighted Oral Abstract Sessions

These sessions highlight research submitted for the ASM Biodefense and Emerging Diseases Research Meeting. Authors are invited by topic to give a 10 to 15 minute presentation of their research followed by a moderated Q&A period.

For specific presentation times and additional details, please see the session information listed by date in this Final Program/Abstracts book.

Discussion Roundtable/Q&A Sessions

One-hour Discussion Roundtable will be held on Monday and Tuesday. These discussions are dedicated to providing attendees with information about selected government programs including how to apply for grants and other financial support.

ASM Press

ASM Press, the book publishing division of the American Society for Microbiology, will be exhibiting its broad selection of texts and references in molecular biology and the microbiological sciences. Stop by to see these and other great offerings from ASM Press. There is a 10% discount on all purchases made at the meeting.
General Information

Registration
Registration will be located in the Hampton Lobby Foyer from Sunday, February 6th – Tuesday, February 8th, of the Omni Shoreham Hotel.

Registration Hours:
- Sunday, February 6 ..................10:00 AM – 7:00 PM
- Monday, February 7 .................7:30 AM – 5:00 PM
- Tuesday, February 8 .................7:30 AM – 3:30 PM
- Wednesday, February 9..............See an ASM staff member for assistance in the Directors Room on the Lobby Level.

Contact Information
To contact ASM staff on site at the conference, please call the Omni Shoreham Hotel, at (202) 234-0700, and ask to be transferred to the ASM Registration Desk located within the Hampton Lobby Foyer (West Registration Desk).

Guests
Non-registered guests are not permitted in session rooms or in poster sessions. However, a guest may attend the Opening Reception on Sunday, February 6th, 6:00 PM – 8:00 PM. (Meeting attendees gain entry to the reception with their registration badge.)

Special Needs
Please contact ASM staff on site at the conference if ASM may provide any special accommodations while you attend the meeting. Please call the Omni Shoreham Hotel, at (202) 234-0700, and ask to be transferred to the ASM Registration Desk located within the Hampton Lobby Foyer (West Registration Desk).

Child Policy
Children are not permitted in session rooms. Childcare may be arranged through the concierge of the Omni Shoreham Hotel.

Omni Shoreham Business Center
A full-service business center is located in the Lobby Level, and is available 24 hours.

Poster Storage
When not displayed, posters may be stored at the Registration Desk. Posters must be clearly marked with the presenter’s name when placed in storage. Please note that registration closes at 3:30 PM on Tuesday, February 8th. All posters must be collected by that time or they will be discarded.

Speaker Ready Room
The speaker ready room will be available during registration hours in the Director’s Room, on the Lobby Level. Limited internet access and laptop internet cables are available in the Speaker Ready Room.

Cameras and Recording
Digital recorders, cameras (including camera phones), and video cameras (including video phones) are prohibited in the poster hall and session rooms. Anyone found photographing, videotaping, or recording in the prohibited areas will be asked to surrender their badge immediately and leave the conference. No refund will be provided. This rule is strictly enforced.

Certificate of Attendance
Certificates of Attendance are available at the Registration Desk during registration hours beginning Tuesday, February 8th. Attendees may also print a Participation Statement of Credit directly from your computer, by accessing the ASM CE Portal (ce.asm.org). You will need your Badge ID from your registration badge to obtain your statement in the CE Portal. The Participation Statement of Credit documents CE Contact Hours for each session you attended at the 9th ASM Biodefense and Emerging Diseases Research Meeting.

NOTE: Certificates of Attendance do not list session information.
Food and Beverage
There are many dining options close to the hotel, some within walking distance, some a short cab ride away. A list of restaurants is available through the hotel concierge. Please note: Lunch will be provided Monday, February 7th, and Tuesday, February 8th, from 12:00 PM-1:00 PM in the Exhibit Hall, Level 1 B.

Lost and Found
Unattended personal belongings will be removed and taken to hotel security.

Meeting Announcements
A handout table displaying previously approved future meeting and other brochures is located in the registration area. A bulletin board will be available for those attendees wishing to post one-page flyers announcing upcoming meetings.

Messages
All mail and communications to meeting registrants should be directed to the individual at the hotel where he or she is staying. The phone number for the Omni Shoreham Hotel is (202) 234-0700.

Smoking Policy
Smoking is prohibited in all areas of the Omni Shoreham Hotel.

Meeting Venue
All scientific presentations and poster sessions will take place at Omni Shoreham Hotel. Scientific presentations and Exhibits/Posters will take place in the Exhibit Hall, Level 1 B.

Student Postdoctoral Fellow Travel Grant Recipients
Congratulations to our ASM Biodefense and Emerging Diseases Research Meeting Student Postdoctoral Fellow Travel Grant Recipients!

The ASM Student Travel Grant Program was established to support the travel of students to ASM meetings. Recipients are selected by the Biodefense Research Program Committee on the basis of the scientific quality of the submitted abstracts.

Christine M. Gonzales
University of Texas Medical Branch, Galveston, TX
Monday Poster Session,
Presentation 100 (I)

Ramesh Koukuntla
BioProtection Systems Corporation, Ames, IA
Monday Poster Session
Presentation 114

Rinosh J. Mani
Oklahoma State University, Stillwater, OK
Tuesday Poster Session,
Presentation 227

Adel M. Nour
Stanford University, Stanford, CA
Monday Poster Session,
Presentations 028 (A), 033 (A)

Adriana S. Patterson
University of California, Santa Barbara, CA
Tuesday Poster Session,
Presentation 184 (D)

Tony Pierson
George Mason University, Manassas, VA
Monday Poster Session
Presentation 119
## 9th Biodefense Exhibits

**Exhibits, posters, lunch, and the Opening Reception will be held in the Exhibit Hall.**

The American Society for Microbiology welcomes the following exhibitors:

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<td>Accelovance</td>
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<td>Applied Maths, Inc.</td>
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<td>ASM Press</td>
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<td>Baker Company, The</td>
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<td>BIAERA Technologies, LLC</td>
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<td>Bioqual, Inc.</td>
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<td>Bio-Synthesis, Inc.</td>
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<td>CH Technologies (USA), Inc.</td>
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<td>Defense Threat Reduction Agency, RD-CB</td>
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<td>DynPort Vaccine Company LLC, A CSC Company</td>
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<td>emkaTECHNOLOGIES, INC.</td>
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<td>Evogen, Inc.</td>
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<td>Germfree Laboratories, Inc.</td>
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<td>List Biological Laboratories, Inc.</td>
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<td>Lovelace Respiratory Research Institute</td>
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<td>Meso Scale Defense</td>
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<td>Microbiology International</td>
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<td>NanoLogix, Inc.</td>
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<td>National Biosafety &amp; Biocontainment Training Program</td>
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<td>National Institute of Allergy &amp; Infectious Diseases</td>
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<tr>
<td>Naval Medical Research Center</td>
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<td>NIH Office of Biotechnology Activities</td>
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<td>OpGen, Inc.</td>
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<td>Paragon Bioservices, Inc.</td>
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<td>Reagent Proteins</td>
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<td>Southern Research Institute</td>
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<td>Tetracore, Inc.</td>
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<td>Transformational Medical Technologies (TMT)</td>
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<tr>
<td>UTMB National Biocontainment Training Center</td>
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Exhibits, posters, lunch, and the Opening Reception will be held in the Exhibit Hall.
Exhibit Hall Information
Scientific exhibits are located in the Exhibit Hall, co-located with poster sessions.

*The Exhibit Hall will be open:*
- Sunday, February 6 ..................6:00 PM – 8:00 PM
- Monday, February 7 .................12:00 PM – 3:30 PM
- Tuesday, February 8 ...............12:00 PM – 3:30 PM

Additionally, lunch will be provided in the Exhibit Hall from 12:00 PM – 1:00 PM on Monday and Tuesday, February 7 – 8, 2011. Attendees can listen to poster presentations, then get the most current information on scientific products and services relating to Biodefense.

Opening Reception in the Exhibit Hall
Sunday, February 6, 6:00 PM – 8:00 PM

Network with colleagues, investigate the amazing variety of exhibits, and visit with industry representatives at your leisure, all while enjoying light hors d’oeuvres and drinks. Name badges required.
**EXHIBITS AND POSTERS — MONDAY**

*Poster Presentations will be on display both days.*

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**Exhibit Hall**  
Omni Shoreham Hotel  
Washington, DC

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<th>Category</th>
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<td>B</td>
<td>39-57</td>
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<td>D</td>
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<td>H</td>
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<td>I</td>
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<td>J</td>
<td>104-107</td>
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<tr>
<td>K</td>
<td>108-111</td>
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</table>
Exhibit Hall
Omni Shoreham Hotel
Washington, DC

*Poster Presentations will be on display both days.
Continuing Education

Objectives
Organizations around the world are involved in research pertaining to biothreat agents, vaccines, detection and diagnostic procedures, animal and plant pathogens, biosecurity facilities, global surveillance, the training of individuals in BL3 and BL4 labs, and other vital topics. As technology changes and research evolves, professionals involved in biodefense are being challenged to move ever faster to respond to the growing threat of bioterrorism.

The ASM Biodefense and Emerging Diseases Research Meeting participants will learn the latest scientific advances in medical intervention for biological terrorism and emerging infectious disease threats, including new pathogens not usually seen in daily medical practices, so that they are able to quickly recognize and appropriately treat patients afflicted with such biothreats.

Target Audience
The ASM Biodefense and Emerging Diseases Research Meeting is intended for scientists, physicians, public health researchers and policy makers who need to be informed about the latest scientific developments.

Continuing Education
The following ASM Biodefense and Emerging Diseases Research Meeting session types offer continuing medical education credit as noted in this Final Program by the CME symbol: 

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<th>Session Type</th>
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<tr>
<td>Focus Sessions</td>
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<tr>
<td>Keynote Session</td>
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<td>Plenary Sessions</td>
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<td>Highlighted Oral Abstract Presentations</td>
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<tr>
<td>Concurrent Symposia</td>
<td>2.0</td>
</tr>
<tr>
<td>Discussion Roundtable</td>
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The American Society for Microbiology (ASM) is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

The ASM designates this live activity for a maximum of 24 AMA PRA Category 1 Credits™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Diplomates of the American Board of Medical Microbiology, Diplomates of the American Board of Medical Laboratory Immunology, and Registrants of the National Registry of Microbiologists may earn AMA PRA Category 1 Credits™ toward re-certification. Each professional should claim credit commensurate with their level of participation in the activity.

Other CE Licenses
A Participation Statement of Credit can be obtained by completing the activity evaluation. This is a detailed statement that documents your participation for each session you attended at this activity.

How to Claim CE Credit
To claim credit for the ASM Biodefense and Emerging Diseases Research Meeting, please visit the CE Portal, ce.asm.org.

The ASM CE Portal allows individuals to:

- Print their own CE or participation certificates for activities they take part in,
- Evaluate the activities they attend,
- Browse the library of ASM CE activities available,
- Review and print a transcript of all ASM CE activities attended, and
- Participate in online learning activities.
Educational Disclaimer

The primary purpose of the sessions at the ASM Biodefense and Emerging Diseases Research Meeting is education. Information presented, as well as publications, technologies, products, and/or services discussed, are intended to inform you about the knowledge, techniques, and experiences of professionals who are willing to share such information with colleagues. A diversity of professional opinions exists in the fields discussed at the Biodefense Research Meeting and the views of the Biodefense Research Meeting faculty are offered solely for educational purposes. Faculty’s views neither represent those of ASM nor constitute endorsement by the association. The ASM disclaims any and all liabilities for damages to any individual attending the ASM Biodefense and Emerging Diseases Research Meeting and for all claims which may result from the use of this information, publications, technologies, products, and/or services at the ASM Biodefense and Emerging Diseases Research Meeting.

Disclosure Statement

As a provider accredited by the Accreditation Council for Continuing Medical Education, the ASM requires that all faculty members participating in continuing education activities sponsored by ASM disclose all relevant financial relationships related to the ASM Biodefense activity. The ASM then takes steps to resolve any identified conflict of interest. A listing of faculty disclosures is available in the Final Program. This policy is intended to make you aware of faculty disclosure so you may form your own judgments about material discussed during the Biodefense and Emerging Diseases Research Meeting.

If you have concerns (e.g., commercially-biased presentations, promotional materials distributed by presenters and/or participants, etc.), please visit the ASM Biodefense and Emerging Diseases Research Meeting Headquarters Office and ask to speak to an ASM staff person so that ASM can respond immediately to your concerns.

Disclosures

Individuals with Disclosures

The following speakers disclosed financial relationships. See CME Relationship Code table on page 14 for code descriptions.

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<th>Session #</th>
<th>Company</th>
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<td>Barrett, Alan</td>
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<td>Chiu, Charles Y.</td>
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<td>Global Viral Forecasting Initiative</td>
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<td>Autoimmune Technologies</td>
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<td>Keim, Paul</td>
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**Individuals with Nothing to Disclosure**

The following are faculty members of the ASM Biodefense and Emerging Diseases Research Meeting who have declared that they have no financial relationship relevant to this activity to disclose.

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**CME Relationship Code Table**

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<td>10. Other Financial Benefit</td>
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001. Clinical Research on Biodefense Pathogens: 
Epidemiology and Diagnostics for Brucellosis and 
Immune Response to Anthrax
Sunday, February 6, 2011  |  12:00 PM – 2:00 PM
Palladian Ballroom

This session will include translational research that applies modern 
immunologic techniques to analyze responses in patients either re- 
ceiving anthrax vaccinations or with anthrax infection. The session will 
also provide clinical descriptions of cutaneous anthrax cases, and cor- 
relation between the immunology and the severity of the infection will 
be presented. The relationship between the response to vaccination 
and the response to infection will be further explored, with a particu- 
lar focus on the cellular responses. This session will ultimately sup- 
port the continued development of the scientific foundation for the 
future development of anthrax countermeasures. Brucellosis is an 
infection of substantial relevance to international public health as well 
as the biodefense community. Recent research into the molecular epi- 
demiology of the pathogen can reveal insight into clinical geographic 
differences. Active surveillance can provide information relevant for 
public health and biodefense. Finally, multiple publications have pro- 
moted the utility of molecular diagnostics for brucellosis, but the ap- 
plication of these tests can be challenging. This session will provide 
a multi-disciplinary forum to discuss the current issues with this com- 
plex infectious disease, ultimately providing further support for the 
development of countermeasures and control programs.

Upon completion of this Focus Session, the participant should be 
able to:

• Identify the clinical manifestations of cutaneous anthrax;
• Describe the immune responses between natural anthrax infec- 
tion and various anthrax vaccines using the same measurement 
techniques;
• Assess the clinical responses with the immunologic responses to 
  anthrax infection;
• Review recent data on the molecular epidemiology of brucellosis, 
  providing an understanding of the applicability of these tech- 
niques;
• Describe performance characteristics of diagnostic tests for bru- 
cellosis applied in a clinical setting, with particular focus on mo- 
  lecular techniques; and
• Explain the challenges inherent in the implementation of molecu- 
  lar diagnostics platforms.

MODERATORS:
MIKELJON NIKOLICH, PhD; Walter Reed Army Inst. of Res., Silver Spring, MD.
NINO TRAPAIDZE, PhD; Natl. Ctr. for Diseases Control and Publ. Health, Tbilisi, Georgia.
Focus Session

002. Impact of Modern Molecular Typing Methods and Whole Genome Sequencing on Bioforensics Epidemiology and Improved Diagnostics of Infectious Diseases Caused by Select Agents

Sunday, February 6, 2011 | 12:00 PM – 2:00 PM
Diplomat Ballroom

The session will present some work done in the frame of the European Biodefense Laboratory Network, which is an effort under the European Defense Agency. The project involves 12 European Biodefense laboratories and aims to the construction of a shared database with high-resolution typing data, employing different techniques, and definition of strain panels for validation of diagnostic tests and identification methods and instruments. The session will also include presentation on EU projects aiming at improved bio-traceability of unintended microorganisms in food and feed chains by using typing methods and whole-genome sequencing as well as typing of Yersinia pestis and Francisella tularensis in a forensic context. Also prospects on Next Generation Sequencing applications toward pathogen genomics, detection, and characterization will be covered.

Upon completion of this Focus Session, the participant should be able to:
• Discuss how different typing methods compare when applied on the same strain;
• Describe different techniques in use for typing of select agents;
• Identify how new molecular diagnostic methods impact the clustering and classification of select agents; and
• Review new improved identification methods for select agents.

MODERATORS:
MATS FORSMAN, PhD; Swedish Defense Res. Agency, Umeå, Sweden.
PETRA C. F. OYSTON, PhD; Biomedical Sciences, DSTL, Salisbury, United Kingdom.

PRESENTATIONS:
12:00 PM
008 Set-Up and Current Work of the European Biodefense Laboratory Network
MATS FORSMAN, PhD; Swedish Defense Res. Agency, Umeå, Sweden.

12:25 PM
009 Whole Genome Sequencing as a Rapid High Resolution Diagnostic Typing Tool When Tracing Bioterror Organisms in the Food and Feed Chain

12:50 PM
010 Typing of Yersinia pestis in a Forensic Context
DAVID M. WAGNER, PhD; Northern Arizona Univ., Flagstaff, AZ.

1:15 PM
011 Typing Francisella tularensis in a Forensic Context
ANDERS JOHANSSON, MD, PhD; Univ. of Umeå, Umeå, Sweden.

1:40 PM
012 Next Generation Sequencing Applications Toward Pathogen Genomics, Detection, and Characterization
PATRICK CHAIN, PhD; Los Alamos Natl. Lab., Los Alamos, NM.

Focus Session

003. Adapting the Public Health Model of Outbreak Investigation for Biodefense

Sunday, February 6, 2011 | 2:15 PM – 4:15 PM
Diplomat Ballroom

This session will consist of biosurveillance efforts with regard to foodborne illness and how they may apply to a bioterrorism scenario. In the event of an epidemic, it is critical to be able to characterize the pathogen agent and identify the source of the outbreak. This is true regardless of whether the outbreak is natural or the result of a bioterror event. However, food-borne illnesses have provided a number of examples of anthropogenic disease outbreaks. This session will focus on the surveillance of disease, the characterization of the etiological agent in an epidemic, and attribution of the outbreak to its source.

Upon completion of this Focus Session, the participant should be able to:
• Discuss biosurveillance with regard to food-borne illnesses;
• Assess methods utilized to characterize pathogens; and
• Identify attribution of an outbreak to its source.

MODERATORS:
WALLACE BUCHHOLZ, PhD; U.S. Army Res. Office, Research Triangle Park, NC.
KARL E. KLOSE, PhD; Univ. of Texas, San Antonio, TX.

PRESENTATIONS:
2:15PM
013 Surveillance, Detection and Investigation of Foodborne Disease Outbreaks in the United States
JOHN J. GUZEWICH, MPH; FDA, College Park, MD.

2:45 PM
014 Rapid and High-Resolution Subtyping of Bacterial Pathogens Associated with Foodborne Outbreaks
ERIC BROWN, PhD; FDA/CFSAN, College Park, MD.

3:15 PM
015 Application of Microbial Forensics for BioCrimes
ROBERT BULL, PhD; FBI, Quantico, VA.

3:45 PM
016 The Armed Forces Health Surveillance Center Global Emerging Infections Surveillance and Response System
MATTHEW C. JOHNS, MPH; Armed Forces Hlth. Surveillance Ctr., Silver Spring, MD.
Focus Session

004. Emerging Plant Diseases and Agricultural Biodefense
Sunday, February 6, 2011  |  2:15 PM – 4:15 PM
Palladian Ballroom

U.S. agriculture is threatened by many diseases that are either already severely affecting our agriculture (for example, citrus greening) or may do so in the near future (for example, stem rust of wheat, strain UG99). Terrorists could easily take advantage of our vulnerability and introduce crop pathogens into the U.S.

Upon completion of this Focus Session, the participant should be able to:

- Identify the importance of plant biosecurity;
- Review diseases that severely threaten US food production;
- Identify parallels between infectious diseases of humans and plants; and
- Review concepts of Biodefense of agriculture.

Moderators:
BORIS A. VINATZER, PhD; Virginia Tech, Blacksburg, VA.
PAUL KEIM, PhD; Northern Arizona Univ., Flagstaff, AZ.

Presentations:

2:15 PM
017 Responding to Plant Biosecurity Threats: A Role for Forensic Plant Pathology
JACQUELINE A. FLETCHER, PhD; Oklahoma State Univ., Stillwater, OK.

2:45 PM
018 Reconstructing Microevolution and Global Spread of Bacterial Plant Pathogens
BORIS A. VINATZER, PhD; Virginia Tech, Blacksburg, VA.

3:15 PM
019 Influence of Epidemiological Characteristics of Arboreal Pathosystems on Development of Mitigation Strategies
TIM GOTTWALD, PhD; USDA, Fort Pierce, FL.

3:45 PM
020 Tracking the Long Distance Movement of High Risk Plant Pathogens in the Atmosphere
DAVID G. SCHMALE III, PhD; Virginia Tech, Blacksburg, VA.

Keynote Session

005. Keynote Session
Sunday, February 6, 2011  |  4:30 PM – 6:00 PM
Regency Ballroom

This past year, the world celebrated the thirtieth anniversary of the declaration that smallpox had been eradicated. It was a triumphant celebration of a remarkable achievement of public health and disease eradication as well as a testimony to the potential of cooperation through the World Health Organization. In 1974, the program itself was instrumental for launching the successful “Expanded Program on Immunization” which has been responsible for a 99% reduction in the incidence of poliomyelitis and, in the Western Hemisphere, in the interruption of transmission of measles and rubella viruses as well as poliomyelitis. Now on the agenda for consideration as to whether the known stocks of smallpox virus should be destroyed as a further step in mitigating the likelihood of smallpox ever recurring. Final decisions are due to be decided at the World Health Assembly in May of this year.

Upon completion of this Keynote Session, the participant should be able to:

- Identify vaccine development efforts; and
- Discuss how Smallpox eradication created new disease or ecologic niches.

Moderators:
CONNIE SCHMALJOHN, PhD; USAMRIID, Fort Detrick, MD.
PAUL KEIM, PhD; Northern Arizona Univ., Flagstaff, AZ.

Presentation:

021 The Eradication of Smallpox: A Continuing Saga
DONALD A. HENDERSON, MD, MPH; Ctr. for Biosecurity of UPMC, Baltimore, MD.
This session will explore the potential of smallpox as a biological weapon, evaluate advances in vaccines and therapeutics as well as investigate the impact that eradication of this virus has had in the creation of a new ecologic niche for other orthopoxviruses. Despite the eradication of smallpox in natural settings, there is concern that stocks of smallpox virus might still exist or that the virus could be recreated using modern technologies. Consequently, smallpox vaccines remain of intense interest. This session will provide an up to date overview of the status of vaccines that have been and are being developed to protect against smallpox and related viruses.

Upon completion of this Plenary Session, the participant should be able to:

- Describe the obstacles encountered to date in developing a universally acceptable smallpox vaccine as well as the need for continued therapeutic development;
- Discuss the obstacles in developing animals models and how this will impact advancement of therapeutics and vaccines; and
- Summarize information pertaining to the US Governments plan to stockpile and distribute smallpox vaccines if needed.

MODERATORS:
MILES CARROLL, PhD; Hlth. Protection Agency, Wiltshire, United Kingdom.
LISA HENSLEY, PhD; USAMRIID, Ft. Detrick, MD.

PRESENTATIONS:

8:30 AM
022 Poxvirus Biology: Targets of Immunity and Antiviral Therapy
BERNARD MOSS, PhD; NIH/NIAID, Bethesda, MD.

9:00 AM
023 Smallpox Preparedness: Review of Antivirals and Considerations for Use
INGER DAMON, MD, PhD; CDC, Atlanta, GA.

9:30 AM
Coffee Break

10:00 AM
024 Determinants of the Increasing Incidence of Human Monkeypox in the Democratic Republic of the Congo
ANNE W. RIMOIN; Univ. of California, Los Angeles, CA.

10:30 AM
025 The HPA’s Role in Smallpox Vaccine Research
SIMON G. P. FUNNELL, PhD; Hlth. Protection Agency, Salisbury, United Kingdom.

11:00 AM
026 Susceptibility of Marmosets (Callithrix jacchus) to Monkeypox Virus
ERIC MUCKER; USAMRIID, Fort Detrick, MD.

11:30 AM
026a Future Perspectives on Subpopulations Genomics Applied to Single Cultures in a Forensic Context
PETER B. JAHRLING, PhD; NIH/NIAD, Fort Detrick, MD.
037 (A) Induction of DNA Damage Signaling Cascade upon RVFV Infection
A. BAER; George Mason Univ., Manassas, VA.

038 (A) Development of Fluorogenic 5' Nuclease Assays for Venezuelan Equine Encephalitis Virus
K. STEPHENS, M. ESONA, M. BOWEN, A. POWERS, K. THURMAN, E. BLACK, R. MEYER, L. DAUPHIN; CDC, Atlanta, GA.

038a (A) Pathogenesis of Eastern Equine Encephalitis Virus in Mice
S. P. HONNOLD, R. BAKKEN, K. SPURGERS, R. ERWIN-COHEN, E. MOSS, R. MAHESHWARI, P. J. GLASS, USAMRIID, Frederick, MD, USUHS, Bethesda, MD.

039 (B) Antibiotic Discovery: A New Method which Focuses on Dormant Bacteria
A. COATES, Y. HU; Helperby Therapeutics Group, London, United Kingdom, Univ. of London, London, United Kingdom.

040 (B) Phylogeography of Francisella tularensis Subspecies Holarctica from the Country of Georgia

041 (B) Development of Immunological Assays for Detection of Salmonella
C. FERAUDET-TARISSE, M. VAISANEN-TUNKELROTT, P. LAMOURRETTE, N. MEZITI, C. CRÊMINON, H. VOLLAND; CEA, Gif sur Yvette, France.

042 (B) Telemetry Characterization of F. tularensis SCHU S4 Aerosol Infection in Naive and LVS Vaccinated Cynomolgus Macaque

043 (B) The Plant Pathogen Pseudomonas syringae pv. Tomato as a Model for Plant Pathogen Source Tracking

044 (B) Multiple Low Dose Bacillus anthracis Ames Inhalation Exposures in the Rabbit

045 (B) In Vitro Characterisation Studies of Burkholderia mallei Prior to Use in an Aerosol Infection Model

046 (B) Identification of Molecular Targets of the LI-37 Peptide that Regulate Francisella Biofilm Production
S. AHMAD, L. S. AMER, M. L. VAN HOEK; George Mason Univ., Manassas, VA.

047 (B) Tissue Burden Demonstrates Drug Efficacy in an Intravenous Mouse Model of MRSA
K. A. OVERHEIM, T. BASREL, R. SHERWOOD; Lovelace Respiratory Res. Inst., Albuquerque, NM.

048 (B) Genotypic and Phenotypic Associations in Yersinia pestis Collected in the Caucasus
M. P. NIKOLICH, W. FAN, Y. LIU, L. E. LINDLER; Walter Reed Army Inst. of Res., Silver Spring, MD.

049 (B) Yersinia pestis Infection of Macrophages from Hosts with Low and High Susceptibility
D. PONNUSAMY, K. CLINKENBEARD; Oklahoma State Univ., Stillwater, OK.

050 (B) A Review of the Clinical Presentation and Case Definitions for Pediatric Brucellosis Cases in Azerbaijan

051 (B) Experimental Respiratory Burkholderia pseudomallei Infection in BALB/c Mice

052 (B) A High-Resolution Phylogenomic Framework for Escherichia coli O157:H7

053 (B) Multiplex PCR for Species-Level Identification of Bacillus anthracis and Detection of Plasmid Presence
M. A. ROJAS, K. STUPEC; 1BEI, Manassas, VA, SCIC, Frederick, MD.

054 (B) Quo Vadis on Your Query for Q Fever?

055 (B) Development of a Rhesus Macaque Model for Francisella tularensis

056 (B) Proteomic Analysis of Bronchoalveolar Lavage Fluid Proteins from Mice Infected with Francisella tularensis
057 (B) Genetic Diversity and Regional Distribution of Human and Animal Brucella Isolates in Georgia

W. CHAPMAN, A. BACETTY; DTRA BTRP, Ft. Belvoir, VA.

059 (D) Thermostable Single Domain Antibodies for the Detection of Bacillus anthracis
S. A. WALPER 1, R. GLAVERE 2, J. LIU 1, A. LEE 1, D. ZABETAKIS 1, G. ANDERSON 1, E. GOLDMAN 1; Naval Res. Lab., Washington, DC, 2Nova Res. Inc, Alexandria, VA.

060 (D) Real-Time 16S rRNA PCR to Monitor Growth of Ciprofloxacin Resistant Bacillus anthracis
L. J. HARTMAN, P. D. CRAW, D. A. NORWOOD, D. A. KULESH; USAMRIID, Ft. Detrick, MD.

061 (D) Use of Antibody-Coated Magnetic Beads in the Concentration of Biothreat Samples
J. JAISSE, P. CRAW, C. ROSSI, T. MINOGUE; USAMRIID, Ft. Detrick, MD.

062 (D) Assessment of Biomatrica’s CloneStable for Improved Stabilization of Diagnostic Biothreat Samples

063 (D) A Novel Microfluidic Platform for Building Rapid Response Capabilities Through the Rapid Detection of Viable Foodborne Pathogens
N. CORNEAU 1, C. LUEBBERT 1, M. DUPLESSIS 1, S. BIDAWID 1, J. M. FARBÉR 1, K. MORTON 1, X. X. DAI HOA 1, L. CLIME 1, T. VERES 1; 1Bureau of Microbial Hazards, Food Directorate, Hlth. Canada, Ottawa, Canada, 2Functional Nanomaterials Group, Industrial Materials Inst., Natl. Res. Council, Boucherville, Canada.

064 (D) Lethal Factor Toxemia and Anti-Protection Antigen Antibody Activity in Naturally-Acquired Cutaneous Anthrax
C. P. QUINN 1, A. E. BOYER 1, C. A. BEESLEY 1, M. GALLEGOS-CANDELA 1, C. K. MARSTON 1, L. X. CRONIN 1, R. C. LINS 1, R. A. STODDARD 1, M. J. HOS-SAIN 1, A. CHAKRABORTY 1, M. RAHMAN 1, S. LUBY 1, W. SHIEH 1, S. ZAKI 1, J. R. BARR 1, A. R. HOFFMASTER 1; 1CDC, Atlanta, GA, 2Batelle Analytical Services, Atlanta, GA, 3Intl. Ctr. for Diarrhoeal Disease Res., Mohakhali, Bangladesh, 4Inst. of Epidemiology Disease Control and Res., Mohakhali, Bangladesh.

065 (D) Comparison of OpenArray Nano-PCR to Established Real-Time PCR Assays for Biothreat Detection
K. CHASE, A. TROMBLEY, T. MINOGUE; USAMRIID, Frederick, MD.

066 (D) Comparison of Bacterial Culture and Real-Time (RT) PCR Platform for the Diagnosis of the Cutaneous Anthrax Infection: Prospective Study of the Volunteers in Georgia
T. KUCHULORIA 1,2, Z. ZHENG 1, M. ZAKALASHVILI 1, L. MALANIA 1, N. TSERTSAVADZE 1, M. ENDELADOZE 1, N. GELASHVILI 1, R. RIVARD 4, R. MODY 4, S. TSANAVA 3, M. J. HEPBURN 4, P. IMNADZE 3; 1Tbilisi State Univ., Tbilisi, Georgia, 2Clinical Res. Unit, TMC, Tbilisi, Georgia, 3Natl. Ctr. for Disease Control and Publ. Hlth., Tbilisi, Georgia, 4Infectious Pathology, AIDS and Clinical Immunology Res. Ctr., Tbilisi, Georgia, 5Rustavi Central Hosp., Rustavi, Georgia.

067 (D) RT-PCR/ESI-MS for Rapid Detection of Biothreat (BT) and Common Respiratory Pathogens in Bronchoalveolar Lavages (BALs)
K. JENG 1, H. WON 1, L. BLYN 1, S. C. PETERSON 1, J. P. HARDICK 1, K. CARROLL 1, A. VALSAMAKIS 1, R. E. ROTHMAN 1, C. A. GAYDOS 1; Johns Hopkins, Baltimore, MD.

068 (D) A Comparison between Impedance-Based and Pressure-Based Plethysmography for Aerosol Dosimetry Calculations
J. KLINE 1, P. DABISCH, L. PITT; USAMRIID, Frederick, MD.

069 (D) Detection of Bacillus anthracis and Yersinia pestis in Sputum Using Real-Time PCR
K. MITCHELL 1, M. KELLMAN 1, J. SHELDON 1, M. SHAIL 1, C. KELLY-CIRINO 1, C. EGAN 1; 1New York State Dept. of Hlth., Latham, NY, 2CDC, Atlanta, GA.

070 (D) Development of a Combined Elisa Microarray and Activity Assay Platform for Sensitive and Quantitative Detection of Biodefense Toxins
Y. ZHANG 1, S. KALB 1, J. BARR 1, J. LOU 1, J. D. MARKS 1, S. M. VARNUM 1; 1Pacific Northwest Natl. Lab., Richland, WA, 2CDC, Atlanta, GA.

071 (E) Application of Genotyping, Microarray and Sequencing Technologies to Detect Biothreat Agents from Complex Environmental Samples
C. JAING, J. THISSEN, J. WOLLARD, A. HINCKLEY, P. J. JACKSON; Lawrence Livermore Natl. Lab, Livermore, CA.

072 (E) A New Recombinant Ricin Agent-Like Material Simulant
S. L. PARKER, R. D. BUCKINGHAM; Life Sci. Div., Dugway Proving Ground, Dugway, UT.

073 (E) Viral Hemorrhagic Fever in Ukraine
I. LOZYSKY 1, O. TARASYUK; Lviv Res. Inst. of Hygiene and Epidemiology of Ukrainian Ministry of Hlth., Lviv, Ukraine.

074 (E) Influence of Growth Media and Washing on the Spectral Laser Induced Fluorescence Signature of Biological Simulant in a Standoff Detection Context
C. LAFLAMME 1, J. SIMARD 1, S. BUTEAU 1, P. LAHAJE 1, G. ROY 1, P. MATHIEU 1, B. DÉRY 1; 1Institut National de Recherche Sci. et Tech., Gif-sur-Yvette, France.

075 (E) Production of MAbs Directed against Bacillus anthracis Spore and Development of Immunological Assays
N. MOREL 1, D. DANO, P. LAMOURETTE, C. CRÉMINON, H. VOLLAND; CEA, Gif-sur-Yvette, France.
076 (E) Mass Spectrometry Based Functional Assay for Rapid Detection of Ricin
M. ANTOINE, N. HAGAN, J. LIN, A. FELDMAN, P. DEMIREV, JHU-APL, Laurel, MD.

077 (F) Genome Engineering in Bacillus anthracis Using Cre Recombinase
A. P. POMERANTSEV, S. H. LEPPLA; NIH/NAID, Bethesda, MD.

078 (F) The Role of Gene Fragmentation in Orthopoxvirus Evolution
E. L. HATCHER, R. C. HENDRICKSON, E. J. LEFKOWITZ; UAB, Birmingham, AL.

079 (F) Effect of Sequencing Errors on Rare Variant Detection in Bacterial Samples
V. Y. FOFOANO1, J. HOWARD 2, T. CONSTANTIN 3, M. SHIN 3,4; Eureka Genomics, Houston, TX, 1Univ. of Houston, Houston, TX, 2Investgen, Hercules, CA, 3Eureka Genomics, Hercules, TX, 4Wyss Inst. for Biologically Inspired Engineering, Cambridge, MA.

080 (G) Multifaceted Potentiation of Antibiotics via Silver Salts
J. WINKLER1, R. MORONES 1, C. SPINA 1,4, J. COLLINS 1,4; Boston Univ., Boston, MA, 1Program in Molecular Biology, Cell Biology, and Biochemistry, Boston, MA, 2Boston Univ./HHMI, Boston, MA, 3Wyss Inst. for Biologically Inspired Engineering, Cambridge, MA.

081 (G) Discovery of New Antimicrobial Compounds Inhibiting Burkholderia pseudomallei
S. GOLDMAN, G. LIU, N. KIM; Evolva, Inc., Palo Alto, CA.

082 (G) Pharmacokinetics of a Monoclonal Anthrax Anti-Toxin Antibody in Healthy and Infected Cynomolgus Macaques
E. LEFFEL, V. RIDDLE, M. MELDORF, J. BOURDAGE; PharmAthene, Inc., Annapolis, MD.

083 (G) Broad-Spectrum Bactericidal Activity of Full-Length Immobilized Antimicrobial Peptides

084 (G) Ongoing Search for Broad Spectrum Antimicrobials

085 (G) High-Throughput Assays Identify Small-Molecule Inhibitors of Alphaviruses
K. SPURGERS1, C. R. HURT 2, V. R. LINGAPPA 3, P. J. GLASS 1; USAMRIID, Frederick, MD, 1Prosetta Bioconformatics, Inc., San Francisco, CA.

086 (G) CD28 Mimetic Peptide Treatment for Superantigen and Endotoxin-Induced Lethal Shock
A. S. CROSS1, G. RAMACHANDRAN 1, S. M. OPAL 2, G. ARAD 1, R. KAEMPFER 1; 1Univ. of Maryland Sch. of Med., Baltimore, MD, 2Brown Univ., Providence, RI, 1The Hebrew Univ., Hadassah Med. Sch., Jerusalem, Israel.

087 (G) Inhibition of the Yersinia pestis Type-III Secretion System by Small Molecules
D. CARMANY1,2, M. GUELTA 1, T. HENDERSON 2; 1Edgewood Chemical Biological Ctr., APG, MD, 2Battelle, Columbus, OH.

088 (G) In Vitro and In Vivo Efficacy of Novel Broad-Spectrum Antiviral Compounds
E. STAVALE 1, K. ANTHONY 2, D. FERNANDEZ 1, D. REED 1, K. WARFIELD 1, M. J. AMAN 1, B. KLOSE 2, U. RAMSTEDT 2; 1Integrated Biotherapeutics, Gaithersburg, MD, 2Unither Virology, Silver Spring, MD.

090 (G) Fosmidomycin and Derivatives as Antimicrobial Agents against Francisella tularensis Subsp. Novicida
E. MCKENNEY 1, E. UH 1, E. JACKSON 2, G. SAN JOSE 1, R. COUCH 1, C. DOWD 1, M. VAN HOEK 1,2; George Mason Univ., Manassas, VA, 3George Washington Univ., Washington, DC.

091 (H) Post-Exposure Vaccination with Smallpox Vaccines Is Protective in Low-Dose, but Not High-Dose, Monkeypox Infected Black-Tailed Prairie Dogs
M. S. KECKLER, D. S. CARROLL, J. S. SALZER, M. B. TOWNSEND, K. L. KAREM, I. K. DAMON; CDC, Atlanta, GA.

092 (H) Ebola Virus Glycoprotein Fc Fusion Protein Confers Protection against Lethal Challenge in Vaccinated Mice
K. KONDURU, S. B. BRADFUTE 1, J. JACQUES 1, M. MANANEGGREENWARAN 3, S. NAKAMURA 1, S. MORSHED 1, S. C. WOOD 1, S. BAVARI 1, G. G. KAPLAN 1, 2CBER/FDA, Bethesda, MD, 2United States Army Med. Res. Inst. of Infectious Diseases, Ft. Detrick, MD, 3CDRH/FDA, Silver Spring, MD.

093 (H) Delayed Challenge of BALB/c Mice Demonstrates Significant Vaccine-Induced Protection against Aerosolized Yersinia pestis CO92
K. A. OVERHEIMP, N. KIKENDALL 1, J. WILDER 1, I. FISHER 1, C. STIDLEY 1, T. BRAS2L 1, E. BARR 1, R. SHERWOOD 1; Lovelace Respiratory Res. Inst., Albuquerque, NM, 1Univ. of New Mexico, Albuquerque, NM.

094 (H) Development of a Rabbit Vaccination and Inhalational Challenge Model for Evaluation of Next Generation Vaccine Candidates for Francisella tularensis
S. MARTIN1, W. SOSNA 3, K. McCURDY 3, R. TUTTLE 3, D. PINSON 3, M. PARMELY 1, J. SHERAER 1, C. CROUTCH 1, L. WOLFRAIM 1; 2DynPort Vaccine Co., Frederick, MD, 3Midwest Res. Inst., Kansas City, MO, 1Univ. of Kansas Med. Ctr., Kansas City, KS.

095 (H) Generation of Fowlpox-Based Reombinants Expressing A27L, A33R, B5R and L1R Vaccinia Virus Genes as Safer Smallpox Vaccine

096 (H) Development of BSL-2 Neutralization Assay for Filoviruses: Proof of Concept Study
J. JACQUES, K. KONDURU, M. MANANEGGREENWARAN, G. G. KAPLAN; CBER/FDA, Bethesda, MD.

097 (H) Construction and Immunogenicity of a Modified Vaccinia Virus L1 Gene-Based DNA Vaccine

098 (H) Assessment of Viraemia in Vaccinated and Unvaccinated Primates after Aerosol Challenge with Monkeypox
099 (I) Characterization of Antibodies Specific for Hemagglutinin and Neuraminidase Proteins of the 1918 and 2009 Pandemic H1N1 Viruses
Y. LIU 1, X. LIU 1, J. FANG 2, X. SHEN 2, W. CHEN 2, X. LIN 1, H. LI 2, W. TAN 1, Y. WANG 1, P. ZHAO 1, Z. QI 1; 1Second Military Med. Univ., N. Shanghai, China, 2Tongji Univ. Hosp., Shanghai, China, ©CDC of Fujian Province, Fuzhou, China, ©CDC, Beijing, China, 3China CDC, Beijing, China.

100 (I) Antibacterial Role for Natural Killer Cells in Host Defense to Bacillus anthracis
C. M. GONZALES 1, C. B. WILLIAMS 1, S. T. MOEN 1, V. L. POPOV 1, W. B. BAZE 1, J. W. PETERSON 1, J. J. ENDSLEY 2; 1Univ. of Texas Med. Branch, Galveston, TX, 2Univ. of Texas, M.D. Anderson Cancer Ctr., Bastrop, TX.

101 (I) Internalization of Bacillus anthracis Spores by Cells Resident in the Human Lung May Contribute to Dissemination

102 (I) Serological Immune Response to Cutaneous Anthrax Infection and Booster of Live Attenuated Anthrax Vaccine
N. TRAPAIDZE 1,2, A. DEBES 1, W. WEBSTER 1, S. LITTLE 1, N. CHITADZE 1, T. KUCHULORIA 1, S. SAGHINADZE 1, N. TSERTSVADZE 1, R. RIVARD 1, R. MODY 1, S. TSANAVA 1, P. IMNADZE 1, E. DYSON 4, A. SIMPSON 4, M. HEPBURN 4; 1Natl. Ctr. for Disease Control and Publ. Hlth., Tbilisi, Georgia, 2Techn. Management Co., Tbilisi, Georgia, ©USAMRIID, Ft. Detrick, MD, ©DSTL, Porton Down, United Kingdom.

103 (I) Mechanistically-Based Computational Model of the Host Immune Response to Biological Warfare Agents: Application to Tularemia
C. HACK, E. J. FLEMING, P. E. ANDERSON, M. K. MAKLEY, P. J. ROBINSON, J. M. GEARHART; AFRL, Wright-Patterson AFB, OH.

104 (J) Killing of Staphylococcus haemolyticus on Metallic Copper Surfaces
C. ESPIRITO SANTO 1,2, D. QUARANTA 1, P. V. MORAIS 1, G. GRASS 2; 1Univ. de Coimbra, Coimbra, Portugal, 2Univ. of Nebraska, Lincoln, NE.

105 (J) Advanced Management of Biological Threats (AMBIT): Raising Awareness Towards Biological Incidents in Primary Care and Public Health
M. RICHTER, C. BARTELS, W. BIEDERBICK; Robert Koch-Instit., Berlin, Germany.

106 (J) Legal Framework for Decontamination and Containment of Biological Weapons in the Former Soviet Union Countries: Case Study of Defense Threat Reduction Agency’s Anthrax Decontamination of Voz. Island, Uzbekistan
A. S. UMAROV, V. SUTTON, B. SHERWIN, S. BEBOUT, A. KATILIUTE; Texas Tech Univ. Sch. of Law, Lubbock, TX.

108 (K) Toward a Better Referee: Tracking Simulant Disseminations Using Molecular Dyes

109 (K) Accelerating R&D with Target Product Profiles
W. K. MILHOUSE 1, T. UNNASCH 1, D. McCLUSKEY 1, A. CANNONS 1, A. VAN OLFEN 1, D. LIM 1, J. McCLUSKEY 1, T. POSTLETHWAITE 2, A. ZULICH 3, C. WICK 1; 1USF Coll. of Publ. Hlth., Tampa, FL, 2USF Draper Labs, Tampa, FL, ©AMSRD-ECB-RT-DD, APG, MD.

110 (K) Clinical and Epidemiologic Characteristics of Cutaneous Anthrax Infection in Georgia
T. KUCHULORIA 1,2, N. TSERTSVADZE 1, M. ENDELADZE 4, N. GELASHVILI 4, R. RIVARD 4, R. MODY 4, S. TSANAVA 1,2, M. J. HEPBURN 4, N. TRAPAIDZE 1,2, P. IMNADZE 1,2; Tbilisi State Univ., Tbilisi, Georgia, ©Clinical Res. Unit, TMC, Tbilisi, Georgia, ©Natl. Ctr. for Disease Control and Publ. Hlth., Tbilisi, Georgia, ©Infectious Pathology, AIDS and Clinical Immunology Res. Ctr., Tbilisi, Georgia, ©Rustavi Central Hosp., Rustavi, Georgia, ©USAMRIID, Ft. Detrick, MD.

111 (K) Development and Validation of Animal Models to Support Testing of Vaccines and Therapeutics
B. ASTROFF, C. CROUTCH, J. RAYNER, R. TUTTLE; Midwest Res. Inst., Kansas City, MO.

**Highlighted Oral Abstract Presentations**

**008. Vaccine Viruses**

**Monday, February 7, 2011 | 3:00 PM – 4:00 PM Diplomat Ballroom**

Upon completion of this session, the participant should be able to:

- Identify and describe vaccines and critical immune response concepts related to biodefense and emerging diseases.

**MODERATOR:**

**MARTHA E. BLOOM, MD; DIR/NIAID/NIH, Hamilton, MT.**

**PRESENTATIONS:**

**3:00 PM**

**112 Efficacy of IMVAMUNE: A Third Generation Smallpox Vaccine Candidate in a Lethal Monkeypox Virus Nonhuman Primate Inhalation Model**

T. BRASEL, K. AGANS, T. BAILEY, S. STORCH, A. CAWTHON; Lovelace Respiratory Res. Inst., Albuquerque, NM.

**3:15 PM**

**113 Evaluation of the Nasal and Sublingual Mucosa as Sites for Rapid Induction of Immunity to Ebola**

M. CROYLE 1, J. CHOI 1, S. SCHAFFER 1, L. ZHANG 2, T. JUELICH 2, A. FREIBERG 6; 1Univ. of Texas, Austin, TX, 2Univ. of Texas Med. Branch, Galveston, TX.

**3:30 PM**

**114 A Virus-Like Particle-Based Vaccine Candidate as a Countermeasure for Rift Valley Fever Virus**

3:45 PM

**115 Pre-Existing Immunity to Vaccinia: Does It Affect the Efficacy of Recombinant MVA Vectored Vaccines?**

J. BREWOO\(^1\), T. POWELL\(^2\), C. PARTIDOS\(^3\), D. STINCHCOMB\(^1\), J. OSORIO\(^1\); \(^1\)Inviragen, Madison, WI, \(^2\)Inviragen, Fort Collins, CO, \(^3\)Univ. of Wisconsin, Madison, WI.

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3:40 PM

**120 A Live Attenuated Strain of *Yersinia pestis* KIM5+ as a Vaccine against Plague**

W. SUN\(^1\), D. SIX\(^2\), X. KUANG \(^1\), K. L. ROLAND\(^1\), C. R. H. RAETZ \(^2\), R. CURTISS III \(^1\); \(^1\)The Biodesign Inst., Tempe, AZ, \(^2\)Duke Univ. Med. Ctr., Durham, NC.

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### HIGHLIGHTED ORAL ABSTRACT PRESENTATIONS

**009. Vaccines Bacteria**

Monday, February 7, 2011 | 3:00 PM – 4:00 PM Palladian Ballroom

*Upon completion of this session, the participant should be able to:*

- Describe the experimental approaches being evaluated to develop protective vaccines against bacterial biothreat agents;
- Identify approaches to vaccine development including delivery platforms and novel adjuvant systems which can be applied to other vaccines; and
- Discuss issues to be considered when moving vaccines from the experimental phase through to licensing.

**MODERATOR:**

PETRA C. F. OYSTON, PhD; DSTL, Salisbury, United Kingdom.

**PRESENTATIONS:**

**3:00 PM**

**116 Passive Transfer of Serum Collected from Humans and Animals Vaccinated with a Recombinant Plague Vaccine (rf1v) Protects Mice from aerosol Challenge with *Yersinia pestis***

P. FELLOWS\(^1\), J. PRICE\(^1\), W. LIN\(^2\), A. ROM\(^2\), S. MARSHALL\(^1\), L. HOLLAND\(^2\), M. BOLANOWSKI\(^1\), D. NYAKITI\(^2\), C. PARTIDOS\(^3\), W. P. MCNAIR, MD; \(^1\)BiolStat Solutions, Mount Airy, MD, \(^2\)Dynport Vaccine Co., LLC, Frederick, MD, \(^3\)Ill Res. Inst., Chicago, IL, \(^4\)BioStat Solutions, Mount Airy, MD.

**3:10 PM**

**117 Cellular Immune Responses to Anthrax, Plague and Tularemia Vaccines***

I. J. THOMPSON, E. DYSON, M. STOKES, N. BAILEY, J. PRIOR, A. SIMPSON; DSTL, Salisbury, United Kingdom.

**3:20 PM**

**118 Evaluation of a Novel Vaccine for Post-Exposure Prophylaxis against Anthrax in Guinea Pigs***

P. A. DABISCH\(^1\), D. NYAKITI\(^1\), J. YEAGER\(^1\), V. SAVRANSKY\(^2\), L. LEMIALE\(^2\), B. IOBIN\(^2\), S. PARK\(^2\), G. NABORS\(^2\), M. SKIADOPoulos\(^2\), M. L. PITT\(^1\); \(^1\)USAMRIID, Frederick, MD, \(^2\)Emergent Biosolutions Inc., Rockville, MD.

**3:30 PM**

**119 Francisella novicida Outer Membrane Vesicles: Novel Vaccine and Possible Role in Virulence***

T. PIERSON\(^1\), D. MATRAKAS\(^1\), V. MOROZOV\(^2\), W. ZHOU\(^1\), M. L. VAN HOEK\(^1\); \(^1\)George Mason Univ., Manassas, VA, \(^2\)Inst. of Theoretical and Experimental Biophysics, Russian Academy of Sci., Pushchino, Russian Federation.
Symposium Session

011. *Coxiella burnetii*: An Emerging Biothreat  
Monday, February 7, 2011 | 4:15 PM – 6:15 PM  
Diplomat Ballroom

This symposium will begin by exploring the natural history of *C. burnetii* and the link to, and description of Q fever in humans. An important milestone for *C. burnetii* research, culturing techniques using cell-free growth will be discussed, along with comparative genomics and virulence mechanisms. The session will also include studies on the recent outbreak of Q fever in the Netherlands, and conclude with a description of efforts to survey genotypes of *C. burnetii* across space and time.

Upon completion of this Symposium Session, the participant should be able to:

- Discuss the natural patterns and processes of *C. burnetii* and Q fever;
- Describe the current tools necessary for studying this pathogen in the laboratory, the environment, and during an outbreak; and
- Identify where new research is needed.

**MODERATORS:**

PETRA C. F. OYSTON, PhD; DSTL, Salisbury, United Kingdom.
TALIMA PEARSON, PhD; Northern Arizona Univ., Flagstaff, AZ.

**PRESENTATIONS:**

4:15 PM
125 Q Fever in the Netherlands: Queries Raised and Queries Answered
YVONNE VAN DUYNHOVEN, PhD; Nat. Inst. of Publ. Hlth. and the Environment, Bilthoven, Netherlands.

4:40 PM
126 The Clinical Spectrum of Q Fever
THOMAS J. MARRIE, MD; Dalhousie Univ., Halifax, Canada.

5:05 PM
127 Q Fever in the USA: A Rare Infection or a Bioweapon in Your Backyard?
ROBERT MASSUNG, PhD; CDC, Atlanta, GA.

5:30 PM
128 Phylogeography and Subtyping of *Coxiella burnetii*
TALIMA PEARSON, PhD; Northern Arizona Univ., Flagstaff, AZ.

5:55 PM
129 Q Fever and War: Past, Present, and Future
JOSHUA D. HARTZELL, MD; Walter Reed Army Med. Ctr., Washington, DC.

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Symposium Session

012. Have Diagnostics: Will Travel?  
Monday, February 7, 2011 | 4:15 PM – 6:15 PM  
Palladian Ballroom

This session will address the reality of implementing non-nucleic acid (NA) ligand based multiplex detection technologies in field, bedside and resource poor environments. Honest appraisals of the need for reagent supply, ligand ruggedness (and potential regenerability—is it needed or are they single use throw away), instrument durability should enable the audience to understand the strengths and weaknesses of the systems. Within biodefense arenas, how can the systems be fooled? Does the advent of synthetic and armored NA and their potential for mis-use drive a search for more complex multi-faceted appraisals of a potential agent (e.g. antibodies to quaternary structures?) to avoid false positives?

Upon completion of this Symposium Session, the participant should be able to:

- Review the diversity of non-nucleic acid ligand based (antigen.marker detection) multiplex technologies;
- Describe the reality of implementing the systems in various environments;
- Identify hurdles in developing the respective technologies and methods;
- Identify obstacles to the smooth operation in field (if they have made it out), particularly harsher environments for long periods; and
- Explain Achilles heels that need to be addressed for the systems of the future.

**MODERATORS:**

JEAN L. PATTERSON, PhD; Southwest Fndn. for Biomedical Res., San Antonio, TX.
ANDREW HAYHURST, PhD; Southwest Fndn. for Biomedical Res., San Antonio, TX.

**PRESENTATIONS:**

4:15 PM
130 Low-Cost, LED-Based xMAP Analyzer for Multiplex Diagnosis and Environmental Detection of Biological Agents
AMY L. ALTMAN, PhD; Luminex Corp., Austin, TX.

4:40 PM
131 Programmable Bio-Nano-Chips and Advanced Diagnostic Devices for the Point of Need
NICOLAS CHRISTODOULIDES, PhD; Rice Univ., Houston, TX.

5:05 PM
132 The Development of Broad-Spectrum Viral Microarray Assays for Point-of-Care Settings
CHARLES Y. CHIU, MD, PhD; Univ. of California, San Francisco, CA.
Many pathogens that are of concern as potential bioweapons can infect the host via different routes. Often the disease manifestation, and therefore the ensuing immune response, is different at these varied sites of infection, e.g., dermal infection versus pulmonary infection. It is important to understand the specific immunological hurdles and/or conditions present at these different sites of infection to develop more effective vaccines, therapeutics and prophylaxes against these important pathogens.

Upon completion of this Symposium Session, the participant should be able to:

• Assess the immunological features in different organs that may contribute or exacerbate disease; and
• Identify specific examples of pathogens that target either mucosal or peripheral tissues (liver, spleen) and the mechanisms by which these pathogens have overcome host immunity at these sites to cause disease.

MODERATORS:
MARSHALL E. BLOOM, MD; DIR/NIAID/NIH, Hamilton, MT.
REBECCA ANDERSON, PhD; Rocky Mountain Lab/NIAID/NIH, Hamilton, MT.

PRESENTATIONS:
4:15 PM
135 Organ Specific Regulation of Innate Immunity: Implications and Applications
EYAL RAZ, MD; Univ. of California, La Jolla, CA.

4:45 PM
136 Differential Host Response for Control of Francisella tularensis in the Lung and Spleen
REBECCA ANDERSON, PhD; Rocky Mountain Lab/NIAID/NIH, Hamilton, MT.

5:15 PM
137 Salmonella and the Gut Mucosal Barrier: Dichotomies of Help and Harm
MANUELA RAFFATELLU, MD; Univ. of California, Irvine, CA.

4:45 PM
138 Immunopathogenesis of Dengue Virus Infections
ALAN L. ROTHMAN, MD; Univ. of Massachusetts Med. Sch., Worcester, MA.
Tuesday, February 8, 2011

**Plenary Session**

**015. Jamming Nanomachines**

Tuesday, February 8, 2011 | 8:30 AM – 12:00 PM  
Regency Ballroom

This session will present a range of examples of molecules exhibiting intradomain and interdomain movements and describe the potential (or lack of) for interference.

*Upon completion of this Plenary Session, the participant should be able to:*

- Review the diversity of biothreat nanomachines (coat proteins, toxins, etc.);
- Describe the dynamics of molecular movements; and
- Identify potential novel targets for antivirals, antitoxins, etc.

**MODERATORS:**

JEAN L. PATTERSON, PhD; Southwest Fndn. for Biomedical Res., San Antonio, TX.


**PRESENTATIONS:**

**8:30 AM**

143 Blocking Botulism: A Journey into Modules and Modulators  
MAURICIO MONTAL, MD, PhD; Univ. of California, La Jolla, CA.

9:30 AM

Coffee Break

10:00 AM

144 Plugging Toxin Channels  
JOHN COLLIER, PhD; Harvard Univ. Med. Sch., Boston, MA.

10:30 AM

145 Models of Viral Assembly: Ebola and Lassa  
ERICA OLLMANN SAPHER, PhD; The Scripps Res. Inst., La Jolla, CA.

11:00 AM

146 Toxin Thermal Instability as a Therapeutic Target  
KEN TETER, PhD; Univ. of Central Florida, Orlando, FL.

11:30 AM

147 Atomic Model of the Human Adenovirus by cryoEM  
Z. HONG ZHOU, PhD; UCLA, Los Angeles, CA.

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**Poster Session**

**016. Tuesday Poster Session**

Tuesday, February 8, 2011 | 1:00 PM – 3:00 PM  
Exhibit Hall

**PRESENTATIONS:**

148 (A) Genetic and Phenotypic Changes Associated with Passage of Eastern Equine Encephalitis Virus and Japanese Encephalitis Virus Under Selective Pressures  

149 (A) Hematopoietic Progenitor Cells in Dengue Virus Infection  
G. PERNG, S. NOISAKRAN, H. HSAIO, K. B. CLARK, F. VILLINGER, A. ANSARI; Emory Univ., Atlanta, GA.

150 (A) Development of Differential Molecular and Immuno-Assays for Identification and Characterization of Filovirus Sub-Types  

151 (A) EV-075-2 Has a Novel Host-Based Mode of Action and Is Effective against Influenza and Several Hemorrhagic Fever Viruses  
A. S. SORENSEN, G. SALERNO, R. HERMOSILLA, P. ALBERTS, C. E. SCULLY, G. OLINGER, R. CARRION, N. WILLIAMS; Evolva SA, Reinach, Switzerland, 2USAMRIID, Fort Detrick, MD, 3SFBR, San Antonio, TX, 4KWS BioTest, Bristol, United Kingdom.

153 (A) Dengue Virus Infection and Modulation of Human Endothelial Cells  
N. A. DALRYMPLE, E. R. MACKOW; Stony Brook Univ., Stony Brook, NY.

154 (A) NFκB Mediated Modulation of Host Response Following Infection by Rift Valley Fever Virus  
A. NARAYANAN, C. B. BAILEY, F. KASHANCHI; George Mason Univ., Manassas, VA.

155 (A) Oxidative Stress and Stress-Induced Host Responses Following RVFV Infection  
A. NARAYANAN, C. B. BAILEY, T. POPOVA, S. TURELL, C. BAILEY, F. KASHANCHI; George Mason Univ., Manassas, VA, 2USAMRIID, Fort Detrick, MD.

156 (A) Influence of Influenza on Adenoviral Infection Detection in Basic Military Trainees at Lackland Air Force Base  

157 (A) Cowpox Virus as a Model of Human Hemorrhagic Smallpox  
R. F. JOHNSON, A. JOHNSON, L. SMITH, J. PARAGAS, J. E. BLANEY, P. B. JAHRLING; NIH, Bethesda, MD.

158 (B) Determination of Inactivation of C. botulinum Neurotoxin via In Vitro Activity Assay  
M. E. JOHANSEN, K. E. KEESTERSON, R. BRUCE, M. ROBINSON; Jacobs Techn., Dugway, UT, 2Dugway Proving Ground, Dugway, UT.
159 (B) Neurotoxicity and Pathogenesis of 6 B. anthracis Related Environmentally Isolated Bacteria Members of the B. cereus Group

S. SAMAN, Y. VISNICK, P. SAMAN, S. SAMAN; Mass. Bay Community Coll., Wellesley, MA.

160 (B) Production of 70 MAbs Directed against One or Several Strains of Salmonella

P. LAMOURETTE, C. FERUAUD-TARISSE, C. CRÉMINON, H. VOLLAND; CEA, Gif sur Yvette, France.

161 (B) Characterization of the Natural History of Inhalational Melioidosis in Non-Human Primate Models

J. YEAGER, P. A. DABISCH, P. FACEMIRE, D. WAAG, M. L. M. PITT; USAMRIID, Fort Detrick, MD.

162 (B) Proficiency Testing for Biological Agents

J. L. GRAY, W. D. RAWLINSON; 1RCPA QAP, Sydney, Australia, 2The Prince of Wales Hosp., Sydney, Australia.

163 (B) Cardiac Changes in the Rabbit Model Following Administration of Anthrax Lethal Toxin

W. S. LAWRENCE, J. R. MARSHALL, D. L. ZAVALA, L. E. WEAVER, W. B. BAZE, T. MOEN, E. B. WHORTON, J. W. PETERSON; 1Univ. of Texas Med. Branch, Galveston, TX, 2Univ. of Texas M.D. Anderson Cancer Ctr., 3Bastrop, TX.

164 (B) Impairment of Macrophage Bioenergetics by Bacillus anthracis Derived Nitric Oxide

M. CHUNG, T. G. POPOVA, A. NARAYANAN, C. L. BAILEY, S. G. POPOV; George Mason Univ., Manassas, VA.

165 (B) Microbial Forensic Investigation of a Yersinia pestis Outbreak in Afghanistan


166 (B) Zeptamole Detection of Anthrax Edema Factor Activity by LC-ESI-MS/MS


167 (B) Molecular Typing of Yersinia pestis Outbreak Strains from the West Nile Region of Uganda

L. B. RESPICIO, B. M. YOCKEY, M. E. SCHRIEFER, J. M. PETERSEN; CDC, Ft. Collins, CO.

168 (B) Identification of SNPs Responsible for Antibiotic Resistance in Biothreat Agents by DNA Microarrays and Illumina Sequencing

J. B. THISSEN, C. JAING, L. VERGEZ, F. BOURGUET, K. MCLAUGHLIN, S. MABERY, P. J. JACKSON; LLNL, Livermore, CA.

169 (B) Susceptibilities of Novel Antimicrobial Compounds against Multiple Bioterrorism Agents

A. CROOK, K. PEARSON, N. JONES, R. GODWIN, J. VIPOND, A. D. G. ROBERTS; HPA, Salisbury, United Kingdom.

170 (B) Pneumonic Tularemia in Rabbits: Utility of X-Rays and Laboratory Diagnostics in Predicting Outcome

D. S. REED, T. DUNSMORE, L. SMITH, A. TRICHEL, K. S. COLE, E. BARRY; 1Univ. of Pittsburgh, Pittsburgh, PA, 2Univ. of Maryland, Baltimore, MD.

171 (B) Susceptibility of Bacillus anthracis Isolates from a UK Outbreak in Intravenous Drug Users against a Range of Antimicrobial Agents and a Novel Compound

K. PEARSON, A. PANG, J. VIPOND, A. D. G. ROBERTS; HPA, Salisbury, United Kingdom.

172 (B) Characterization and Aerosol Uniformity of a Whole-Body Exposure System

S. FREDERICK, P. A. DABISCH; USAMRIID, Frederick, MD, 2USAMRIID, Frederick, MD.

173 (B) An In Silico Model of Endotoxic Shock

M. MAKEY, E. HACK, J. GEARHART; HIF 711th HPW WPABF, Dayton, OH.

174 (B) Attenuation and Protective Efficacy of a Type A Strain of Francisella tularensis Complemented for Loss of O-Antigen

C. RYDER, G. BERG, J. LI, T. J. INZANA; Virginia Tech, Blacksburg, VA.

175 (B) Francisella novicida QseC and Novel Motility Phenotype Is Inhibited by Norepinephrine

E. SCHIFFHAUSER, E. MCKENNEY, M. L. VAN HOEK; George Mason Univ., Manassas, VA.

176 (B) Evaluation of Real-Time PCR Assays for the Detection of Brucella Spp. in Human Clinical Samples in Georgia


177 (D) Fast and Reliable Identification of Bacteria Based Biological Warfare Agents by the Maldi Biotyper

T. ELSSNER, T. MAIER, M. KOHRZEWAS; 1Bruker Detection, Leipzig, Germany, 2Bruker Daltonik, Bremen, Germany.

179 (D) Development of an Internal Positive Control Molecular Inversion Probe Assay


180 (D) Determination of Regions of Variability in Bacillus Species Using Optical Mapping as Diagnostic Indicators

C. BALDWIN, S. COYNE, T. MINOGUE; USAMRIID, Frederick, MD.

181 (D) Association of Early Lethal Factor Levels with Survival Time and a Point of No Return in Rhesus Macaques and NZW Rabbits with Inhalation Anthrax

182 (D) Evaluation of Phenotypic Characterization Methods for Diagnostic Implications Using *Bacillus* Species
A. A. SHEA\(^1\), C. D. BALDW\(^1\), M. A. SHIPLEY\(^1\), T. D. MINOGUE\(^1\), M. J. WOLCOTT\(^1\); \(^1\)USAMRIID, Frederick, MD, \(^2\)Ke’aki Techn., LLC, Frederick, MD.

183 (D) Lethal Toxin Complex and Total Lethal Factor Levels in Rhesus Macaques and N2Z Rabbits with Inhalation Anthrax
M. G. CANDELA\(^1\), R. C. LINS\(^1\), C. P. QUINN\(^1\), A. R. WOOLFITT\(^1\), G. MEISTER\(^1\), J. R. BARR\(^1\), A. E. BOYER\(^1\); \(^1\)CDC, Atlanta, GA, \(^2\)CDC/Battelle, Atlanta, GA, \(^3\)Battelle BioMed. Res. Ctr., Atlanta, GA.

184 (D) Rapid, On-Chip Electrochemical Detection of Salmonella
A. S. PATTERSON, B. S. FERGUSON, H. T. SOH, K. W. PLAXCO; Univ. of California, Santa Barbara, CA.

185 (D) Development of Monoclonal Antibodies against Plasminogen Activator Pla of *Versinia pestis*

186 (D) Development of Inhibitor Resistant Real-Time PCR Methods for Biothreat Agent Detection
A. TROMBLEY, A. ZOVANYI, T. MINOGUE; USAMRIID, Frederick, MD.

187 (D) Real-Time Assays for *hms* and *ip2* in a High Pathogenicity Island Required for Virulence in *Versinia*

188 (D) Multiplexed Diagnostic Assays Applied to the Detection of Biothreat Agents in Complex Food Matrices
A. CARRILLO, J. OLIVAS, A. ROSA, P. MARAGHI ARANI; Lawrence Livermore Natl. Lab., Livermore, CA.

189 (E) Multiplex Detection of Category A and B Biothreat Pathogens
M. JONES, A. TAYLOR, C. OSWALD, R. CRISP; Idaho Techn., Salt Lake City, UT.

190 (E) Development and Characterization of Novel Monoclonal Antibodies to *Coxiella burnetii*
S. HAYWARD, J. RANCHES, E. FULTON; Defence Res. and Dev. Can. – Suffield, Medicine Hat, Can. –

191 (E) Field Based Real-Time PCR Detection of Biothreat Pathogens without Sample Extraction or Purification
A. BIRD\(^1\), D. KADAVY\(^1\), A. VINAS\(^2\), L. ALLEN\(^1\), N. WESTFALL\(^1\), K. HOOSIEN\(^1\), M. RENDON\(^1\), C. CHRISTENSEN\(^1\), J. GARDNER\(^1\), R. TRAUSCHT\(^1\), M. VAUGHN\(^1\), R. CRISP\(^1\); Idaho Techn., SLC, UT, \(^2\)Signature Sci., Austin, TX, \(^3\)Southwest Fdn. for BioMed., San Antonio, TX, \(^4\)Battelle BioMed. Res. Ctr., Columbus, OH.

192 (E) Development of a Real-Time Quantitative PCR Assay to Detect of *Cryptosporidium parvum* Oocysts in Soil
Z. LIANG\(^1\), A. KEELEY\(^1\); \(^1\)Natl. Res. Council Associate, Ada, OK, \(^2\)U.S. EPA, Ada, OK.

193 (E) Evaluation of the PLEX-ID Biothreat Kit for Use with Environmental Air Samples
N. MULHOLLAND\(^1\), C. HARTER\(^1\), N. WAYBRIGHT\(^1\), M. FRINDER\(^2\), D. RUBIO-APARICIO\(^2\), R. LOVARI\(^1\), YASUDA\(^2\), R. HOUSLEY\(^1\), L. BLYNN\(^2\), ECKER\(^2\), R. SAMPATH\(^2\); \(^1\)Midwest Res. Inst., Rockville, MD, \(^2\)Ibis Biosci., Carlsbad, CA.

194 (E) An In Vitro Comparison of Two Types of Viral Throat Swabs for Sampling Orthopoxviruses

195 (F) A Combined Computational/Experimental Approach for Specificity Profiling of Lead Therapeutic Agents
D. ROE, P. ANDERSON, S. ELMER, V. DESAPIO, Y. LIGHT, J. SCHOENIGER; Sandia Natl. Labs, Livermore, CA.

196 (F) Tool for Identifying Sequence Variations that Correlate with Virus Phenotypic Characteristics
B. E. PUCKETT\(^1\), M. L. LIU\(^2\), V. HUNT\(^1\), L. ZHOU\(^1\), J. DIETRICH\(^3\), S. ZAREMBA\(^1\), E. B. KLEM\(^1\), R. H. SCHEUERMANN\(^1\); \(^1\)Univ. of Texas Southwestern Med. Ctr., Dallas, TX, \(^2\)Southern Methodist Univ., Dallas, TX, \(^3\)Northrop Grumman Hlth. Solutions, Rockville, MD.

197 (G) Assessing the Therapeutic Efficacy of Intravenous Levofoxacin for the Treatment of Inhalational Anthrax in New Zealand White Rabbits
S. B. YEE\(^1\), D. N. DYER\(^2\), S. A. ORR\(^1\), M. L. M. PITT\(^1\); \(^1\)USAMRIID, Ft. Detrick, MD, \(^2\)Clinical Res. Management, Hinckley, OH.

198 (G) Comparison of Gentamicin Dose Schedule for Treatment of Infection from Aerosolized *Versinia pestis*
W. C. LIN, K. E. SIEFKAS, S. HU, B. A. GINGRAS, L. E. HOLLAND; IIT Res. Inst., Chicago, IL.

199 (G) Efficacy Evaluation of DEF201 in the Mouse Model of Venezuelan Equine Encephalitis Virus Infection
T. BABAS\(^1\), A. RIPPEON\(^1\), K. HAGELIN\(^1\), D. GOHEGAN\(^1\), J. ENNIS\(^2\), L. RHODES\(^1\), J. TURNER\(^2\), P. SILVERA\(^1\); \(^1\)Atlanta Res. and Ed. Fndn., Atlanta, GA, \(^2\)Southern Res. Inst., Frederick, MD.

200 (G) Efficacy of a Single Intravenous Administration of Anthrax Immune Globulin (AIG) in New Zealand White Rabbits
N. KAMAL\(^1\), R. BARNEWALL\(^1\), C. WILHELM\(^1\), E. MCGUINNESS\(^1\), C. P. QUINN\(^1\); \(^1\)Atlanta Res. and Ed. Fdn., Atlanta, GA, \(^2\)CDC, Atlanta, GA, \(^3\)Battelle BioMed. Res. Ctr., Columbus, OH.

201 (G) Immune Augmentation of Chemotherapy against Tularemia
M. SUTHERLAND, R. TROYER, S. DOW, J. BELISLE; Colorado State Univ., Ft. Collins, CO.

202 (G) Liposomal Botulinum Toxin Heavy Chain Targets Neuronal Cells via the Ganglioside Receptor

203 (G) Antibiotic Delivery Platform Enables Increased Intracellular Delivery and Killing of Virulent *Brucella abortus*

204 (G) Good Laboratory Practices (GLP) in High Biocontainment Laboratories: Challenges and Solutions
205 (G) Silver Nanoparticles Inhibit Vaccinia Virus Infection by Preventing Viral Entry
J. C. TREFRY, D. P. WOOLEY; Wright State Univ., Dayton, OH.

206 (G) Cyclohextrin Derivatives as Novel Antimicrobial Agents against Francisella
J. HIX, E. KARGINova, V. A. KARGINova, M. L. VAN HOEK; George Mason Univ., Manassas, VA, Innovative Biologics, Inc., Herndon, VA.

207 (H) Longevity of Vaccine Protection against Pneumonic Plague in BALB/c Mice

208 (H) Comparative Efficacy of Recombinant Anthrax Vaccine against Intramuscular Challenge with Anthrax Spore
J. Chun, H. Kim, G. Rhie, C. Yoo, H. Oh; NIH, Seoul, Korea, Republic of.

209 (H) F1/V Plague Vaccine Protects Cynomolgus Macaques but not African Green Monkeys from Death after Lethal Aerosol Plague Challenge Despite Inducing a Humoral Response

210 (H) Development and Pre-Clinical Evaluation of a Recombinant Ricin Vaccine (RVEc)
R. B. Reisler; Behalf of the Ricin Vaccine Integrated Product Team; USAMRIID, Frederick, MD.

211 (H) Update of an Attenuated Smallpox Vaccine LC16m8 Research
H. Yokote, T. Hanada, A. Sato, S. Maruno; Kaketsuken, Kumamoto, Japan.

212 (H) Evaluation of F. tularensis Type A Derived Live Attenuated Vaccines in the Rabbit Model
E. M. Barry, D. S. Reed, B. J. Mann, K. Cole, A. E. Santiago; Univ. of Maryland, Baltimore, MD, Univ. of Pittsburgh, Pittsburgh, PA, Univ. of Virginia, Charlottesville, VA.

213 (I) Growth Media Affects Francisella tularensis SCHU S4 Virulence in Aerosol Challenged LVS-Vaccinated Cynomolgus macaques

214 (I) Flow Cytometric Quantification of Lung Natural Killer Cell Activity Associated with TLR-3 Signalling Pathway Activation

215 (I) Assessment of Smallpox Vaccine-Induced Antibodies and Resultant Survival Following Aerosolised Monkeypox Infection

216 (I) 5 Colour Immunophenotyping to Characterize the Immune Response after Smallpox Vaccination and Aerosol Challenge with Monkeypox

217 (J) Emphasis on Impact: An Analysis of BSL-4 National Laboratory Biosafety Measures in Environmental Impact Statements
L. Lawrence; Texas Tech Sch. of Law, Lubbock, TX.

218 (J) Quantification of Bacillus anthracis Spores on Complex Surfaces
J. J. Calamiris; U.S. Army, USANCA, Fort Belvoir, VA.

219 (J) BiGRUDI: Mobile Diagnostics for Multiplex Detection of Biothreat Agents
H. Ellerbrok; Robert Koch Inst., Berlin, Germany.

220 (K)Preparing Biodefense Professionals: MS in Biotechnology Concentration in Biodefense and Certificate in National Security

221 (K) The Bundeswehr Rapidly Deployable Biolab: High-Tech Microbiological Diagnostics for Biodefense Operations Abroad
R. Wölfel; Bundeswehr Inst. of Microbiol., Munich, Germany.

222 (K) Collaborative Efforts between the Oklahoma Public Health Laboratory and Emergency Responders in Ensuring Public Safety
N. Zitterkopf-Khoury, M. Mcdermott, J. Murray; Oklahoma State Dept. Hlth., Oklahoma City, OK.

223 (K) Information Concerns for Biosecurity Labs
J. Mustin; Texas Tech Univ. Sch. of Law, Lubbock, TX.
Highlights:

017. Pathogen Host Interactions I

Upon completion of this session, the participant should be able to:
- Identify various means by which pathogens cause diseases.

MODERATOR:
DRUSILLA L. BURNS, PhD; CBER, FDA, Bethesda, MD.

PRESENTATIONS:

3:00 PM
224 Development of Pneumonic Plague is Dependent on the Immunomodulating Effects of the *Yersinia pestis* Yersiniabactin Siderophore
H. LEE-LEWIS¹, R. D. PERRY ², J. D. FETHERSTON ², D. M. ANDERSON ¹; ¹Univ. of Missouri, Columbia, MO, ²Univ. of Kentucky, Louisville, KY.

3:10 PM
225 Effect of LVS Vaccination and Growth Media on *Francisella tularensis* SCHU S4 Aerosol-Induced Serum Cytokine Response in Non-Human Primates
A. J. RIETZ ¹, M. VALDERAS ¹, Y. LIU ¹, J. HUTT ², E. J. ZINTER ¹, T. WU ², R. LYONS ²; ¹Lovelace Respiratory Res. Inst., Albuquerque, NM, ²Univ. of New Mexico, Albuquerque, NM.

3:20 PM
226 Cellular Immune Responses to Three Licensed Anthrax Vaccines
N. CHITADZE ¹, T. KUCHULORIA ¹, S. SAGHINADZE ¹, N. TSERTSVADZE ¹, M. CHUBINIDZE ¹, R. RIVARD ¹, S. TSANAVA ¹, P. IMNADZE ¹, H. DYSON ², T. LAWS ³, A. SIMPSON ³, M. HEPBURN ³; ¹Natl. Ctr. for Disease Control and Publ. Hlth. (NCD), Tbilisi, Georgia, ²Lovelace Respiratory Res. Inst., Albuquerque, NM, ³USAMRIID, Ft. Detrick, MD.

3:30 PM
227 Biology of *Francisella tularensis* Subspecies *Holarctica* in Tick Vectors
R. J. MANI, J. ABBEY, M. V. REICHARD, K. F. KOCAN, K. D. CLINKENBEARD; Oklahoma State Univ., Stillwater, OK.

3:40 PM
228 Stability of Virulent *Coxiella burnetii* Strains Grown in Host Cell-Free Media
G. J. KERSH, L. D. OLIVER, K. A. FITZPATRICK, J. S. SELF, R. F. MASSUNG; CDC, Atlanta, GA.

Highlights:

018. Pathogen Host Interactions II

Upon completion of this session, the participant should be able to:
- Describe molecular attributes of pathogens that contribute to diseases.

MODERATOR:
LISA HENSLEY, PhD; USAMRIID, Ft. Detrick, MD.

PRESENTATIONS:

3:00 PM
229 Discovery of an Ebola-Like Filovirus in Iberian Peninsula Cave Bats
G. PALACIOS ¹, A. NEGREDO ², N. SAVI ², I. LIPKIN ¹, A. TENORIO ²; ¹Columbia Univ., New York, NY, ²Centro Natl. de Microbiologia, Madrid, Spain.

3:10 PM
230 Evolution of Viral Quasispecies During Interspecies Transmission Events
M. BORUCKI ¹, J. ALLEN ¹, S. MESSENGER ², C. TORRES ¹, T. SLEZAK ¹; ¹Lawrence Livermore Natl. Lab, Livermore, CA, ²CA Dept. Publ. Hlth., Livermore, CA.

3:20 PM
231 Evaluation of the Innate Immune Response of Human Brain Endothelial Cells to Henipavirus Infection
A. N. FREIBERG ¹, T. YUN ¹, B. TIGABU ¹, T. JUELICH ¹, B. LEE ²; ¹Univ. of Texas Med. Branch, Galveston, TX, ²Univ. of California, Los Angeles, CA.

3:30 PM
232 Cell Specific Innate Immune Recognition of Rift Valley Fever Virus
M. ERMLER ¹, E. YERUKHIM ¹, K. FITZGERALD ², C. H. KING ¹, A. G. HISE ¹; ¹Case Western Reserve Univ., Cleveland, OH, ²Univ. of Massachusetts, Worcester, MA.

3:40 PM
233 Identification of Host Factors that Interact with Bunyavirus Nucleoproteins
L. ALTAMURA ¹, M. BRAMBLE ¹, N. VAN DEUSEN ¹, J. MOYER ¹, X. YE ², J. BLONDER ², T. VEESTRA ², C. SCHMALJOHN ¹; ¹USAMRIID, Ft. Detrick, MD, ²Natl. Cancer Inst., Fort Detrick, MD.
019. Antiviral Therapeutics
Tuesday, February 8, 2011 | 3:00 PM – 4:00 PM
Ambassador Ballroom

Upon completion of this session, the participant should be able to:
- Identify and explain the therapeutic research concepts related to biodefense.

MODERATOR:
COLLEEN B. JONSSON, PhD; Southern Res., Louisville, KY.

PRESENTATIONS:

3:00 PM
234 PANACEA Broad-Spectrum Antiviral Therapeutics

3:10 PM
235 Therapeutically Targeting Hantavirus Induced Endothelial Cell Permeability
E. R. MACKOW, E. GORBUNOVA, T. PEPINI, I. GAVRILOVSKAYA; Stony Brook Univ., Stony Brook, NY.

3:20 PM
236 Zaire Ebolavirus Infection Requires Access to and Is Blocked by Drugs Targeting Late Endosome-Golgi Trafficking Pathway
M. F. SAEED, R. A. DAVEY; Univ. of Texas Med. Branch, Galveston, TX.

3:30 PM
237 Development of a Smallpox Antiviral Drug for the Strategic National Stockpile

3:40 PM
238 Broad Spectrum Antiviral Therapeutic Based on Iminosugar Derivatives

020. Optimizing Clinical Care of Viral Hemorrhagic Fevers
Tuesday, February 8, 2011 | 4:15 PM – 6:15 PM
Diplomat Ballroom

Viral hemorrhagic fevers pose unique challenges for patient management because of their acute presentation and the limited treatment options currently available. This session will review existing medical interventions for viral hemorrhagic fevers and will explore the promise of new treatments such as antibody-based or anti-sense therapeutics. Parallels with sepsis interventions being implemented in Africa as well as potential issues or pitfalls in transporting and treating patients with viral hemorrhagic fevers will be discussed. In addition, the long term health consequences of patients that have survived severe hemorrhagic fever caused by infection with Ebola virus will be described.

Upon completion of this Symposium Session, the participant should be able to:
- Identify current medical care or practices for treatment of Viral Hemorrhagic Fevers;
- Describe long term health impact from Ebola virus infections; and
- Describe new therapies for the treatment of filoviruses.

MODERATORS:
LISA HENSLEY, PhD; USAMRIID, Ft. Detrick, MD.
MATTHEW L. LIM, MD; WHO, Geneva, Switzerland.
MATTHEW HEPBURN, MD; USAMRIID, Ft. Detrick, MD.
JOSEPH N. FAIR, PhD, MPH; Global Viral Forecasting, San Francisco, CA.

PRESENTATIONS:

4:15 PM
239 Current Medical Care of Patients with Viral Hemorrhagic Fevers
GIUSEPPE IPPOLITO; INMI, Rome, Italy.

4:35 PM
240 Improving Clinical Care for Hemorrhagic Fever Viruses
JAMES V. LAWLER, MD; NIAID, Ft. Detrick, MD.

4:55 PM
241 Ebola Virus Survivors from Bundibugyo, Uganda
DANIELLE V. CLARK, MPH; NIH, Ft. Detrick, MD.

5:15 PM
242 Profiling the Human Hemoral Response to Ebolavirus Sudan Gulu
LESLIE LOBEL, MD, PhD; Ben Gurion Univ., Beersheva, Israel.

5:35 PM
243 Development of an Immunoprotectant for Ebola Virus Infections
GENE OLINGER; USAMRIID, Ft. Detrick, MD.

5:55 PM
243a New and Emerging Therapies for the Treatment of Filoviruses
HEINZ FELDMANN, MD, PhD; Rocky Mountain Lab., Hamilton, MT.
Symposium Session
021. Molecular Discovery of Virus-Host Interactions
Tuesday, February 8, 2011  |  4:15 PM – 6:15 PM
Palladian Ballroom

Viruses exploit host factors to replicate. In turn the host cell combats viral infection with its own intrinsic immune system. Functional genomics represents a powerful means for discovering such viral-host interactions. These approaches can be employed in the discovery of novel targets for small molecule discovery. Likewise small molecule screens can reveal critical host-pathogen interactions. Topics will include a discussion of screen design, caveats, and interpretation, as well as follow up studies of candidate genes and small molecules found in these screens.

Upon completion of this Symposium Session, the participant should be able to:

• Review current state-of-the-art in methods employed in the identification of host targets involved in host-pathogen interactions;
• Describe various approaches in screening designs for the identification of critical host targets; and
• Assess various approaches in validation of the critical host targets.

MODERATORS:
COLLEEN B. JONSSON, PhD; Univ. of Louisville, Louisville, AL.
MIKE BRAY, MD, MPH; OCR/OD/NIAID/NIH, Bethesda, MD.

PRESENTATIONS:
4:15 PM
244 Strategies to Discover Small Molecules as Probes of Virus-Host Interactions
COLLEEN B. JONSSON, PhD; Univ. of Louisville, Louisville, KY.

4:40 PM
245 Genetic Strategies to Investigate Host-Virus Interactions
ABRAHAM L. BRASS, MD, PhD; Ragon Inst. at Massachusetts Gen. Hosp., Charlestown, MA.

5:05 PM
246 Knock Down Approaches Identified Kinases and Phosphotases as Host Targets for Multiple Highly Pathogenic Viruses
SINA BAVARI, PhD; USAMRIID, Fort Detrick, MD.

5:30 PM
247 Identification of Small Molecules that Suppress MicroRNA
KUAN-TEH JEANG, MD, PhD; NIH, Bethesda, MD.

5:55 PM
248 Development of ER Targeting Liposomes for Potential Use in Broad Spectrum Antiviral Therapy
NICOLE ZITZMANN, PhD; Univ. of Oxford, Oxford, United Kingdom.

Symposium Session
022. Innate Immunity and the Host Response to Infection
Tuesday, February 8, 2011  |  4:15 PM – 6:15 PM
Ambassador Ballroom

This session will cover pathogen-associated microbial patterns (PAMPs) receptor sensing of bacteria and viruses and the different pathways triggered by these receptors; inflammasome activation in infection; innate immune system first responder cell interactions with pathogens, and other related innate immunity topics.

Upon completion of this Symposium Session, the participant should be able to:

Discuss the innate immune system responses to pathogens, the mechanisms used by pathogens to evade these responses, and protective/detrimental sequelae of the innate immune response.

MODERATORS:
DRUSILLA L. BURNS, PhD; FDA, Bethesda, MD.
MAHTAB MOAYERI, PhD; NIH, Bethesda, MD.

PRESENTATIONS:
4:15 PM
249 The Nucleotide-Binding, Leucine-Rich, Repeat Containing Protein (NLR) Family+ Select Agents
JENNY P. Y. TING, PhD; Univ. of North Carolina, Chapel Hill, NC.

4:40 PM
250 The NLR P3 Inflammasome in Infection and Inflammation
FANYAZ SUTTERWALA, MD, PhD; Univ. of Iowa, Carver Coll. of Med., Coralville, IA.

5:05 PM
251 IL-1 Cytokines During Mycobacterium tuberculosis Infection
KATRIN MAYER, PhD; NIH, Bethesda, MD.

5:30 PM
252 Subversion of Signal Transduction Pathways in Macrophages by Yersinia pestis
JAMES B. BLISKA, PhD; Stony Brook Univ., Stony Brook, NY.

5:55 PM
253 Bacillus anthracis Relies on Secretion of Anthrax Toxin to Impair the Scavenger Functions of Myeloid Cells to Establish Successful Infection
SHIHUI LIU, PhD; NIH, Bethesda, MD.
023. Medical Countermeasure Development in the U.S. Government

Tuesday, February 8, 2011  |  6:30 PM – 7:30 PM
Regency Ballroom

This is an opportunity to discuss how the U.S. government is trying to remove chokepoints in the process of development of medical countermeasures for biological threats by working together in portfolio management through the Integrated Portfolio, reducing the backlog in animal testing capabilities, and engagement with industry and academia for development of flexible advanced manufacturing platforms.

Upon completion of this Discussion Roundtable Session, the participant should be able to:

- Distinguish areas of commonality between the Human Health Services and Department of Defense medical countermeasure programs;
- Describe the Integrated Portfolio approach to managing the candidate pipeline for various countermeasures for biological agents such as Ebola;
- Review recent initiatives to support advanced development and manufacturing core services; and
- Assess the implications of the need to perform efficacy testing in animal models on future infrastructure requirements and plans to address these requirements.

MODERATORS:
RICHARD JAFFE, PhD; ANSER, Inc., Washington, DC.
PHILIP J. FERRO, PhD; BARDA, Washington, DC.

PRESENTATIONS:

254 The Integrated Portfolio for CBRN Medical Countermeasures
CAROL D. LINDEN, PhD; Biomedical Advanced Res. and Dev. Authority, Washington, DC.

255 National Biotechnology Centers for Advanced Product Development (NBCADP) Medical Countermeasure T&E Facility
GEORGE V. LUDWIG, PhD; U.S. Army Med. Res. and Material Command, Fort Detrick, MD.

256 PHEMCE Medical Countermeasure Review
GEORGE W. KORCH; Dept. of Hlth. and Human Services, Washington, DC.
Wednesday, February 9, 2011

Plenary Session

024. New Interventions and the Challenges of Emerging Infections
Wednesday, February 9, 2011 | 8:30 AM – 12:00 PM
Regency Ballroom

New and re-emerging diseases pose a challenge to public health and new routes are required to solve the problems they pose. This session covers recently described infections, particularly those associated with central Asia, which can affect both civilians and military personnel. Talks will cover both the infections and some of the new translational research being applied to increase understanding and develop interventions.

Upon completion of this Plenary Session, the participant should be able to:

- Describe the global perspective on new and emerging diseases;
- Report on the recent outbreaks in Central Asia affecting civilians and military personnel;
- Review application of translational research to develop interventions against emerging diseases; and
- Describe emerging challenges in antimicrobial resistance.

MODERATORS:
MILES CARROLL, PhD; Hlth. Protection Agency, Wiltshire, United Kingdom.
STUART NICHOL, PhD; CDC, Atlanta, GA.

PRESENTATIONS:

8:30 AM
257 Virus Evolution in the Real World: Global Perspective
STUART NICHOL, PhD; CDC, Atlanta, GA.

9:20 AM
258 Emerging Arbovirus Disease in Central Asia
ROGER HEWSON, PhD; Hlth. Protection Agency, Salisbury, United Kingdom.

9:40 AM
Coffee Break
10:10 AM
259 Emerging Bacterial Pathogens: Rickettsia and Q Fever
ROEL COUTINHO, PhD; RIVM, Biltoven, Netherlands.

10:30 AM
260 Emerging Challenges in Antimicrobial Resistance
PATRICK MCDERMOTT, PhD; Food & Drug Admin., Laurel, MD.

10:50 AM
261 Emergence of Highly Pathogenic TBE Viruses
ALAN BARRETT, PhD; Univ. of Texas Med. Branch, Galveston, TX.

11:10 AM
262 Anthrax: Investigation of a New Presentation
TIM BROOKS, PhD; Hlth. Protection Agency, Wiltshire, United Kingdom.

11:30 AM
263 Anthrax: Experimental Therapeutics
STEPHEN MORRIS, PhD; Biomed. Advanced Res. and Dev. Authority, Washington, DC.
028 (A)

Varicella-Zoster Virus Infection Triggers Formation of the NLRP3 Inflammasome Complex

A. M. NOUR; Stanford Univ., Stanford, CA.

Innate cellular immunity is the immediate host response against pathogens and activation of innate immunity also modulates the induction of adaptive immunity. The NOD-like receptors (NLRs) are a family of intracellular receptors that recognize conserved patterns associated with intracellular pathogens but information about their role in the host defense against DNA viruses is limited. Here we report that varicella zoster virus (VZV), an alpha-herpesvirus which is the causative agent of varicella and herpes zoster, induces formation of the NLRP3 inflammasome and the associated processing of the proinflammatory cytokine, IL-1β, by activated caspase-1, in infected cells. NLRP3 inflammasome formation was induced in VZV-infected human THP1 cells, which is a transformed monocyte cell line, primary lung fibroblasts and melanoma cells. AIM2 (Absent in Melanoma gene-2) is an interferon-inducible protein that can form an alternative inflammasome complex with caspase-1 in virus-infected cells. Experiments in VZV-infected melanoma cells showed that NLRP3 protein recruits the adaptor protein ASC and caspase-1 to form an NLRP3 inflammasome complex independent of AIM2 protein and free radical reactive oxygen species ROS release. NLRP3 was also expressed extensively in infected skin xenografts in the SCID mouse model of VZV pathogenesis in vivo. We conclude that NLRP3 inflammasome formation is an innate cellular response to infection with this common pathogenic human herpesvirus.

030 (A)

Development of Rabbitpox Virus and Cowpox Virus Real-Time PCR Assays

J. M. SHIFFLET, S. RADHAKRISHNAN, K. LANGENBACH; American Type Culture Collection, Manassas, VA.

Background: Vaccinia virus is a prevalent tool in research because it’s easily manipulated into viral vectors, can be handled at BSL-2, occupies a wide host range and is inexpensive to propagate. The 41 recognized strains of Vaccinia virus have minimal variation at the genomic level and cross contamination of viral stocks is a potential problem. BEI Resources was tasked with the development of Rabbitpox virus and Cowpox virus real-time PCR assays in order to distinguish the viruses from other common laboratory strains of Orthopoxviruses. Methods: The Rabbitpox virus assay was designed based on a whole genome multiple alignment of near neighbors. The Cowpox virus primers designed by S.N. Shchelkunov, et al (2005) were adapted for our use in real-time PCR by the addition of a newly designed specific probe. The specificities of the primer and probe sequences were verified using NCBI BLAST and tested against the 21 available Orthopoxviruses and 5 host cell lines at ATCC. Positive control plasmids for each assay were designed by inverting every other 10 nucleotides within the target region, excluding the primer and probe binding regions. This resulted in a positive control target with the same GC content and melting temperature as the “wild type” target. Results: The Rabbitpox virus and Cowpox virus assays are specific to the detection of Rabbitpox or Cowpox viruses respectively by both the NCBI database and wet lab testing against the more common laboratory strains. The assays reliably detect down to 100 molecule copies. Additionally, a spike-in study demonstrated that 10 copies of Rabbitpox virus could be detected and discriminated from near Vaccinia virus strain MVA or Monkeypox virus strain 7-61 WRAIR. Conclusion: The popularity of Vaccinia virus strains in research requires a sensitive method to detect near-neighbor contaminants to insure the integrity of experiments. Here, we have shown the development of a novel Rabbitpox virus real-time PCR assay and the adaptation of a Cowpox virus real-time PCR assay.

031 (A)

Prevalence and Symptoms of Kobuvirus and Hepatitis-E Virus Infections from Porcine Population in Northern Spain

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Objectives: a) Determine the prevalence of Kobuvirus and Hepatitis-E virus infections, and their respective association to possible symptoms. b) HEV potential as a zoonotic agent. Material and Methods: The study was conducted in the intensive porcine production region of northern Spain. A total of 132 samples were collected at random from different farms in the region. Animals were grouped into two groups to match general farm management and age criteria: suckling and weaning groups, 0-4 and >4-8 weeks, respectively. Body temperature was recorded and faecal samples were scored as diarrhoeic or normal. Kobuvirus and Hepatitis-E were investigated by reverse transcription-polymerase chain reaction (RT-PCR). RT-nested PCR was used of HEV RNA detection, where as a real-time RT-PCR was carried out using TaqMan probes for porcine Kobuvirus (EU787450). Results: Kobuvirus and Hepatitis-E virus were detected with an overall prevalence of 48.7% and 24.2%, respectively. Kobuvirus virus infections with mild increase in body temperature (39+0.7°C) were associated only in piglets aged <5 weeks with odds ratios (ORs) equal to 3.3. HEV shedding in faecal samples was homogenously distributed in both ages, showing no significant association with age or faecal consistency (OR 0.87 and 0.99, respectively). A selection of HEV positive stools were further analyzed by partial sequencing for HEV capsid protein gene. A high nucleotide identity (91%) between human and swine sequences.
in genotype 3 are being observed. Further studies involving farm personnel and more genomic sequencing are being planned. Conclusions: a) The potential transmission of HEV to persons working in piglets intensive production units is to be considered seriously, and preventive measures are to be applied. b) A statistically significant increase in body temperature in nursing piglets shedding Kobuvirus, indicates an active host response to natural infection occurring in lactating period.

032 (A)
Development of the Inhalational Monkeypox Model in the Cynomolgus Macaque
R. BARNEWALL, P. VALES, A. ROBERTSON, K. KNOSTMAN, J. BIGGER; Battelle, Columbus, OH.
Abstract: A smallpox inhalation animal model is needed to be able to assess the effectiveness of vaccines and therapeutics against smallpox. A surrogate to variola virus (the causative agent of smallpox) must be used since there are only two World Health Organization approved repository sites for variola (CDC Atlanta, and NPO Novosibirsk, Russia). Monkeypox virus (MPV) is an excellent surrogate for variola because it causes a disease that is clinically similar to smallpox. To this end, a Cynomolgus macaque inhalation monkeypox model was characterized and developed to test future novel smallpox vaccines and therapeutics under the FDA animal rule. Materials & Methods: Six cynomolgus macaques (4 male and 2 female) were challenged with MPV strain Zaire 79 using a head-only exposure system. Monkeys were randomized to three dose groups of 2 animals for target doses of 3 x 10^4 pfu (Group 1), 1 - 2 x 10^5 pfu (Group 2), and > 3 x 10^5 pfu (Group 3). Virus aerosols were generated using a 3-jet Collison nebulizer and sampled using a gelatin filter. Post challenge, filters were dissolved in EMEM and quantified. Monkeys were observed twice daily for 21 days post challenge for: lesion development (counts); percent body weight declination; body temperature changes; incidence of abnormal clinical observations; viral quantification from blood and buccal swabs. Necropsy and histopathology was performed on all monkeys that were euthanized or died. Results: Monkeys in Group 1 received inhaled doses of 2.5 x 10^4 and 4.3 x 10^4 pfu, Group 2 received 1.2 x 10^5 and 2.9 x 10^5 pfu, and Group 3 received 3.9 x 10^5 and 9.3 x 10^5 pfu. All Group 1 animals survived and none of the Groups 2 or 3 survived. There was an apparent dose dependency in the severity of the lesion counts, clinical presentation virus (genomes) recovered from lung (and to a lesser extent brain and heart). Gross necropsy was indicative of pneumonia; histology pending. Conclusions: Cynomolgus inhalation MPV model was developed that has a clinical course, lesions and pathology similar to human infection.

033 (A)
The Role of the Viral Protein ORF32 in Varicella Zoster Virus Pathogenesis
A. M. NOUR, A. M. ARVIN; Stanford Univ., Stanford, CA.
Varicella-Zoster virus (VZV) is the causative of human herpes zoster and shingles. The VZV genome is homologous to human simplex herpesvirus-1 (HSV-1); VZV has five open reading frames (ORFs) 1, 2, 13, 57, and 32) not present in HSV-1. ORF32 encodes for ~16 kda hydrophilic protein, which is modified posttranslationally by one of the two VZV ser/thr kinases, ORF47. Using bioinformatics analyses, we hypothesized that ORF32 modulates the ser/thr phosphatases. In initial experiments, we tested the subcellular localization of ORF32; our subcellular fractionation and immunostaining results showed that ORF32 is localized to both of the nuclear and the cytoplasm of VZV infected cells. After that, we cloned, expressed and purified ORF32 for use in an ORF32 affinity purification column. With this approach, we identified ORF32 cellular factor interacting proteins from U2OS cells; an epithelial cell line reported to require ORF32 for normal VZV replication. Two different ser/thr phosphatase complexes, were among the candidates for proteins interacting with ORF32 that were identified by mass-spectrometry.

034 (A)
Development of a Murine Nose-Only Inhalation Model of Influenza Infection
L. E. BOWEN1, J. E. TROMBLEY1, K. HARRIS2, J. K. BOHANNON3, J. A. BOYDSTON1, M. C. EICHELBERGER2; 1Southern Res. Inst., Birmingham, AL, 2U.S. FDA, Bethesda, MD.
Background: Most influenza mouse studies use intranasal instillation as the route of infection. However, this does not sufficiently mimic natural infection of inhaled particles. A nose-only inhalation model of PR8 infection in BALB/c mice was developed to compare the 50% lethal dose (LD50) for virus delivered by inhalation versus instillation. Methods: Two studies with five groups of eight female mice were conducted. Mice were infected with PR8 influenza virus by nose-only inhalation in one study and by intranasal instillation in the other. In the intranasal study received 0, 14, 170, 1500, or 2200 plaque forming units (PFU) while mice in the instillation study received 1.6, 8, 40, 200 or 1000 PFU. Results: For the inhalation study, 31 of 32 infected mice were found dead or euthanized in moribund condition by Day 12. The estimated inhalation LD50 using Probit analysis was 8.7 PFU and the mean time to death was 7.7 days. For the instillation study, 19 of 40 mice were found dead or euthanized by Day 9. The estimated instillation LD50 using Probit analysis was 51.6 PFU and the mean time to death was 8.2 days. Conclusion: Our results show that mice are more sensitive to inhaled virus than virus delivered by intranasal instillation. The murine nose-only inhalation model of influenza infection can be used to infect large numbers of animals simultaneously with well characterized, homogenous PR8 aerosol in a controlled and reproducible manner which is critical to lethal dose testing and the development of antiviral therapeutics and vaccines candidates.

035 (A)
Proteomic Characterization of the Rift Valley Fever Virus NSs-Host Protein Interactions
Rift Valley Fever Virus (RVFV), a mosquito borne member of the Bunyaviridae family, is listed as a select agent for both the HHS and USDA. RVFV has a significant effect on livestock, causing spontaneous abortions and death. In approximately 1% of human cases it causes inflammation of the brain, spinal cord, and meninges, retinitis with visual impairments, and liver necrosis with hemorrhaging. Currently there are no FDA licensed vaccines or therapeutics for RVFV infection. Our research is aimed at determining critical RVFV host interactions to identify therapeutics targeted against the host. RVFV is a negative stranded RNA virus that encodes three segments; large (L), medium (M) and small (S). The S segment codes for the NSs protein which plays a major role in viral pathogenesis. NSs protein exists in both the nucleus and cytoplasm and forms distinct filamentous structures in the nucleus. It is known to suppress transcription of host mRNA as well as function as an interferon antagonist. Due to the importance of NSs to pathogenicity, we aimed to identify novel host proteins that interact with NSs. Through Mass Spectrometry we identified multiple novel NSs interacting proteins, including Protein Arginine Methyltransferase 5 (PRMT5). Interestingly, Methylation Modification Prediction Server (MeMo) predicted multiple arginine methyltransferase sites and PRMT5 methylated NSs peptides in vitro. Inhibition of PRMT5 through Arox or siRNA resulted in decreased viral replication. Current studies are focused on identifying the site(s) of NSs methylation and the effects of methylation on
Hantavirus Regulation of Endothelial Cell Responses

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Background: Hantaviruses primarily infect endothelial cells (ECs), which form the primary fluid barrier of the vasculature, and cause 2 vascular permeability based diseases, HFRS and HPS. Although hantaviruses are not lytic, hantavirus infection of ECs provides a primary means for hantavirus infection and contribute to pathogenesis. Methods: We compared transcriptional and functional changes of pathogenic ANDV and HTNV infected ECs to responses elicited by nonpathogenic TULV. RNA analysis, EC permeability and adherence assays were used to analyze receptor and pathway specific changes of hantavirus infected ECs. Results: Pathogenic hantaviruses dramatically enhance the permeability of infected ECs via VEGFR2-Src signaling pathways. Pathogenic hantavirus inhibition of specific microRNA functions further alters EC permeability. We found that permeability responses are controlled by changing functions and levels of αβ3 integrins, Robo1/4 and syndecan receptors which regulate VEGFR2 responses. Regulatory receptor changes occurred concomitantly with the adherence of quiescent platelets to the surface of HTNV and ANDV infected ECs. Collectively, changes in both EC receptors and miRNAs were found to alter normal VEGFR2 signaling and permeability responses following hantavirus infection. Conclusions: Viral regulation of αβ3 integrins, Robo and VEGFR2 receptors as well as microRNAs which normally regulate the paracellular permeability of ECs provide mechanisms for hantaviruses to increase capillary leakage and at least in part contribute to hantavirus pathogenesis. Since ECs are fundamentally altered by hantavirus infection the responses of infected ECs, which are the ultimate targets of all capillary permeabilizing signals, need to be considered in evaluating and targeting hantavirus induced capillary leakage therapeutically.

Development of Fluorogenic 5’ Nuclease Assays for Venezuelan Equine Encephalitis Virus

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Background: Venezuelan equine encephalitis virus (VEEV) is a member of the Family Togaviridae, genus Alphavirus. The CDC has classified VEEV as a Category B Select Agent due to its potential for large-scale dissemination with resultant illness. Due to its diversity and availability of fully sequenced genome data, there are no published real-time reverse transcription-polymerase chain reaction (RT-PCR) assays for rapid detection of VEEV in literature. Methods: Twelve geographically-diverse VEEV isolates were sequenced using primer walking methods aligned with VEEV strain sequences available in GenBank and used to develop a consensus sequence. A total of 20 primer/probes sets were designed to target conserved regions of the viral genome, including the nonstructural and structural protein genes. The phylogenies were then inferred by Neighbor-joining analysis using MEGA 4.0 software. Results: We determined phylogenetic relationships among the represented VEEV strains. In addition, we were able to perform a comprehensive analysis to identify conserved regions of the viral genome for real-time RT-PCR assay development. These results represent the first steps of a future diagnostic assay development for VEEV. Conclusions: The VEEV genome data and the real-time PCR primer/probe sets from this study will provide the basis for future diagnostics directed toward rapid detection and identification of medically important VEEV.

Pathogenesis of Eastern Equine Encephalitis Virus in Mice

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Eastern equine encephalitis (EEE) virus, an arbovirus, is an important human and veterinary pathogen belonging to one of seven antigenic complexes in the genus Alphavirus, family Togaviridae. EEE virus is considered the most deadly of the mosquito-borne alphaviruses due to the high case fatality rate associated with clinical infections, reaching as high as 75% in humans and 90% in horses. In patients that survive, the neurologic sequelae are often devastating. Although natural infections are acquired by mosquito bite, EEE virus is also highly infectious by aerosol. This fact, along with the relative ease of production and stability of this virus, has led it to being identified as a potential agent of bioterrorism. To characterize the early events in the pathogenesis of EEE virus FL93-939 when the virus is inoculated by various routes, we compared the clinical parameters and viral titers in the blood and target tissues. Twelve-week-old female Balb/c mice were infected by intranasal or aerosol route. Mice were then euthanized at sacrificed time points and tissues harvested for viral titer determination. Although both groups of animals exhibited similar clinical signs after inoculation, the percentage of animals affected was significantly different. Additionally, in those mice challenged by the aerosol route, 80% of the animals had a detectable viremia at 24 hours post infection (hpi), compared to only 30% in the intranasal group. However, the most significant difference was in the brain, where EEE virus was present in 40% of the animals at 6 hpi in the aerosol group, and was not
**Antibiotic Discovery: A New Method which Focuses on Dormant Bacteria**

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**Background:** New classes of antibiotics are needed. All current classes have been discovered by the Fleming method which targets log phase bacteria. These compounds are powerful agents against multiplying bacteria, but are relatively weak against non-multiplying or dormant bacteria. The presence of non-multiplying bacteria and spores increases the period of chemotherapy required for a cure, and this increases the emergence of resistance. Other infections such as Burkholderia spp or Bacillus antracis spores, are remarkably antibiotic tolerant, with latent subpopulations of bacteria.

**Methods:** By targeting non-multiplying bacteria throughout the discovery process, a small chemical compound was identified with potent activity against non-multiplying *Staphylococcus aureus* and *Bacillus cereus* spores. **Results:** Numerous hits were obtained, of which one, called HT61, is quinolone-derived with a molecular mass of about 400 Daltons. It is active against non-multiplying bacteria, including methicillin sensitive and resistant as well as Panton-Valentine leukocidin-carrying *Staphylococcus aureus*. It also kills mupirocin resistant MRSA. In addition, it is active against *Bacillus cereus* spores. The mechanism of action of the drug is depolarisation of the cell membrane and destruction of the cell wall. In comparison to conventional antibiotics, HT61 kills non-multiplying cells more effectively, 6 logs versus less than one log for major marketed antibiotics. No resistant phenotype was produced during 50 serial cultures over a one year period, and is now in clinical trials. **Conclusion:** This is a new concept for antibiotic discovery. By targeting non-multiplying bacteria throughout the discovery process, many new classes of antibiotics have been identified, of which one has been developed through preclinical and is now in clinical trials.

**Phylogeny of Francisella tularensis Subspecies Holartica from the Country of Georgia**

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**Background:** *Francisella tularensis* is a causative agent of tularemia, displays subspecies-specific differences in virulence, geographic distribution and genetic diversity. The rapid and widespread dispersal of *F. tularensis* subs. *holartica* (found throughout the Northern Hemisphere), is not well understood due to a lack of phylogenetic knowledge, particularly for populations in the European/Asian juncture and East Asia. In this study, we describe the phylogeny of isolates from the country of Georgia, which lies along the European/Asian juncture. **Results:** From Georgia, we describe a new population in subclade B.Br.013/014 that is genetically and geographically distinct from other subclade B.Br.013/014 populations found in Europe. Specifically, the Georgian strains belong to a subclade B.Br.013/014 lineage that is basal to the European subclade B.Br.013/014 lineages and isolates that comprise this new Georgian lineage are also found in the Ukraine. We identify considerable genetic diversity within this Georgian lineage that is comparable to the extent of diversity in the subclade B.Br.013/014 populations found in Europe. **Conclusion:** The Georgian population expands our knowledge of *F. tularensis* subsp. *holartica* phylogeny. Its uniqueness argues for additional phylogenetic studies of Asian and East European *F. tularensis* for a more comprehensive understanding of the rapid radiation of *F. tularensis* subsp. *holartica*.

**Development of Immunological Assays for Detection of Salmonella**

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**Background:** *Salmonella* spp. is one of the most important foodborne pathogens in humans. Contamination of foods or water by these bacteria is common throughout the world and affects an estimated 1.4 million people each year in the USA, according to the CDC. Therefore, the ability to detect *Salmonella* is essential for ensuring food safety and public health. The conventional procedure for detection and identification of *Salmonella* is laborious and time consuming taking 3-5 days. Various rapid methods have been reported, but they use an enrichment step to reach the sensitivity required by legislation. The objective of our study is to develop a rapid, accurate and simple method (without enrichment step) to detect *Salmonella*. **Methods:** Seventy monoclonal antibodies directed against *Salmonella* were produced and characterized in our lab (see Abstract by P. Lamourette). We have tested all these mAbs for the specific detection of *Salmonella* (*Salmonella typhimurium*, *Salmonella enteritidis* and 5 other strains) by different immunological assays: sandwich enzyme immunoassay using microtiter plates and immunochromatographic assay. **Results:** We have developed 3 immunoaassays: i) specific for detection of *Salmonella typhimurium*, ii) specific of *Salmonella enteritidis* and iii) detecting different strains of *Salmonella*. The sensitivity of these immunoaassays is between 10³ and 10⁵ CFU/ml in less than 15 hours, and optimization is still ongoing. Immunochromatographic assays, performed in 20 min, were less sensitive with optimal detection limit around 10⁵ CFU/ml. **Conclusions:** Our study confirms the feasibility of rapid tests either for laboratory application (15 hours) or for field application (20 min), but the sensitivity of our tests (without enrichment step), despite being promising, is still insufficient for legislation requirements. Further improvements are under investigation, among them the combined use of immunomagnetic separation and immunodetection tests.

**Telemetry Characterization of F. tularensis SCHU S4 Aerosol Infection in Naïve and LYS Vaccinated Cynomolgus Macaque**

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**Background:** *F. tularensis* infects humans via several routes; though bacterial strain type and route of infection govern disease severity, primary pneumonic tularemia has the highest mortality rate. We characterized the natural course of aerosolized tularemia in telemetry-implanted non-human primates (NHP) that were naïve (N) or vaccinated (V) with *F. tularensis* Live Vaccine Strain (LVS). **Methods:** Two groups of primates (N and V) received a target presented aerosol dose of ~ 1000 CFU SCHU S4. Animals experienced the full course of disease and were euthanized if moribund. The following parameters were collected pre and post-exposure (PE): clinical observations, telemetry [heart rate (HR), respiratory rate, and body temperature (BT)], clinical chem...
istry, hematology, bacteremia, organ bacterial burden, and histology. Results: BT in N NHP increased 48 hr PE (>2.5°C) and remained elevated until ~120 hr after which BT declined until moribund. BT in V NHP had a milder increase (<1.5°C) with no decline at 120 hr. HR in N NHP increased ~12 hr PE (>50 bpm) and remained elevated until moribund; HR became dissociated from diurnal rhythm by 48 hrs. A mild increase in HR was found in V NHP ~ 63 hr PE and diurnal rhythm remained. Clinical disease was less severe in V NHP and survival was significantly extended. Conclusions: In telemetry-implemented NHPs, BT represented a clear and real-time biomarker indicative of disease with HR providing a strong supportive biomarker. Telemetry and other clinical parameters supported less severe disease in V NHP. BT and HR from telemetry are reliable indicators of disease state, and could also be used to initiate therapeutic intervention in Cynomolgus macaques. This project was funded in whole or in part with federal funds from NIAID, NIH, DHHS, Contract No. HHSN266200500040C.

043 (B)

The Plant Pathogen Pseudomonas syringae pv. Tomato as a Model for Plant Pathogen Source Tracking


Background: Recently, bacterial plant pathogens have accidentally been introduced into the USA causing billions of dollars of losses. To develop efficient means to identify the source of such pathogen introductions, it is imperative to investigate microevolution and phylogeography of bacterial plant pathogens. As a model for bacterial plant pathogen microevolution and phylogeography we have used the tomato pathogen Pseudomonas syringae pv. tomato (Pto). Methods: The genomes of five pathogen isolates were completely sequenced using 454 and Illumina technology. 89 additional isolates were analyzed with genome derived single nucleotide polymorphism (SNP) markers. Results: We find that the most common isolates of Pto are as genetically monomorphic as the human pathogens Yersinia pestis or Salmonella Typhi, suggesting a recent evolutionary origin. In particular, isolates from North America and Europe are more similar to each other than to isolates from South America and Africa indicating frequent pathogen movement between these world regions. Three non-synonymous mutations were identified in the flagellum gene fliC. Strains with one of the three mutations have replaced former populations of the pathogen almost completely within 20 years in Europe and North America. The other two mutations are currently present only in Colombia. Importantly, strains with mutated fliC trigger less plant defenses and are thus probably more virulent. Conclusions: It is thus critical for plant biosecurity to limit pathogen movement even for pathogens that already have a worldwide distribution to avoid spread of new pathogen variants. Approaches similar to the one described should be applicable to other bacterial plant pathogens for source tracking and molecular epidemiological investigations.

044 (B)

Multiple Low Dose Bacillus anthracis Ames Inhalation Exposures in the Rabbit


Background: Credible multiple exposure dose-response relationships must be generated to support cleanup decisions following a Bacillus anthracis release to more accurately assess the risks to residual low level contaminations. To begin to address these unique re-occupancy/re-use scenarios, an innovative and complex inhalation exposure study was conducted. Methods: Thirty male New Zealand White rabbits were implanted with D70-PCT transmitters and exposed to aerosolized B. anthracis (Ames strain) spores once a day for five straight working days each week for three weeks (up to 15 times each). The targeted inhaled doses ranged from 100 to 10,000 colony forming units (CFU). A negative control group was exposed to multiple targeted inhaled doses of 10,000 irradiated spores. The rabbits were monitored during the three exposure weeks and for three weeks after the last exposure for clinical signs of disease, mortality, changes in body temperature, cardiovascular function, hematology, C-reactive protein, bacteremia, toxemia, and seroconversion. Results: A dose-response relationship was observed in all the parameters measured. All seven rabbits exposed to multiple targeted inhaled doses of 100 CFU survived the entire study, as did all rabbits in the negative control group. Six of seven rabbits survived multiple targeted inhaled doses of 1,000 CFU, while only three of seven rabbits survived multiple targeted inhaled doses of 10,000 CFU. Interestingly, one of the surviving rabbits exposed to multiple inhaled doses of 10,000 CFU became bacteremic, toxemic, and seroconverted, but did not die on study. Conclusions: This data suggests multiple B. anthracis inhaled doses of 100 CFU or lower do not affect the physiological parameters measured in the rabbit model.

045 (B)

In Vitro Characterisation Studies of Burkholderia mallei Prior to Use in an Aerosol Infection Model


Background: Burkholderia mallei, the aetiologic agent of glanders, is a Gram-negative, non-motile, intracellular bacterium. The use of B. mallei as a biological warfare agent has been reported historically and as such is now classified as a category B biological agent due to its potential use as a bio-weapon. Despite the highly infective nature of B. mallei there is limited research into the pathogenesis and host response; our work aims to fully characterize the infection in a non-human primate (NHP) model to ultimately evaluate antibiotic efficacy. However, prior to in vivo studies it is essential to fully characterize the bacterium in vitro and here we present the results of these studies. Methods: Bacteria B. mallei NCTC 12938 was obtained as a freeze-dried sample from NCTC, UK. Growth and colony morphology Standard microbial methods were followed for the growth at 37°C for 40-48h, using Mueller-Hinton broth (MHB) and Columbia Horse blood (COH) agar. A comparison between MHB, Tryptone Soya (TSB), Luria-Bertani (LB) and LB+4% glycerol broths was made, along with growth comparisons on COH, Tryptase Soya Agar and LB Lennox agar. Real time PCR Thermomixes were prepared and real time PCR was performed targeting the bimA gene. Gram stain Standard Gram staining technique was used and slides examined microscopically. Results: The B. mallei 12938 Cell Bank has been confirmed as a Gram negative coco-bacillus producing smooth, domed colonies and shown to contain the bimA gene by real time PCR. Colony morphology of B. mallei was found to be regular between 40 and 48h incubation, with colony diameter ranging between 1mm and 3mm. The growth was most consistent on COH agar and in TSB. Conclusion Full in vitro characterisation of pathogens before progressing to NHP studies is essential. Here we confirm that the B. mallei cell banks produced are suitable for further in vivo studies having confirmed growth, morphology and gene target identification by PCR.
046 (B)

Identification of Molecular Targets of the LL-37 Peptide that Regulate Francisella Biofilm Production

S. AHMAD, L. S. AMER, M. L. VAN HOEK; George Mason Univ., Manassas, VA.

Background: Francisella species are gram-negative, facultative intracellular pathogens resulting in the disease of tularemia. We have recently shown that Francisella form biofilms, which are a natural aggregation of bacterial colonies surrounded by an extracellular polymeric matrix and result in an increased survival of the bacteria. Understanding biofilms and developing ways to break down biofilms has important economic and medical applications. LL-37 peptide is a human cathelicidin peptide that exhibits antimicrobial activity against many bacteria, including Francisella. We also have demonstrated that LL-37 peptide exerts antibiofilm activity against Francisella at the EC50 concentration of 0.24 g/ml, which is below the minimal inhibitory concentration for antimicrobial activity. We hypothesize that LL-37 peptide will exert its antibiofilm activity by specifically altering gene expression of biofilm-related genes in Francisella. Methods and Results: Confocal images of F. novicida incubated with LL-37 peptide have shown a decrease in depth and tightness of biofilm formation compared to untreated F. novicida biofilm. We further tested LL-37 peptide’s antibiofilm activity by determining its bacterial genome targets through microarray analysis. Electrophoresis mobility shift assay revealed that LL-37 peptide is able to bind to F. novicida DNA. Francisella contains a two-component regulatory system QseBC that is homologous to the regulatory system found in E. coli for quorum sensing, which contributes to biofilm formation. LL-37 peptide was shown to decrease QseC expression levels in F. novicida by quantitative real-time PCR. Conclusion: These results suggest that LL-37 peptide targets QseC which may regulate biofilm formation in Francisella.

047 (B)

Tissue Burden Demonstrates Drug Efficacy in an Intravenous Mouse Model of MRSA

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Background: Staphylococcus aureus (SA) is a Gram-positive bacterium that persists on human skin and within the mucosal surfaces of the anterior nares. Twenty percent of individuals are persistently colonized, while 60% are intermittently colonized in the nares. Compounding the seriousness of this situation is the increasing incidence of methicillin resistance. Methicillin resistance (MR) is a predictor for resistance to other antibiotics; 60-80% of SA strains are multi-drug resistant. Thus, the need for new treatments is underscored. We developed an intravenous murine infection model for MRSA to aid in the determination of efficacy of new therapeutics. Methods: BALB/c mice were intravenously infected with 7x10^7 CFU of SA Strain USA 300 to determine if reduction in bacterial burden in tissues could be utilized to assess the efficacy of therapeutic drugs. Mice were treated twice daily for 4 days post-challenge with methicillin (M; 205 mg/kg day), vancomycin (V; 411mg/kg day) or sham treated with saline. At 5 days post-exposure blood for hematology and tissues (lung, spleen and kidney) for bacterial burden were collected. Results: All V- and M-treated mice survived and 90% survival was observed in the saline-treated group. No differences between groups were found in body weights or blood parameters (white blood cells, neutrophils, lymphocytes and platelets). Tissue burdens demonstrated significantly lower bacterial numbers in all tissues of animals treated with V as compared to M- or saline-treated animals. Conclusions: An intravenous MRSA infection model successfully differentiated drug efficacy by tissue burden. However, body weights and hematology data were not useful in determining differences between treated and non-treated groups.

048 (B)

Genotypic and Phenotypic Associations in Yersinia pestis Collected in the Caucasus

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Background: Atypical non-epidemic subspecies of Yersinia pestis have been described in the Caucasus region but thus far have not been well characterized genetically. Methods: A collection of Y. pestis strains from the Caucasus was characterized phenotypically and using Single Nucleotide Polymorphism (SNP) analysis and Multiple Variable Number Tandem Repeat Analysis (MLVA). This MLVA approach was previously applied to a Kazakh isolate collection (Lowel et al. 2007) that was also compared in the current study. Results: The majority of strains from the Caucasus region had characteristics of atypical enzootic strains including atypical plasmid profiles. SNP separated typical epidemic strains and atypical variants into distinct clades as described by Achtman et al. (2004) and differentiated genetic groups within Georgian isolates of atypical subspecies caucasica, an additional genetic cluster of Armenian strains within the atypical Y. pestis variants and a clade within epidemic Y. pestis variants represented by two Kazakh isolates. Possible clonal associations among strains was provided by the resolution of MLVA, including within a genetically homogeneous cluster of Georgian strains isolated over a span of decades closely related to atypical type strain Pestoides E. Potentially clonal strains from Azerbaijan and Georgia biotyped as Mediaevalis and clustered with strains from the Kazakh collection that were isolated in the Volga-Ural regions of the pre-Caspian basin, indicating a potentially far-flung transfer. A group of Azerbaijani isolates clustered with other Antiqua strains from Kazakhstan and Kyrgyzia, consistent with its biotype determination. Conclusions: Combining SNP and MLVA provided both deeper phylogenies and strain-level differentiation. The clustering of isolates appeared to associate with described plague foci in the Former Soviet Union region, indicating that this combined genotyping approach may provide geographical associations that MLVA alone cannot.

049 (B)

Yersinia pestis Infection of Macrophages from Hosts with Low and High Susceptibility

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Background: Yersinia pestis (Yp) causes severe systemic disease in natural rodent hosts, whereas in some rodent predator such as dogs only mild disease has been reported. After entering susceptible hosts via fleas, Yp invades and multiplies in host macrophages before systemic dissemination. Thus, macrophages from mice and dogs may handle Yp differently. Methods: To test this hypothesis, mouse RAW264.7 and dog DH82 macrophage-like cell lines were infected with Yp strain KIM6-2053.1+, extracellular Yp cleared by gentamicin treatment, and various parameters measured for 27.5 h post-infection (PI). Results: For RAW264.7 cells, intracellular Yp colony forming units (CFUs) and genomic equivalences (GEs) were initially 7 per macrophage, but they increased to 15 but the CFUs declined to 4 at 27.5 h PI. For both RAW264.7 and DH82 cells, Yp GEs were initially 7 per macrophage with the GEs increasing to 15 but the GEs were initially to 20 at 27.5 h PI; whereas for dog DH82 cells intracellular CFUs and GEs were 5 per macrophage with the GEs increasing to 15 but the CFUs declining to 4 at 27.5 h PI. For both RAW264.7 and DH82 cells, Yp GEs initially increased with CFUs lagging, suggesting that although Yp multiplied in macrophages from both hosts, these bacteria were less culturable. In RAW264.7 cells, Yp CFUs did increase from 12.5 to 27.5 h PI to equal GEs per macrophage, but not in DH82 cells. Yp infected RAW264.7 cells exhibited large vacuolated phagolysosomes and cell lysis, whereas infected DH82 cells showed normal morphology and retained cellular integrity. Conclusions: Yp multiply and retain viability in macrophages for highly susceptible host such as mice facilitating subsequent dissemination, whereas While Yp may initially multiply in macrophages from a less susceptible host such as dogs these Yp
A Review of the Clinical Presentation and Case Definitions for Pediatric Brucellosis Cases in Azerbaijan


Background: Brucellosis remains a major public health concern in Azerbaijan and worldwide. There is a need for further investigation of the pediatric manifestations of this infection. Azerbaijan recently instituted case definitions for public health surveillance of brucellosis. The applicability of these case definitions to pediatric patients was assessed. Methods: A retrospective medical chart review of children with a clinical diagnosis of brucellosis was initiated at the Garyev Children’s Hospital in Baku, Azerbaijan covering the period of 2000-2009. Confirmed high-titer brucellosis cases, defined by positive agglutination test, were compared to a current case definition (fever plus positive agglutination test higher than 1:800 met the case definition). Results: A total of 111 charts were reviewed. Although a substantial percentage of patients were from Baku (43%), children from many regions of Azerbaijan were represented. Children with acute brucellosis were more likely (p<0.001) than chronic brucellosis cases to have fever (95% vs. 68%), sweats (100% vs. 50%) and classification as acute. Acute brucellosis cases were more likely (p<0.001) than chronic brucellosis cases to have severe illness (79% vs. 23%). Although over 80% of patients had symptoms such as fever, myalgias, arthralgias, only 3% children with an agglutination test higher than 1:800 met the case definition. Conclusions: The results to date indicate significant differences in the clinical presentation of acute versus chronic brucellosis in children, suggesting that fever and other symptoms subside with chronic infection. These findings also emphasize the importance of adapting case definitions for pediatric populations. Further refinement of current case definitions are needed to improve surveillance for this infection.

Experimental Respiratory Burkholderia pseudomallei Infection in BALB/c Mice


Background: Burkholderia pseudomallei is the causative agent of melioidosis and is classified as a category B biological agent due to its potential use as a bio-weapon, most likely as an aerosol where respiratory disease would predominate. The objective of this study was to compare mid-log and stationary phase virulence and characterise a relevant respiratory B. pseudomallei infection in BALB/c mice. Materials & Methods: Groups of ten mice were challenged by the aerosol route using a modified Henderson apparatus in conjunction with the Biaera aerosol management platform to various doses of either mid-log or stationary phase B. pseudomallei strain K96243. Bacterial counts within the lungs of survivors fourteen days post challenge were determined and histopathological profiles of the lung, liver, spleen and brain were assessed. Results: It was determined that the presented dose to the lungs ranged between 3 and 3334 colony forming units. Bacterial numbers in the lungs of the surviving subjects reached approximately 5.93E+02 cfu/mg, fourteen days post challenge. Mean time to death was inversely proportional to dose and no obvious difference in virulence was evident between mid-log and stationary phase cultures. At higher doses acute B. pseudomallei infection was rapidly progressive and was characterised by ruffled coat, malaise and immobility, leading to death within two to six days. In contrast, sub acute melioidosis was characterised by hypothermia and anorexia. Lung and spleen tissue appeared to be the primary targets of pathological involvement. Conclusion: These results suggest that the BALB/c mouse is susceptible to both mid-log and stationary phase B. pseudomallei when delivered by the aerosol route. This model system of acute human melioidosis may therefore be suitable for research into B. pseudomallei pathogenesis and for the evaluation of antimicrobials and vaccine candidates against this disease.

A High-Resolution Phylogenomic Framework for Escherichia coli O157:H7

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Background: Understanding the genomic plasticity of E. coli O157:H7 is crucial to the epidemiology, surveillance, diagnostic and forensic communities, because of the rapid emergence of this serotype since its discovery in 1982. To assess the genome plasticity and dynamics, we have sequenced and analyzed 19 strains from ingested produce, the bovine host reservoir and infected patients, some of whom presented with severe and potentially life-threatening complications. Methods: Whole-genome shotgun Sanger sequencing followed by phylogenomic polymorphism discovery analyses in the conserved genomic backbone as well as the dissected mobilome allowed tracking the pathogenome evolution in detail. To investigate the phenotypic variations on the level of individual polymorphisms, we have developed a bioinformatics pipeline for single nucleotide polymorphism (SNP) discovery and validation taking into account the coverage and quality of underlying sequence reads. Results: The phylogenomic analyses revealed numerous novel biomarkers, many of which seem to be intimately associated with the lineage-specific niche-adaptation and pathogenesis of E. coli O157:H7. Markers include more than 1,200 SNPs, by far more than previously available for the typing of this pathogen, as well as novel polymorphisms in the Shiga-toxin converting virulence phages. Conclusions: The established high-resolution phylogenomic framework achieved an unprecedented phylogenetic accuracy.

Abstracts
and resolution and could aid current molecular assays used in public health laboratories in improving risk assessment in response to novel and recently emerging severe E. coli O157:H7 resistance and virulence phenotypes. The discovered polymorphisms within strains derived from a single outbreak suggest the need to study of outbreak populations rather than rely on single outbreak-type strains.

053 (B)

Multiplex PCR for Species-Level Identification of Bacillus anthracis and Detection of Plasmid Presence

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Background: The Bacillus anthracis virulence plasmids pXO1 and pXO2 have critical implications on biosafety and Select Agent status. Thus, the proper identification and characterization of B. anthracis and its plasmid profile is important to the biodefense research community. It was hypothesized that a multiplex PCR assay could simultaneously distinguish B. anthracis from closely related species and determine its plasmid profile. This work presents a novel multiplex PCR assay for species-level identification of B. anthracis and the detection of the pXO1 and pXO2 plasmids. Methods: A multiplex PCR was designed to target a chromosomal mutation specific to B. anthracis, four targets distributed across pXO1, three targets distributed across pXO2, and a highly conserved region of the 165 gene, allowing an internal positive control for each sample. PCR products were run on an Invitrogen 4% agarose E-Gel® and visualized using a Bio-Rad Gel Doc™ XR system. Using DNA from the Ames strain of B. anthracis as a positive control, the multiplex PCR was used to characterize DNA samples extracted from B. anthracis, other Bacillus species, and other bacterial species from many different genera. Results: Based on the presence or absence of the relevant targets, the multiplex PCR assay can produce as many as nine easily separable and distinguishable bands of sizes between 188 and 555 bp. The assay was tested against inclusion and exclusive exclusion panels, with a correlation to previously published and expected results of 100%. The multiplex PCR was then used to test DNA from previously unpublished B. anthracis strains in the BEI collection. Of these, 10 (34.5%) were pXO1+/pXO2+, 9 (31.0%) were pXO1+/pXO2–, 7 (24.1%) were pXO1–/pXO2+, and 3 (10.3%) were pXO1–/pXO2–. Conclusions: The present work presents a novel 9-target multiplex PCR assay capable of species-level identification of B. anthracis via a unique chromosomal marker and the detection of the pXO1 and pXO2 plasmids via multiply redundant targets on each.

054 (B)

Quo Vadis on Your Query for Q Fever?

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Q fever, caused by Coxiella burnetii, is a zoonosis with a worldwide distribution and can affect both humans and animals. Between 2007 and 2010, large and unprecedented outbreaks of Q fever were observed in the Netherlands. It is hypothesized by the veterinary and public health community in the Netherlands that abortion waves on large dairy goat farms played a predominant role in the transmission of C. burnetii to humans. Transmission of C. burnetii by inhalation of contaminated aerosols is thought to be the primary route of infection, as large number of pneumonia cases were reported while there had not been any direct contact with animals or animal products. Here we present results and progression of several research projects, aimed at (1) a better understanding of the most likely veterinary and environmental sources of C. burnetii infection during the outbreaks, (2) development of robust molecular detection and typing methods for C. burnetii, and (3) cultivation of C. burnetii from complex veterinary and environmental sources for whole genome sequencing purposes.

055 (B)

Development of a Rhesus Macaque Model for Francisella tularensis

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Background: Francisella tularensis (F. tularensis), the etiologic agent of tularemia is a category A agent as aerosols of F. tularensis are highly infectious in humans. Licensure of next generation tularemia vaccines will likely involve the FDA Animal Rule. The objective of these studies was to develop the rhesus macaque as a model for human pneumonic tularemia for evaluation of future vaccine candidates. Methods: The lethality and pathophysiological response in nonhuman primates (NHP) following aerosol exposure to F. tularensis SCHU S4 was evaluated using a stage-wise approach. Animals were observed for signs of disease and changes in clinical pathology, temperature and activity. Histopathology was evaluated in all animals that succumbed to disease. The protective efficacy of F. tularensis Live Vaccine Strain (LVS) against aerosolized SCHU S4 was evaluated in a subsequent study. Animals were challenged with 2.5 x 10^5 cfu by aerosol exposure either 36 or 64 days post-vaccination. Endpoints evaluated in the MLV study were also evaluated in the efficacy study. Results: The inhalational LD50 of SCHU S4 in rhesus macaques was estimated to be 1.38 x 10^5 cfu. Nine out of 20 animals succumbed to disease following aerosol challenge with SCHU S4 dosages ranging from 16 cfu to 1.6 x 10^6 cfu. Nineteen out of 20 animals exhibited fever, loss of diurnal rhythm and clinical signs within 1 to 5 days following challenge. In the efficacy study, vaccination with LVS provided 60 to 70% protection in all groups regardless of vaccination route or time to challenge. All control animals succumbed to disease. Changes in body temperature and activity were observed in both vaccinated and control animals. Conclusions: The similarity of clinical signs of tularemia in rhesus macaques compared to humans encourages continued development and use of this animal model in the evaluation of medical countermeasures against tularemia.

056 (B)

Proteomic Analysis of Bronchoalveolar Lavage Fluid Proteins from Mice Infected with Francisella tularensis

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Francisella tularensis causes the zoonosis tularemia in humans and is one of the most virulent bacterial pathogens known. However, little is known concerning the host response during infection. We utilized a global proteomic approach to examine protein changes in bronchoalveolar lavage fluid in mice exposed to F. tularensis ssp. novicida, a strain of F. tularensis known to induce lethal murine tularemia. Proteomic changes were evaluated at 4, 24 and 48 hour time points following lung inhalation with one of three organisms, F. novicida, an avirulent mutant of F. novicida (ΔmglA), and Pseudomonas aeruginosa. A number of proteins were found to be altered following infection, including proteins involved in neutrophil activation, oxidative stress and inflammatory responses. Components of the innate immune response were induced in Ftn-exposed mice, including acute phase response and the complement system, however the timing of their induction is altered in Ftn-exposed mice. Ftn infected mice do not appear to efficiently activate an innate immune response in the first hours of infection. However, within 24 hours and continuing at least until 48 hours after infection, the BALF of Ftn-infected mice show an upregulation of a number of innate immune response proteins. This is in contrast to PA infected animals which demonstrate an innate immune response within the first four hours of infection. Likewise, Ftn ΔmglA
infection initiates an early innate immune response, however this response appears to become weakly attenuated by 24 hours and strongly attenuated by 48 hours following infection.

057 (B)

Genetic Diversity and Regional Distribution of Human and Animal Brucella Isolates in Georgia

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Background: Brucella spp are the most common zoonoses causing substantial morbidity in humans and economic loss in Georgia as well as a potential biological warfare agent. Genetic characterization of Brucella strains by molecular typing methods could become a useful tool for identification of the source of infection. Multiple Locus Variable-number tandem repeats Analysis (MLVA) is a highly discriminatory approach for phylogenetic analysis of Brucella strains. Methods: MLVA-8, targeting eight minisatellite markers with good species identification capabilities, was applied to the typing of 35 isolates of human and animal Brucella obtained in Georgia in 2009 and 2010. Results: Isolates displayed high genetic diversity, with only 2 or 3 strains in one genotype cluster. Some genotypes show restricted geographical distribution, while others were disseminated among different regions of Georgia. One genotype cluster contained animal and human isolates from the same region, revealing a potential source of human infection. Conclusions: Extension of current initial characterization to the analysis of multilocus with higher discriminatory power will provide more specific information about genotypes and their distribution in Georgia.

058 (D)


W. CHAPMAN, A. BACETTY; DTRA BTRP, Ft. Belvoir, VA.

The Cooperative Biological Engagement Program (CBEP) is working on an increasingly global scale building capacity to detect extremely dangerous pathogens and emerging infectious diseases. The Program has operated for over ten years in the former Soviet Union states and recently has begun to work in the Middle East and Africa. Throughout this time CBEP has struggled along with its partners to identify and deploy diagnostic reagents that were well understood by all parties and that would be sustainable over the long run. No one diagnostic approach will be correct for all settings, and the relentless march of technology will continue to provide new approaches to the challenge of detecting and diagnosing dangerous pathogens. In order to address this challenge CBEP has elected to build the Critical Reagents Library (CRL). The aim of this library is to provide a comprehensive compendium of detection methodologies for the World’s most dangerous diseases. The CRL will provide the disease detection community with all available data on the full range of presumptive diagnostics for these high consequence diseases. Rating reagents on a number of parameters the CRL will allow health systems to choose the mix of attributes that best meets their specific needs. The attributes measured will include:

- Cost to procure
- Cost of needed infrastructure
- Ease of transport and storage
- Sensitivity and Specificity
- Ease of use and Operational Conditions
- Quality of manufacture
- Sample type needed
- Expected Results

Different attributes will weigh differently for the myriad of users and scenarios faced in the field. No one approach is perfect. To this end, the CRL serves only to provide the information needed for users to choose what’s best for their needs, not to serves as an authority picking winners and losers.

059 (D)

Thermostable Single Domain Antibodies for the Detection of Bacillus anthracis

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Background: The conserved morphological and genetic features of members of the Bacillus cereus family complicate the accurate detection of B. anthracis with field-based assays. To develop such technologies a sdAb library has been constructed from llamas immunized with spores of B. anthracis Sterne strain. Utilization of a sdAb library will allow for the isolation of detection elements that demonstrate both a high level of specificity and improved stability compared to standard antibodies. Methods: A phage display library was constructed from llamas immunized with the veterinary vaccine for B. anthracis. Selection against bacterial spores produced unique clones identified through monoclonal phage ELISAs and DNA sequence analysis. Target specificity was examined using Luminex assays and direct binding ELISAs with both the target and non-target bacterium. Representative sdAbs were further characterized for thermal stability using circular dichroism and binding activity assayed following successive rounds of heat denaturation and refolding. Results: Several unique sdAbs were isolated that displayed specificity for the B. anthracis target, of which two sdAbs were chosen for further characterization. Both antibodies bound a target protein of approximately 90 kDa that is present in both spore and whole cell material. Both antibodies were able to maintain a majority of their initial binding ability following several rounds thermal cycling while the antibody control lost nearly all binding activity. Conclusions: We have demonstrated that sdAbs can readily be isolated from our llama library that exhibit specificity for B. anthracis. Both sdAbs were able to refold following several cycles of heating, a significant advantage over other antibodies. Combined with production in bacterial cultures, the high specificity and stability of sdAbs makes them suitable for integration into field-based assays and biosensors.

060 (D)

Real-Time 16SrRNA PCR to Monitor Growth of Ciprofloxacin Resistant Bacillus anthracis

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Background: The emergence of antibiotic resistant bio-threat agents poses a serious risk to military readiness. Antibiotic resistance can emerge naturally, during therapy, or through directed bioengineering. Early detection and antibiotic resistance status determination is imperative for appropriate treatment. Methods: A TaqMan-MGB probe was designed for a Bacillus cereus group 16S rRNA standard PCR assay. The real-time PCR assay was optimized
and then used to determine ciprofloxacin resistance (cipror) in Bacillus anthracis. Cipror B. anthracis isolates were incubated in Tryptic Soy Broth (TSB), blood culture media or blood culture media with blood, supplemented with varying concentrations of cipror. Potential cell growth was monitored by DNA extraction and real-time PCR with the B. cereus group 16S assay. Samples were collected hourly and results were compared to the time zero Ct values. Four cipror B. anthracis isolates were grown to establish the minimum time needed to ascertain ciprofloxacin resistance levels. Results: Our preliminary results with this non-traditional antibiotic sensitivity testing have concluded that an approximate minimum inhibitory concentration (MIC) can be determined in as little as 5 hours of incubation. Cipror can further be established by determination of growth at 0.5µg/mL, which is the break point for cipror sensitivity established by the Clinical Laboratory Standards Institute MIC Interpretive Standard. Mutants grew at 0.5µg/mL with Ct values shifting more than 7 cycles earlier, indicating resistance based on over 2 logs of growth. Discussion: With this approach, real-time PCR can easily and rapidly establish a bacterial isolate’s antibiotic resistance status based on simple growth capabilities. The approach is less restrictive than developing assays to detect “specific” point mutations that lead to antibiotic resistance. Furthermore, the current approach provides information on the extent of MIC, which has direct implications for therapeutic intervention.

061 (D)

Use of Antibody-Coated Magnetic Beads in the Concentration of Biothreat Samples

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Background: Rapid and highly sensitive detection of DNA/RNA from environmental or human samples is advantageous for detecting bio-agents of national security importance. In this study, biothreat-specific, antibody-coated beads were used to concentrate samples to increase detection sensitivities. Methods and Materials: CRP antibodies for Bacillus anthracis, Yersinia pestis, Vaccinia, and Venezuelan equine encephalitis (VEE) were covalently linked to Dynabead MyOne Tosylated magnetic beads (Invitrogen). Serial dilutions of blood samples did not show signs of degradation. In fact, PCR analysis of both treated and untreated samples showed earlier crossing thresholds (Cts) as time progressed, possibly indicating bacterial growth. Also, in Francisella samples analyzed by ECL, Clonestable™ samples had one log increased detection by day 28 as opposed to untreated. Conclusions: Biomatrix®'s CloneStable™ utilizes technology that stabilizes biothreat samples for analysis. This will greatly enhance sampling capabilities.

062 (D)

Assessment of Biomatrix’s CloneStable for Improved Stabilization of Diagnostic Biothreat Samples


Background: Preventing the degradation of samples between time of collection and diagnostic analysis is critical. Biomatrix® developed chemistries to improve stability of samples including whole organisms, plasmids, and nucleic acids. One product that utilizes this technology, CloneStable™, was evaluated for stabilizing biothreat samples. Methods: For analysis by PCR, dilutions of Francisella tularensis LVS were prepared in buffer. Aliquots of diluted bacteria were placed in tubes for storage at either room temperature (RT) or 45°C or dried down in presence of CloneStable™ reagent and then stored at those temps. At time intervals up to one month, untreated and rehydrated CloneStable™ samples were extracted using the Qiagen QIAamp DNA Mini kit and analyzed by real-time PCR. Dilutions of Yersinia pestis 0017 (live, attenuated) were prepared in EDTA-treated whole blood. Aliquots were prepared and analyzed as above. For detection by rapid electrochemiluminescence immunoassay (ECL), dilutions of F. tularensis LVS were prepared in buffer. Diluted bacteria were placed in tubes for storage at RT or dried down in presence of CloneStable™ reagent and then stored. At time intervals, untreated and treated samples were analyzed on the BioVeris M1M with the standard shake assay. Results: DNA from Francisella buffer samples stored at RT did not show degradation in either treated or untreated samples. However, samples stored at 45°C showed a one log increase in detection in CloneStable™ samples by day 14. Interestingly, DNA from whole blood samples did not show signs of degradation. In fact, PCR analysis of both treated and untreated samples produced earlier crossing thresholds (Cts) as time progressed, possibly indicating bacterial growth. Also, in Francisella samples analyzed by ECL, CloneStable™ samples had one log increased detection by day 28 as opposed to untreated. Conclusions: Biomatrix®’s CloneStable™ utilizes technology that stabilizes biothreat samples for analysis. This will greatly enhance sampling capabilities.

063 (D)

A Novel Microfluidic Platform for Building Rapid Response Capabilities Through the Rapid Detection of Viable Foodborne Pathogens


The national listeriosis outbreak that occurred in Canada in the summer of 2008, along with the USA peanut butter Salmonella outbreak are prime examples that foodborne pathogens are a constant threat to the food supply. Current “gold standard” methods for detecting bacterial pathogens in foods rely on lengthy culture-based approaches that can, for example, take up to 10 days for Listeria monocytogenes (Lm). Recent advances in microfluidics technology indicate that both conventional and molecular-based approaches can be used in tandem to provide a rapid and portable solution for the fast detection of pathogenic bacteria. The objective of this project is to develop a portable lab-on-a-chip platform, capable of performing sample preparation leading to the specific and efficient isolation and molecular identification of viable bacterial pathogens within a few hours. We report herein the results obtained in the design, fabrication and testing of a polymer-based, disposable microfluidic system for the rapid isolation and identification of Lm. A stone-based pre-filtration column was designed to remove food debris larger than 100 µm. The pre-filtrate is further cleaned up and concentrated through a polymer-based inertial separation chip. The first generation of the capture
chip successfully traps 50±15 Lm cells/mL with specific antibodies. An aliquot of captured cells are then used for on-chip molecular detection/identification (PCR and microarray hybridization), while other cells are directed onto an agar plate to confirm viability and for further forensic investigation. These portable units will provide industry with a rapid turnaround time, to detect the presence or absence of viable foodborne pathogens.

064 (D)
Lethal Factor Toxemia and Anti-Protective Antigen Antibody Activity in Naturally-Acquired Cutaneous Anthrax


Background: Naturally occurring anthrax outbreaks are reported frequently in many locations across the world particularly where widespread vaccination of livestock is not practiced. Natural human exposures to Bacillus anthracis occur from handling infected domestic animals or their contaminated products. Three outbreaks of cutaneous anthrax were reported in North Western Bangladesh from August-October 2009. An extensive epidemiologic investigation of the largest of these outbreaks was conducted by ICDDR,B, the Government of Bangladesh, and CDC. Acute and convalescent sera from 26 cutaneous cases were obtained. Anthrax toxins and anti-toxin responses were determined. Methods: Swabs and biopsies from cutaneous lesion vesicles were obtained when possible for culture, M’Fadyean (Mfad) staining and in situ immunohistochemical (IHC) detection of bacilli. Quantitative analyses were done on acute and convalescent serum for anthrax lethal factor (LF), anti-protective antigen (anti-PA) IgG and anthrax lethal toxin neutralization activity (TNA). Results: B. anthracis was detected by either Mfad or IHC in 6 of 20 case samples. LF levels were detected in 18 of 26 cases and ranged from 0.005-1.264 ng/ml. Anti-PA IgG and TNA were detected in sera from the same 18 cases and ranged from 4.0-679.5 µg/ml and 27-593 units, respectively. Patients without acute LF also lacked convalescent anti-PA/TNA. Conclusions: This is the first report of LF toxemia in cutaneous anthrax and its association with subsequent toxin antibody responses. Toxin-based serological tests provide reliable diagnosis of cutaneous anthrax.

065 (D)
Comparison of OpenArray Nano-PCR to Established Real-Time PCR Assays for Biothreat Detection

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Background: Nucleic acid detection is frequently used for biological threat agents. PCR allows for sensitive and rapid analysis; however, the ability to multiplex target detection is often limited by primer and probe interactions. Life Technologies’ OpenArray platform allows for multiple agent detection in a microarray format at with alacrity and sensitivity comparable to conventional PCR technologies. This platform uses a 33 nanoliters reaction volume which provides a higher degree of parallel interrogations compared to capillary-based real-time PCR. This study compares the linearity and limit of detection of the OpenArray technology with currently established real-time PCR assays. Methods: Nucleic acid from five bioterror agents was serially diluted from 1ng to 1fg and robotically spotted onto an OpenArray slide, containing 37 primer and probe combinations previously designed and optimized at USAMRIID. The slide was then cyclically scanned. Resulting data was compared to previously established capillary-based PCR detection of biowarfare target nucleic acid. Results: Limits of detection for the organisms ranged from 50pg down to 500fg. There was no cross-reactivity detected among any of the assays. Results for the analogous PCR assays are 500fg to 10fg. Statistical limit of detection for respective bioterror organisms will also be presented. Conclusions: The OpenArray platform allows for multiplexed detection of bioweight agents with the ability to perform 3072 independent PCR reactions. The use of the robot allows for rapid sample loading with minimal time and no user inconsistencies. The limits of detection and speed are within a log of current PCR methods; however, the ability to run a large number of assays in parallel compensates for any slight loss in sensitivity.

066 (D)
Comparison of Bacterial Culture and Real-Time (RT) PCR Platform for the Diagnosis of the Cutaneous Anthrax Infection: Prospective Study of the Volunteers in Georgia

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Background: Rapid anthrax molecular diagnostic testing platforms have significant applicability, but they have not been extensively assessed in prospective clinical studies. Methods: We compared skin swab culture vs. polymerase chain reaction (PCR, Roche, Lightcylcer) prospectively in cutaneous anthrax patients. Blood cultures and skin swabs were collected at multiple time points after enrollment. Results: To date, 11/20 subjects were positive by both swab culture and PCR. An additional 4/20 subjects were positive only by PCR. Average duration of the skin lesion prior to presentation was 7 days. A total of 14 subjects were already receiving antibiotic therapy on enrollment with the average duration of 1 day (range 1-7 days). The proportion of PCR positive samples on days 2-3, 4-7 and 8-11 after enrollment was 7/15 (47%), 4/11 (36%) and (1/4) 25% accordingly. None of the collected blood cultures were positive Conclusions: PCR demonstrated substantial utility as a diagnostic test in cases of cutaneous anthrax, particularly when the volunteer had already received antimicrobial therapy. Further optimization of anthrax molecular diagnostic platforms will improve the biodefense response to this pathogen.

067 (D)
RT-PCR/ESI-MS for Rapid Detection of Biothreat (BT) and Common Respiratory Pathogens in Bronchoalveolar Lavages (BALs)

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Background: New diagnostic technologies for respiratory pathogens must be capable of accurate, high throughput and rapid differentiation of BT organisms from common pathogens. Base composition characterization by electrospray ionization mass spectrometry (ESI-MS) (Abbott Molecular) of amplified nucleic acids from human samples allows for pathogen identification within eight hours after collection. We investigated the performance of PCR coupled to ESI-MS for distinguishing BT agents from common bacterial, fungal, and viral respiratory pathogens in clinical samples from subjects with suspected respiratory infections. Methods: In an ongoing study, 27 BALs collected from patients at the Johns Hopkins Hospital were processed using gold standard bacterial, viral, and fungal testing in the clinical microbiology laboratory. Samples were blindly spiked with nucleic acids from BT organisms (B. anthracis, Y. pestis, F. tularensis from BEI) or water and processed by
A Comparison between Impedance-Based and Pressure-Based Plethysmography for Aerosol Dosimetry Calculations

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Background: Throughout the course of an aerosol exposure, changes in an animal’s respiratory parameters can influence both how much aerosol is delivered and where it is deposited in the respiratory tract. Therefore, real-time monitoring of respiratory parameters is fundamental to increasing biological agent dosing accuracy as compared to the use of estimates. The aim of the present study was to compare respiratory data obtained using impedance-based implants to those acquired using pressure-based plethysmography systems.

Methods: Three non-human primates (NHPs) were surgically implanted with PCTR implants from Data Sciences International. After recovery, each NHP was anesthetized and placed inside a head-out plethysmograph.

The signals from the impedance plethysmography implant, a head-out pneumotachograph signal, and the pressure signal from the biased-flow exposure chamber were recorded both preexposure and during an aerosol exposure. The respiratory parameters derived from each signal were used to estimate an inhaled dose for each plethysmograph, and compared.

Results: The shapes and artifacts evident in the raw signals transfer from one system to another - respiratory pauses or perturbations were easily discerned on the signals from the impedance plethysmography implant, the head-out pneumotachograph signal, and the pressure signal from the biased-flow exposure chamber. Estimated inhaled doses derived from each of the three methods were not significantly different. Conclusions: Impedance plethysmography, head-out pneumotachography, and plethysmography based on the pressure signal from the biased-flow exposure chamber all provide similar measurements of respiratory minute volume. Thus, it is feasible to use any of the three methodologies for the calculation of inhaled doses in inhalation studies. However, some methods better allow respiratory parameters to be monitored in real-time, facilitating the detection of variations in minute volume or anomalies that could influence the overall inhaled dose.

Detection of Bacillus anthracis and Yersinia pestis in Sputum Using Real-Time PCR

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Background: Bacillus anthracis, the causative agent of anthrax, and Yersinia pestis, the causative agent of plague, are two Category A bacterial agents due to their potential use as a biological weapon. Because of the high mortality rates associated with both agents, rapid detection in multiple human clinical matrices is essential to initiate appropriate medical treatment.

Methods: Currently, the CDC Laboratory Response Network (LRN) has developed real-time PCR assays for detection of these agents in blood and respiratory swab specimens. These biothreat agents may also be present in other respiratory specimen types such as sputum, but the complex nature of this specimen type has been problematic for extraction of DNA. In this study we describe an effective pretreatment method for sputum specimens that allows for efficient DNA extraction. Data will be presented to demonstrate that utilization of this pre-treatment does not affect the sensitivity of detection of B. anthracis and Y. pestis DNA.

Results: In this study, sputum samples were digested with sputolysin prior to DNA extraction. DNA was extracted using both an automated and manual method, the Magna Pure Compact and the Qiagen kit, respectively. After performing both extraction methods we were able to show equivalent detection levels for both B. anthracis and Y. pestis by using primer and probe set specific for each organism. Similar results for sensitivity were obtained using both the manual and automated extraction methods, suggesting either would be acceptable for use with these LRN real-time PCR assays in this matrix. Conclusions: Our study provides a quick and efficient method for the rapid detection of both B. anthracis and Y. pestis in sputum samples.

Application of Genotyping, Microarray and Sequencing Technologies to Detect Biothreat Agents from Complex Environmental Samples

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Background: Detection and characterization of threat agent signatures in complex environmental samples are critical areas for homeland security. Current detection technologies have their advantages and limitations. In this study, we compared Taqman and MLVA genotyping methods and DNA microarray techniques with high throughput Illumina and 454 sequencing to determine the limits of each technology to accurately characterize different threat agents.

Methods: We analyzed purified genomic DNA, DNA-spiked environmental samples and non-spiked complex soil and/or environmental air samples. The samples were spiked with B. anthracis or B. thuringiensis DNA to determine the feasibility of using each method to characterize the DNA
content of such samples. We also applied high throughput sequencing meth-ods and our pan-pathogen detection microarrays to total DNA isolated from BioWatch filters and environmental soil samples to determine the feasibility of using such an experimental approach to identify threat agent target DNA sequences relative to the environmental samples. Results: Our B. anthracis MLVA, Taqman canonical SNP assays and Pan-pathogen detection array are highly sensitive, capable of detecting 10-100 copies of B. anthracis when spiked into soil or aerosol samples. All three techniques positively identified B. thuringiensis from environmental filter samples that have been exposed to a B. thuringiensis-based spraying program to control Gypsy Moth. Using Il-lumina sequencing, we observed a seasonable effect on microbial content of BioWatch aerosol samples collected from a specific location over the four seasons. Conclusion: This study provided valuable information concerning the relative utility of the different methods for directly detecting and characterizing different threat pathogens and for identifying genetic signatures specific to these microbes.

072 (E)

A New Recombinant Ricin Agent-Like Material Simulant
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Ricin has proven to be the most commonly encountered threat material in ac-tual bio-threat situations. There exists the need to closely simulate this ma-terial in detection, decontamination, and proficiency testing. Most current close simulants that accurately mimic ricin are derived from active ricin iso-lated from the castor bean plant (Ricinus communis). Isolating ricin to pro-duce the modified simulants presents unique regulatory problems as ricin is both a select agent and a listed toxin under the Chemical Weapons Convention. In addition the antigenic activity of these “detoxified” ricin simulants is often significantly reduced; the response of antibodies to some formalin-modified ricin simulants is often less than 0.1% of the native toxin. We re-port the expression and production of a recombinant-derived simulant that is non-toxic by design so that no post-production modification, purification, or testing is needed to ensure that ricin toxicity is absent from simulant ma-terial. The recombinant protein accurately mimics ricin in its reactivity, size, and stability. The reaction of the simulant protein is essentially equal on both a molar or concentration basis to native ricin on common antibody-based de-tection systems like hand-held assays and ELISAs. It is a fusion of the ~30,000 dalton non-toxic A-chain to a ~30,000 dalton carrier that facilitates purification.

073 (E)

Viral Hemorrhagic Fever in Ukraine
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Ukraine has several endemic, enzootic viruses that are etiologic agents of acute seasonal febrile diseases, with Old World hantaviruses serving as one of the most likely causes of febrile illness associated with renal syndrome. ELISA-based analyses (conducted in the 1980s to perform widespread screening for hantavirus-specific antibodies in tissues collected from the pri-mary reservoir, rodents) detected antibodies to hantaviruses in six rodent species. Those with the highest antibody titers were members of the family Cricetidae, including My. arvalis and Cl. glareolus. Among the Muridae, hantavirus-specific antibodies were detected in Ap. flavicollis, Ap. agrarius, Mus musculus and Ap. sylvaticus. IFA (used to assess sera obtained from more than 1000 healthy donors) revealed hantavirus antibody specificity, with prevalence ranging from 1.4% to 5.5%. IFA was also performed with sera ob-tained from febrile patients with illness of unknown etiology. 95 cases of sus-pected HFRS were identified retrospectively of 484 patient sera. In comparing antibody titers to various Old World hantaviruses, serological examination primarily detected antibody specificity to PUUV rather than Hantaan virus (HTNV). Antibody titers to HTNV greater than those indicated for PUUV were only detected in HFRS patients from the Zakarpatska region of Ukraine. In summary, outbreaks of HFRS were revealed in Zakarpatska and Ivan-Frankovska regions, with potential occurrence of sporadic cases in all regions of Ukraine. HFRS morbidity correlated with detection of hantavirus-specific antibodies in small mammals and human populations. The remaining uniden-tified febrile illnesses associated with hemorrhage suggest the presence of other viruses in Ukraine, such as CCHFV. With further use of existing and newly engineered molecular diagnostic tools we plan to enhance knowledge of the ecology of this virus and define incidence of febrile illness caused by these pathogens.

074 (E)

Influence of Growth Media and Washing on the Spectral Laser Induced Fluorescence Signature of Biological Simulant in a Standoff Detection Context
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Background: Aerosolized pathogenic microorganisms represent a threat for the populations. Long range detection of such menaces is greatly attractive because it provides a spatial and dynamical description of areas being con-taminated within seconds. A technique based on spectrally resolved Laser-In-duced Fluorescence (LIF) LIDAR (Light Detection And Ranging) has been used as a potential approach to detect and classify a bacterial cloud. However, it is anticipated that the growth media used for the culture of microorganism contributes to the spectral signature observed and interfere with the classi-fication process. This work aims to assess the magnitude of this interference. Methods: Three different bacterial simulant strains (Bacillus globigii (BG), Bacillus thuringiensis (BT) and Erwinia herbicola (EH)) were grown for a 24 hour period in three different growth media (tryptic soy broth (TSB), nutrient broth (NB) and brain and heart infusion (BHI)). At the end of the incubation period, three types of samples were prepared and freeze-dried from each cultu-re: 1) the culture itself 2) the growth medium only and 3) a triple-washed sample of the culture. The resulting materials have been aerosolized in a lab-size LIF LIDAR aerosol chamber in order to obtain their respective spectral signatures using an excitation at 355 nm. Results: By using visual inspection and principal component analysis, signature variations were observed be-tween the three kinds of sample for most combinations of growth media/bac-teria. Conclusions: This study shows that the culture media used to grow the bacteria influences the cultures’ spectral signatures and must therefore be taken into account in the detection strategy and when producing a database of fluorescence spectra.

075 (E)

Production of MAbs Directed against Bacillus anthracis Spore and Development of Immunological Assays
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Background: Bacillus anthracis is a spore-forming bacterium that causes an-thrax in humans and in others mammals. The concerns for the use of Bacil-lus anthracis spore by biotorrem highlighted the necessity for rapid and easy test for the detection and identification of Bacillus anthracis spore in environmental samples. Several approaches have been proposed to detect Bacillus anthracis spore, including immunoasays, nucleic acid based detect-ion methods and mass spectrometry analyses. However immunological
methods appear more suited for rapid and simple detection. We produced series of monoclonal antibodies allowing sensitive and specific detection of *Bacillus anthracis* spore and we propose different detection formats (two site immunosassays on plate, immunochromatographic assays). **Methods:** Mabs were raised in mice by immunizing with formaldehyde-inactivated *Bacillus anthracis* spores and were screened by ELISA on spore coated plates. Mabs were characterized by western blot and ELISA for their cross-reactivity with a panel of *Bacillus cereus* and *Bacillus thuringiensis* isolates. Using antibodies with appropriate properties we have developed different immunological procedures including sandwich enzyme immunosassay on microtitre plates and immunochromatographic assay. **Results:** We have isolated 48 mAbs directed against *Bacillus anthracis* spores among which 15 were highly specific for *Bacillus anthracis* isolates. We have established different immunosassay tests allowing detecting between 10^4 and 10^3 CFU/ml in less than 5 hours on microtitre plates and around 10^3 CFU/ml in less than 30 min by immunochromatographic assay. **Conclusions:** By means of immunosassay test it is possible to rapidly and easily detect *Bacillus anthracis* spores with a high specificity and good sensitivity.

**076 (E)**

**Mass Spectrometry Based Functional Assay for Rapid Detection of Ricin**

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**Background:** Ricin, isolated from the seeds of *Ricinus communis*, is a potent cytosolic toxin. The ricin A-chain inactivates ribosomal protein synthesis by hydrolysing an N-glycosidic bond of adenosine in 28S rRNA. A functional-based assay for ricin detection and quantification using mass spectrometry (MS) was recently reported. This CDC-developed assay incorporates a 3-prong approach for ricin detection. The protocol has been successfully tested, however it is slow, manual, and needs specific antibodies. We have adapted and streamlined the functional-assay protocols into a robust, fieldable and automated detection assay with the primary goal of rapid ricin detection in crude castor bean extracts. **Method:** We utilize the CB-TOF system developed at JHU-APL, a system for rapid triaging of white powders. In our approach, we expand the number of synthetic substrates to two single-stranded DNA oligonucleotides. Ricin was mixed with the substrate in buffer. The reaction was optimized as a function of time and temperature. Positive ion MALDI MS was used to monitor the adenosine loss from the substrate after appropriate sample preparation steps: desalting and mixing with matrix. **Results:** Initially, a proteomics-based protocol for ricin detection was developed that included direct detection of the intact toxin by MALDI MS and its proteolytic peptides after rapid enzymatic digestion. This method is sensitive and very rapid but not appropriate for mixtures unless antibody extraction is performed first. As a complement to this protocol we have developed the MALDI MS functional assay, reported here. In this assay we bypass the application of ricin-specific antibodies allowing the assay to be directly applied to other ribosome-inactivation toxins. The assay is easy to perform, requires a controlled heating device and takes about 150 min end-to-end. Detecting a signal from the two DNA substrates improves the assay robustness. **Future work:** We plan to demonstrate the assay with other toxins, and further optimize protocols for automated CB-TOF analysis.

**077 (F)**

**Genome Engineering in Bacillus anthracis Using Cre Recombinease**

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Genome engineering may include deletion, insertion, or replacement of DNA regions in genomes that are under investigation. Here we describe approaches that efficiently excise selected regions from the *Bacillus anthracis* chromosome or the large virulence plasmids pXO1 and pXO2. These approaches used homologous recombination to introduce two direct bacterio- phage P1 loxP sites flanking the DNA region targeted for deletion. Cre recombinase action at the loxP sites excised the intervening DNA, leaving a single loxP site within the targeted genomic region. In this way, we mutated several *B. anthracis* genes and identified a new pXO1 minireplicon. A single loxP site generated as described above was also used successfully for site-specific integration of DNA either to complement a mutated gene or to insert a new genetic element. Finally, to replace a selected *B. anthracis* DNA region (e.g., htrA), two oppositely oriented loxP sites were inserted flanking the targeted DNA region. The incoming DNA segment (e.g., a spectinomycin resistance cassette) was separately inserted between two oppositely oriented loxP sites on a temperature-sensitive plasmid and introduced into the recipient *B. anthracis* strain. Transient expression of Cre recombinase caused replacement of the chromosomal htrA gene with the spectinomycin resistance cassette from the plasmid.

**078 (F)**

**The Role of Gene Fragmentation in Orthopoxvirus Evolution**

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**Background:** Variola virus is the causative agent of smallpox, a disease which was potentially lethal to humans. It has been eradicated in nature, however it is still considered to be a potential agent of bioterrorism. In addition to Variola, the genus Orthopoxvirus contains several other members which are capable of causing disease in humans. Orthopoxviruses appear to be evolving through the loss of genes which were present in an ancestral virus. There is a possibility that one of these viruses could develop into a more severe disease, either through genetic engineering as a bioterrorist agent or natural evolution of a more lethal phenotype. We believe that remnants of evolutionary processes, such as gene truncations and fragments, contain patterns which can be elucidated and can tell us about the biology of the viruses. **Methods:** Gene sets were predicted for completely-sequenced viruses in the genus Orthopoxvirus. Gene sets were derived using sequence similarity as well as gene synteny, or similar location in the genomes, to establish the relationships between them. Genes which were present in some genomes but appear to be truncated, fragmented, or absent in others were further scrutinized to determine biologically-relevant patterns of gene loss. **Results:** Truncated genes and gene fragments serve as evidence of part of the evolutionary process in the Orthopoxviruses, and are the cause of a significant portion of the differences between the viral genomes. **Conclusions:** It appears that a gene’s sequence is lost through random mutations, presumably after removal of selection pressure to maintain that gene’s function. By clarifying the ways that these viruses are changing over time, we better understand poxvirus biology, and their potential threat as human diseases.

**079 (F)**

**Effect of Sequencing Errors on Rare Variant Detection in Bacterial Samples**

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Presence of rare variants in bacterial samples could act as a sample’s fingerprint and be important in investigation and prosecution of bioterrorism attacks or attempts. We have conducted computational simulations and experimental validation of detecting ultra-rare variants (up to 1:1,000 mutant to wildtype mixing ratios) for PCR amplified regions from Mycobacterium tuberculosis. Results suggest that High Throughput Sequencing approaches, such as Illumina’s Genome Analyzer, have the sequencing depth necessary...
for identifying rare SNPs present in as low as 1/1,000th of the sample. However, distinguishing true rare variants from false positives is a major challenge. Herein we present a study of the sources and types of sequencing and data analysis algorithms associated errors that give rise to false positives. We also present several strategies for reducing the effects such errors have on sensitivity and specificity of rare variant identification.

080 (G)
Multifaceted Potentiation of Antibiotics via Silver Salts
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Background: Bacterial infections represent a public health issue and a potential threat to national security. New, broad-spectrum therapies and the improvement of currently used agents are critical to address this issue. Combination therapies represent one way to improve and expand the current arsenal of antibacterial agents. The compound silver nitrate has been used as an antimicrobial agent for centuries. In this work we explored the potential of silver nitrate as an adjuvant therapy for antibiotics. Methods: Silver nitrate’s bactericidal properties individually and in combination with antibiotics were determined against Escherichia coli using colony-forming unit survival assays. Minimum inhibitory concentrations (MIC) were assessed using broth microdilution assays in 96-well plates. Results: The MIC for AgNO3 against Escherichia coli was determined to be 20 μM. Cells treated with AgNO3 (30 μM) in culture showed a 3-log10 reduction in colony forming units over three hours. Killing by low concentrations of the antibiotics ampicillin, kanamycin, and norfloxacin was enhanced 1000-fold by the addition of subinhibitory levels of AgNO3. In combination with a subinhibitory concentration of AgNO3, the MIC for vancomycin against Escherichia coli was reduced approximately 4-fold. Conclusions: Silver nitrate demonstrates significant synergy in combination with other antibiotics against bacterial cells, thereby supporting its application as a potential adjuvant therapy. Silver also enhances the susceptibility of Gram-negative bacteria to the drug vancomycin, thereby broadening vancomycin’s antibacterial spectrum. We are currently expanding our work to include other organisms and exploring the phenotype in animal models.

081 (G)
Discovery of New Antimicrobial Compounds Inhibiting Burkholderia pseudomallei
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Background: The focus of the research program described here is the identification of new small molecules with antimicrobial activity against the pathogen Burkholderia pseudomallei (Bps). There have been few new antibiotics discovered with new modes of action and very few of those have been granted FDA approval for therapeutic use in the past 20 years. Most antibiotics in use today are based on naturally occurring compounds with new chemical entities acquired through medicinal chemistry based modification of known compounds. Evolva, Inc. has engaged in this discovery program using both natural products discovery efforts, our proprietary synthetic biology platform and in silico computational efforts. Three Methods of Antimicrobial Compound Discovery: 1) A highly combinatorial platform technology consisting of ligating the genes of biochemical pathways and cDNA collected from organisms that have antibacterial activity assembled into yeast artificial chromosomes is used to produce novel chemistries. 2) Natural products libraries are assayed for growth inhibition of Burkholderia thailandensis using optical density based assays. 3) In silico efforts are completed using proprietary computational software used to model the interaction between EF-tu and known inhibitors. A virtual library of over 8 million compounds is tested and a final group of selected compounds is tested in living system assays. Preliminary Results: Evolva, Inc., has identified several compounds from synthetic biology assays, natural product extracts, and in silico efforts. Methods and results will be presented for a selection of molecules from each effort. Preliminary Conclusions: The different approaches to the discovery of new antimicrobial molecules have each yielded candidates. This multipronged approach may be part of the solution for creating an improved pipeline of antimicrobial molecules.

082 (G)
Pharmacokinetics of a Monoclonal Anthrax Anti-Toxin Antibody in Healthy and Infected Cynomolgus Macaques
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Background: Valortim® (MDX-1303) is a fully human monoclonal antibody being developed to protect against inhalation anthrax by targeting protective antigen. The objective of this study was to compare the pharmacokinetics (PK) of Valortim following a single intravenous injection in non-challenged cynomolgus macaques (cynos) to that in cynos infected with B. anthracis (BA). Methods: Thirty healthy cynos were given 1, 5, 10, 20, or 40 mg/kg Valortim on Day 1, in the non-challenged study. Forty-eight cynos were challenged with BA spores and given 5, 10, 20, or 40 mg/kg Valortim when antigenemic. Serum was collected over 56 days in non-challenged and 5 days in challenged animals for PK measured by ELISA. Non-compartmental analysis was conducted on the concentration-time profiles in all cynos. Results: Healthy animals had approximately a 45-fold increase in Cmax for a 40-fold increase in dose between 1 and 40 mg/kg and an approximately 34-fold increase in area under the curve (AUC). Dose normalized Cmax, AUC values, mean clearance, volume of distribution, and half-life were generally similar across doses of 1 to 40 mg/kg. Infected animals had approximately a 10-fold increase in Cmax for an 8-fold increase in dose and an approximately 8- to 9-fold increase in partial AUC for an 8-fold increase in dose between 5 and 40 mg/kg groups. Due to limited numbers of measurable observations in the terminal phase for cynos in all dose groups, other PK parameters were not calculated. Conclusions: Pharmacokinetic parameters in non-challenged cynos were similar to those observed in anthrax-infected cynos treated with Valortim. This could have important implications for allowing an effective dose translation to a human dose. This work was supported by federal funds from the Biomedical Advanced Research and Development Authority (BARDA), Department of Health and Human Services (HHS), and the National Institute of Allergy and Infectious Diseases, National Institutes of Health, HHS, under Contract No. HHSN272200800040C.

083 (G)
Broad-Spectrum Bactericidal Activity of Full-Length Immobilized Antimicrobial Peptides

Background: Substrates with antimicrobial functionality have utility medical applications, food safety, decontamination, and prevention of materials degradation. Here, the activity of cationic antimicrobial peptide (AMPs) covalently-attached to amine functionalized surfaces was examined. Methods: SMAP29 variants synthesized with an N- or C-terminal cysteine, were directionally attached via a maleimide-NHS ester heterobifunctional linker to control orientation. A microplate-based kinetic assay was used to measure the activity at various peptide concentrations as a function of time; viable plate counts were used to determine bactericidal activity and correlated to the kinetic assay results. Results: Broad-spectrum activity has been demonstrated...
against both Gram-positive and Gram-negative pathogenic bacteria including *Acinetobacter baumannii* and *Staphylococcus aureus*, as well as *Bacillus anthracis* spores. Immobilized peptide potency was cell-dependent; however, the peptides exhibited activity for all organisms in a dose-dependent manner, reaching a critical concentration that inhibited growth completely. Compared to peptides in solution, a combination of increased concentration and longer exposure time was required for activity. The role of immobilized peptide orientation relative to the solid substrate was also investigated, revealing that the orientation with greatest activity was organism dependent. This was in contrast to solution activity in which SMAP with the C-terminal cysteine was more active against the range of bacteria evaluated. **Conclusions:** We have demonstrated that antimicrobial functionality can be imparted to surfaces through covalent, directional attachment of SMAP29. The research presented here lays the foundation for a new generation of non-leaching antimicrobial treatments. Since AMPs are not expected to induce resistance, they are a potential alternative to antimicrobial agents currently in use that may promote emergence of resistant microorganisms.

084 (G)  
**Ongoing Search for Broad Spectrum Antimicrobials**  
**Background:** An objective of the NIAID Biodefense Program is to obtain new antimicrobials for use against bioterrorist pathogens and emerging infectious agents. As part of this program, our institution has screened a library of 10,000 drug-like compounds against category A Select Agents and has begun similar screening against various drug resistant pathogens. **Methods:** An automated high-throughput microdilution screening assay was used to search for lead compounds in a Diversity Drug Collection. Compounds were obtained in DMSO and diluted in appropriate media to 16 μg/mL (*Bacillus anthracis* Ames, *Yersinia pestis* CO92, *Brucella abortus* 2308) or 8 μg/mL (*Francisella tularensis* Schu S4) for initial screening. Additional similar screening has begun against various drug resistant strains including MRSA, VRSA, VRE, and *Acinetobacter baumannii*. Minimal inhibitory concentration (MIC) assays were performed on compounds that showed activity in the initial screen using a two-fold dilution range of 16.0-0.0625 μg/mL. A colorimetric growth detection system was used for the assay for all organisms except *F. tularensis*. **Results:** Of 10,000 compounds thus far screened, 692 (6.9%) have been found to inhibit *B. anthracis* at ≤ 16 μg/L; 141 (1.4%) for *Y. pestis*; 2,047 (20.5%) for *F. tularensis* and 461 (4.6%) for *B. abortus*. Forty nine of the compounds were found to be inhibitory for all four of the select agents, suggesting the potential for broad spectrum activity. So far, screening of 500 compounds from the MyriaScreen library against the four drug resistant strains has revealed a similar pattern with about a 1-2 % hit rate. **Conclusions:** Completion of the screening of a 10,000 compound library against four category A and B agents has revealed many possible drug development leads with broad spectrum activity. Extending this screening to drug resistant category C agents has shown that the broad spectrum nature of several of these compounds includes activity against the class C agents.

085 (G)  
**High-Throughput Assays Identify Small-Molecule Inhibitors of Alphaviruses**  
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Venezuelan, eastern, and western equine encephalitis viruses, members of the genus *Alphavirus*, are causative agents of debilitative and sometimes fatal encephalitis. Although human cases are rare, these viruses pose a threat to military personnel, and to public health, due to their potential use as bioweapons. Currently, there are no licensed therapeutics for treating these infections. In collaboration with Prosetta Bioconformatics, Inc., several small-molecule compounds were identified that exhibit activity against multiple alphaviruses. The primary screen utilized a cell-free system (CFS) that recapitulates the alphavirus capsid assembly pathway. Compounds were identified that inhibit alphavirus capsid assembly in vitro. At USAMRIID these compounds were screened in high-throughput, live-virus assays. Compounds were mixed directly with virus prior to infection, or added to the cell culture medium before and after infection. As an end-point measurement, viral antigen was detected using antibodies against alphavirus E2 glycoproteins in a cell-based ELISA. Additionally, virus-induced cytotoxicity and apoptosis were assayed. These experiments helped differentiate between compounds that act directly on extracellular virions, or act during the intracellular portion of the viral lifecycle. Several compounds effectively inhibited virus infection when present in the cell culture medium but did not exhibit antiviral activity when incubated with virions before infection. Surprisingly, other compounds effectively inactivated extracellular virions, but did not influence infection when added to cell culture medium. As these compounds were initially identified in a CFS of capsid assembly, these results suggest a role for target proteins involved in capsid assembly and also other steps of the viral life cycle. Further studies will examine the mechanism of action of these compounds.

086 (G)  
**CD28 Mimetic Peptide Treatment for Superantigen and Endotoxin-Induced Lethal Shock**  
**Background:** Superantigens (SAg), such as *Staphylococcus aureus* (SA) enterotoxin B, (SEB) and *Streptococcus pyogenes* (SP) pyrogenic exotoxins (SPE) A, B and C, activate >20% of T cells with the release of proinflammatory cytokines and subsequent lethal shock. One of us (RK) found that dissimilar SAg structures share a conserved region that binds to CD28 co-stimulatory molecules. We now test the hypothesis that a peptide (AB103) that prevents CD28 dimerization protects against lethal shock not only against SAg exposure but also to endotoxin (LPS) which primes SAg lethal responses. **Methods:** AB103 was administered i.v. to Balb/C mice at 30 min before and up to 12 hr after challenge with SEB or live infection with SP that produce SPE A, B and C, and to mice subjected to polymicrobial sepsis after cecal ligation/puncture (CLP). **Results:** AB103 at -30 min protected 100% (5/5) of mice against SEB/D-galactosamine (D-gal) and 66% (8/13) of mice against LPS/D-gal. vs 0% (0/5) and 15% (2/13) of untreated controls respectively. Since mice are relatively resistant to SA, we gave mice live SP im. AB103 protected 27/30 (90%) when given before infection and 7/15 (47%) when given up to 5 hr after vs. 3/30 (10%) in untreated mice. Since few bacteria disseminated beyond the local injection site in untreated mice, we hypothesize that death was largely due to toxemia. Individual AB103-treated mice developed ileus against each of the SPEs but not to all three. While 11/12 (92%) of untreated mice died after CLP, 16/21 (71%) survived if treated with AB103 at the time of CLP and 5/8 (63%) when AB103 was given 8 hr post-CLP. Survival was associated with increased bacterial uptake in liver and spleen and no difference in plasma cytokines. **Conclusions:** We conclude that AB103 blocks multiple SAgS, enabling hosts to generate antibody against the conserved regions in SAgS. AB103 appears to have specific activity against endotoxin independently of CD28.
087 (G)

**Inhibition of the Yersinia pestis Type-III Secretion System by Small Molecules**

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*Yersinia pestis* is a zoonotic pathogen responsible for plague. The pathogen is classified as a Category A Select Agent by the CDC due to its high infectivity and lethality. The bacterium uses a Type III secretion system (T3SS) to deliver virulence factors into target cells. The system contains a single ATPase, YscN, thought to be responsible for the transport of virulence factors from the bacterium into the mammalian host. This ATPase has been shown previously to be essential for virulence in animal models. Previously, small molecules have been identified that target the activity of YscN and inhibit the T3SS in bacterial cultures. These small molecules do not inhibit the growth of HeLa cells and conform to the Lipinski Rule of 5 for potential therapeutics. In the current work, we have tested the ability of these molecules to inhibit the T3SS during infection of HeLa cells with *Y. pestis*.

088 (G)

**In vitro and in vivo Efficacy of Novel Broad-Spectrum Antiviral Compounds**

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Antiviral drug discovery has often revolved around development of inhibitors against virus-specific targets or mechanisms. This method has proved fruitful in discovering antiviral drugs against intended targets, but not necessarily for development of inhibitors against a broad spectrum of viruses. Development of therapeutics against host cell factors involved in the viral life cycle are highly desirable since these types of inhibitors are expected to possess broad-spectrum antiviral activity given the reliance of a wide range of viruses on a limited number of cellular mechanisms. IBT is using pre-clinical/non-GLP BSL-2 surrogate virus systems as a substitute for viral targets. Development of influenza A and B vaccines, but increases in naïve and vaccine-contraindicated populations complicate treatment. Limited post-exposure vaccination studies and the prairie dog model’s unique incubation period led us to examine the protective effectiveness of post-exposure vaccination in MPXV-infected prairie dogs. Methods: In the high-dose studies, we infected 40 animals with 10^6 pfu of MPXV (170x LD50) and vaccinated with Dryvax®, Acambis 2000®, or IMVAMUNE® at 1 or 3 days post-exposure. In the low-dose study, 20 animals were infected with 10^5 pfu of MPXV (2x LD50) and vaccinated 1 day later. Group comparisons were made for weight loss, rash illness, amount of viral DNA in blood by RT-PCR, and amount of infectious virus in oral swabs by tissue culture. Antibody responses were also compared by ELISA, cellomics and protein microarray. Results: In the high-dose studies we observed 100% mortality for all groups when vaccinated 1 or 3 days post-exposure with no differences between vaccinated and unvaccinated animals for any morbidity parameters. There were also no differences in antibody titers except for higher antibody titers (10^4) on Day 14 post infection in animals vaccinated 1 day post-exposure with IMVAMUNE® than all other groups (10^3). Conversely, in the low-dose study, no mortality or rash illness was observed in Dryvax® and Acambis 2000® vaccinated animals but mortality (25% and 33%) and rash illness (10 and 20 lesions) were observed in IMVAMUNE® vaccinated and unvaccinated animals. Preliminary analysis shows higher neutralizing antibody titers from Day 14 in select vaccinated animals when compared to unvaccinated animals. Conclusion: The results described above are relevant to public health preparedness for either a natural outbreak or a bioterrorism event involving OPXV.
Ebola Virus Glycoprotein Fc Fusion Protein Confers Protection against Lethal Challenge in Vaccinated Mice

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Ebola virus is a Filoviridae that causes hemorrhagic fever in humans and induces high morbidity and mortality rates. Filoviruses are classified as “Category A bioterrorism agents”, and currently there are no licensed therapeutics or vaccines to treat and prevent infection. The Filovirus glycoprotein (GP) is sufficient to protect individuals against infection, and several vaccines based on GP are under development including recombinant adenovirus, parainfluenza virus, Venezuelan equine encephalitis virus, vesicular stomatitis virus (VSV) and virus-like particles. Here we describe the development of a GP Fc fusion protein as a vaccine candidate. We expressed the extracellular domain of the Zaire Ebola virus (ZEBOV) GP fused to the Fc fragment of human IgG1 (ZEBOVGPFc) in mammalian cells and showed that GP undergoes the complex furin cleavage and processing observed in the native membrane-bound GP. Mice immunized with ZEBOVGPFc developed T-cell immunity against ZEBOV GP and neutralizing antibodies against replication-competent VSV-G deleted recombinant VSV containing ZEBOV GP. The ZEBOVGPFc vaccinated mice were protected against challenge with a lethal dose of ZEBOV. These results show that vaccination with the ZEBOVGPFc fusion protein alone is sufficient to induce protective immunity against ZEBOV in mice. Our data suggested that Filovirus GP Fc fusion proteins could be developed as a simple, safe, efficacious, and cost effective vaccine against Filovirus infection for human use.

Delayed Challenge of BALB/c Mice Demonstrates Significant Vaccine-Induced Protection against Aerosolized Yersinia pestis CO92

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Background: Y. pestis is the causative agent of plague, a disease that can manifest as pneumonic or bubonic forms. Pneumonic plague is the deadliest form and rapidly progresses in the host. This progression is believed to be a result of the ability of Y. pestis to evade or suppress the host immune system. Long-term immunity post-vaccination has not been studied in relation to efficacy in a standardized mouse model. Methods: We assessed the long term efficacy and humoral immunity of a sub-unit vaccine in the BALB/c mouse model of pneumonic plague. Mice were given two intramuscular vaccinations (primary, Day 0, and boost, Day 21), bled monthly for antibody evaluation and challenged with 50 LD50 Y. pestis CO92 at 182 days, 274 days or 365 days post-primary vaccination. IgG anti-Yp. pestis levels were measured by ELISA. Results: 92% of BALB/c mice challenged 182 days post primary vaccination survived challenge while 87.5% and 83.7% of those challenged on Day 274 and Day 365 respectively survived. Serum antibody levels peaked ~6 months post-vaccination, regardless of challenge group; and thereafter declined. Conclusions: Vaccinated BALB/c mice showed 83.7% or greater survival 182, 274 and 365 days after the primary vaccination. These data demonstrate that this vaccine is efficacious 365 days post primary vaccination in a murine model of pneumonic plague, with only a slight, non-significant, reduction in protection as compared to earlier challenges. Serum antibody data demonstrated that these mice had peak antibody levels ~6 months post vaccination. Higher anti-Yp. pestis antibody levels were associated with survival.

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Development of a Rabbit Vaccination and Inhalational Challenge Model for Evaluation of Next Generation Vaccine Candidates for Francisella tularensis

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Background: Francisella tularensis (F. tularensis), the etiologic agent of tularemia is a category A agent as aerosols of F. tularensis are highly infectious in humans. Licensure of next generation tularemia vaccines will likely involve the FDA Animal Rule. The objective of this study was to develop the New Zealand White (NZW) rabbit as a model for evaluation of F. tularensis vaccine candidates. Methods: The lethality and pathophysiological response in NZW rabbits following aerosol exposure to F. tularensis SCHU S4 was evaluated using a stage-wise approach. Blood was collected at multiple time points for detection of bacteremia and histopathology of lungs, liver, spleen and kidney was assessed. The efficacy of F. tularensis Live Vaccine Strain (LVS) administered via the subcutaneous route or by scarification was also evaluated in the NZW rabbit model. Rabbits were challenged with aerosolized SCHU S4 (7LD50) six and nine weeks post-vaccination. Survival was monitored for 28 days. Results: The inhalational median lethal dosage was estimated to be 31 cfu of SCHU S4. Rabbits succumbing to SCHU S4 infection died between four and nine days post-challenge. Tissue damage was observed in the lungs, liver and spleen. Vaccination with LVS protected 10-40% of rabbits from death following exposure to SCHU S4. Conclusions: The NZW rabbit is highly susceptible to aerosolized SCHU S4. The pathophysiological response in rabbits appears to be consistent with reports of human inhalational tularemia. Although vaccination with LVS did not protect the majority of animals, use of the NZW rabbit as a model for testing future vaccine candidates for F. tularensis continues to be of interest as future vaccine candidates will be required to demonstrate attenuation and efficacy comparable to LVS.

Generation of Fowlpox-Based Recombinants Expressing A27L, A33R, B5R and L1R Vaccinia Virus Genes as Safer Smallpox Vaccine

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Background: After smallpox eradication in 1980, the vaccination program with Vaccinia virus (V) was discontinued. The threat of intentional re-introduction of Variola virus (VARV) by bioterrorists remains of concern because of the easy transmission, high mortality and low or absent smallpox immunity in the majority of the human population. Furthermore, no antisera are licensed against smallpox and the administration of antiviral Cidofovir® requires hospitalization. Although attenuated VV strains (MVA, NYVAC, LC16m8) have been isolated, a smallpox vaccine is needed, based on selected VV structural genes expressed by a safer vector. Methods: Four constructs based on fowlpox virus (FPV) genome were generated by inserting separately L1R and A27L IMV-specific genes and A33R and B5R EEV-specific genes which were found to elicit a protective immunity in animal models of smallpox infection. The recombinants were produced by homologous in vitro recombination in replication- permissive chick embryo fibroblasts (CEF), and characterized by RT-PCR, Western blot (WB) and immunofluorescence (IF). The transgene expression was evaluated by infecting monkey (vero), human (293w) and avian (CEF) cells, and safety and immunogenicity will be
pared in mice to the conventional Dryvax®, Lederle, Wyeth and the attenuated MVA and NYVAC smallpox vaccine strains. Results: L1R, A27L A33R and B5R transcripts were expressed in all cells infected with the recombinants and the corresponding proteins identified by WB were localized in the cytoplasm in proximity of viral factories by IF. As expected, new recombinant FPV progeny was not detected in replication-restricted VERO and MRC-5 cells, while FPV recombinants were produced in CEF where an extensive cytopathic effect was observed. Conclusions: These results confirm that FPV recombinants can express selected VV genes for the elicitation of a protective immunity in the absence of vector replication.

096 (H)

Development of BSL-2 Neutralization Assay for Filoviruses: Proof of Concept Study

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Background: Ebola virus is a Filoviridae that cause severe hemorrhagic fever in humans and nonhuman primates with high morbidity and mortality rates up to 90%. Due to the required BSL-4 containment, it is difficult to use infectious Filoviruses to evaluate preclinical and clinical vaccine studies. We hypothesized that replication-competent VSV-G-deleted recombinant VSV containing the Filovirus glycoprotein (rVSV-FiloGP) could be used to develop a BSL-2 neutralization assay that mimics Filovirus neutralization. Methods: We constructed an rVSV-FiloGP containing the Zaire Ebola virus (ZEBOV) glycoprotein (rVSV-ZEBOVgp). This virus grew in VeroE6 cells and produced plaques that were smaller than wt VSV. Using a plaque reduction assay in VeroE6 cells, we analyzed specificity, sensitivity, and reproducibility of neutralization of VSV-ZEBOV. Viruses were neutralized with sera from mice vaccinated with a ZEBOV glycoprotein Fc (ZEBOVgp-Fc), which were protected against lethal challenge with ZEBOV, and human anti-ZEBOV neutralizing monoclonal antibody (mAb) KZ52. Results: Normal mouse and human sera did not neutralize rVSV-ZEBOVgp or wt VSV. Sera from C57BL/6 or Balb/c mice vaccinated with ZEBOVgp-Fc but not control FLAG-Fc specifically neutralized rVSV-ZEBOVgp. Human normal sera spiked with different concentrations of mAb KZ52 and EBOV neutralized rVSV-ZEBOVgp but had no effect in wt VSV titers. The neutralization assay was highly reproducible during the 1-year evaluation in our lab. Conclusions: Our data showed that rVSV-ZEBOVgp is a practical tool to evaluate anti-ZEBOV neutralizing antibodies under BSL-2 conditions, which could be used for research purposes and to assess consistency of production and potency of vaccines. Further research will be required to determine whether this BSL-2 Filovirus neutralization assay could be used as a surrogate marker or correlate of protection in preclinical and clinical vaccine trials.

097 (H)

Construction and Immunogenicity of a Modified Vaccinia Virus L1 Gene-Based DNA Vaccine


Although eradication of naturally occurring smallpox disease was reached in 1979, the potential threat of smallpox use in a biological weapon has heightened the need to develop an effective and safe smallpox vaccine. The purpose of our study is to evaluate DNA vaccine expressing the modified L1 gene of vaccinia virus that would elicit strong immunogenicity in mice. The L1 protein is required for vaccinia virus entry into host cells and is target for neutralizing antibody. In the present study, immunogenicity of plasmid DNA vaccine encoding the C terminal truncated form of L1 protein (tL1) was investigated. The tL1 gene was codon modified for optimal expression in mammalian cells. Insertion of SV 40 enhancer into the plasmid backbone increased tL1 expression as shown confocal microscopy and Western blot assay of transfected 293 T cells. We also tested the immunogenicity of a tL1 construct to which an immunoglobulin M (IgM) signal sequence was placed in frame with tL1 gene. The IgM-tL1 construct led to secretion of tL1 into the culture medium. Following immunization of Balb/c mice with tL1, the corresponding neutralizing antiserum titers were markedly increased. These data indicate that a gene-based vaccine of tL1 gene may be a useful candidate to protect against lethal smallpox infection.

098 (H)

Assessment of Viraemia in Vaccinated and Unvaccinated Primates after Aerosol Challenge with Monkeypox


Background: The aim of this study was to assess the protective effect of smallpox vaccination in an aerosol model of Monkeypox (MPXV) using cynomolgus macaques. The vaccines were Imvamune®, utilising a single and prime-boost regime, and ACAM2000™. Previous studies at HPA-Porton have shown that aerosol delivery of Monkeypox can be used to establish a reproducible infection in cynomolgus macaques. A range of parameters were measured throughout the vaccination and infection process including viral load and immunological parameters. Methods: Vaccination and Challenge ACAM2000™ was administered to the mid scapular area by scarification using a bifurcated needle (2.5x10^5-1.25x10^7 PFU). Imvamune® was administered via the s.c. route to the mid scapular area (1x10^7 TCID50). 24 macaques were challenged with Monkeypox virus Zaire 79 via the aerosol route (1x10^2 PFU). Viral Load This was measured in blood by using a real-time quantitative PCR (qPCR) for the MPXV Haemagglutinin (HA) gene. Plaque Reduction Neutralisation Test (PRNT) Heat-inactivated serum samples were assayed for the presence of Vaccina-specific neutralising antibody. Results: Orthopox-specific neutralising antibody levels were found to range from highest to lowest in the following respective order: ACAM2000™ > prime-boost Imvamune® > single dose Imvamune® > TSB only. Viraemia was found to be highest in the TSB vaccinated group followed by single-dose Imvamune® group and prime-boost Imvamune® group, respectively. No viraemia was detected in the ACAM2000™ group. Peak viraemia occurred 3 days earlier in both Imvamune® vaccinated groups when compared to the TSB vaccinated group. Conclusions: Vaccination with ACAM2000™ induced higher levels of neutralising antibody than Imvamune® and appeared to prevent viraemia after challenge. Imvamune® vaccination before aerosolised Monkeypox challenge appeared to reduce peak viraemia and time of onset of viraemia when compared with unvaccinated animals.

099 (I)

Characterization of Antibodies Specific for Hemagglutinin and Neuraminidase Proteins of the 1918 and 2009 Pandemic H1N1 Viruses

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Serologic studies have detected protective immunity against 2009 pandemic H1N1 influenza virus (H1N1-2009) in some people. However, further study of preexisting immunity has been complicated by the complexity of the human immunological background. In this study, we immunized mice with HA- and NA encoding plasmids. The cross-neutralizing activity of the anti-HA antiserum and the effect of the anti-NA antiserum on viral infectivity were evaluated using H1N1-1918- and 2009-pseudotyped particles (pps) and an H1N1-2009 isolate. Antibodies to H1N1-2009 HA (09HA) neutralized pps harboring...
09HA or H1N1-1918 HA (18HA). Similarly, antibodies to 18HA neutralized pps harboring 18HA or 09HA. Antibodies to 09HA and 18HA also neutralized the H1N1-2009 virus with high efficiency. Both antibodies to H1N1-1918 NA (18NA) and H1N1-2009 NA (09NA) enhanced the infectivity of pps harboring 09NA and 18NA. Although anti-09NA and -18NA antibodies significantly reduced cytopathic effects in multiplicity infection assays, conversely, these antibodies enhanced the infectivity of H1N1-2009 in single-cycle infection assays. Our study demonstrates the existence of cross-protection between antibodies against these two antigenically related virus strains and shows that anti-NA antibodies have a dual effect that requires reexamination of their role in human immunity.

100 (I)

**Antibacterial Role for Natural Killer Cells in Host Defense to Bacillus anthracis**

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Bacillus anthracis is the etiologic agent of inhalation, cutaneous and gastrointestinal anthrax and is a category A bioterrorism agent. Vaccination for anthrax in the US involves a complex dosing schedule, global adverse injection site reactions, and is only available to those with a high risk of exposure to *B. anthracis*. New treatment options to augment innate immunity to *B. anthracis* may reduce mortality and slow the spread of disease during a terrorist attack or natural infection. To determine the potential for NK cells to play a role in host defense to *B. anthracis*, we assessed the in vitro antibacterial activity of human NK cells against *B. anthracis* bacilli and spores and determined the outcome of infection in a mouse model following in vivo depletion of murine NK cells. Our results demonstrated that human NK cells kill both extracellular and intracellular *B. anthracis* bacilli but do not have activity against spores. Using electron microscopy, transwell separation, and chemical blockade, we demonstrated that NK cells make intimate contact with *B. anthracis* bacilli and that antibacterial activity is contact and granule dependent. We also observed that functional activity of NK cells is not sensitive to immunosuppression by *B. anthracis* toxins as has been demonstrated for other leukocytes. Murine NK cells have similar antibacterial activity to human NK cells when exposed to *B. anthracis* in our studies. *In vivo* depletion of murine NK cells using anti-asialo GM1 antibody does not alter animal survival following intranasal infection with *B. anthracis* in our studies, but significantly increases the bacterial load in the blood of infected mice. Our studies demonstrated that NK cells may play an important role that contributes to host defense against *B. anthracis*. Immunomodulation to augment NK cell function in early stages of anthrax should be explored further as a clinical intervention strategy to complement antibiotic therapy.

102 (I)

**Seroimmune Response to Cutaneous Anthrax Infection and Booster of Live Attenuated Anthrax Vaccine**

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Background: Understanding immune response to anthrax vaccines remains a priority in biodefense countermeasure development. We compared time-dependent changes of humoral immune responses in recipients of live attenuated anthrax vaccine (LAAV) to that of cutaneous anthrax infection.

Methods: Fifteen confirmed clinical cases of cutaneous anthrax and 14 first-year booster recipients of LAAV were studied at multiple time points after hospital presentation and vaccination, respectively. Seroimmune responses to the recombinant protective antigen (PA), edema factor (EF) and lethal factor (LF) were assessed by ELISA. Results: Anti-PA and anti-LF antibodies from the clinical cases increased 2-3 days after admission and leveled off from between 8-11 days and 24-32 days after admission. By 140-210 after admission, anti-PA and anti-LF titers had decreased slightly. Anti-EF ELISA titers showed a similar profile but with lower titers. LAAV booster vaccine recipients demonstrated an increase in anti-PA titers to similar levels as the cutaneous anthrax group. By 1 year after vaccination, anti-PA titers were near baseline. Anti-LF and anti-EF serological responses, however, did not increase in the LAAV vaccine recipients. Conclusions: Cutaneous anthrax induces antibodies to both PA and LF that remained elevated over time. In contrast, LAAV booster recipients produce a modest antibody response to PA that subsides over time, and no response to LF or EF. These results provide substantial insight for anthrax vaccine and immunodiagnostic development.

103 (I)

**Mechanistically-Based Computational Model of the Host Immune Response to Biological Warfare Agents: Application to Tularemia**

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Background: We are developing a mechanistically-based computational model of the interactions between pathogens and the host immune system. The model correlates pathogenesis with the physiological response in multiple species, enabling a rational, physiologically-based cross-species extrapolation of experimental infectivity data to humans. Typically, neutrophils,
macrophages, and dendritic cells first respond to an infection, then macrophages and dendritic cells present antigens to T and B cells, and cytokines are secreted to provide the signals which orchestrate this response and the subsequent adaptive response. **Methods:** The model structure incorporates cellular members of innate and adaptive immunity as well as cytokines. Our computational approach allows the actions of these members to be enhanced or suppressed to simulate mechanisms of immune subversion. The interaction of *Francisella tularensis* with host immune cells was simulated. Simulated production of pro-inflammatory cytokines by infected macrophages and dendritic cells was suppressed by varying degrees in a strain-dependent fashion. **Results:** Time course profiles of neutrophils, macrophages, dendritic cells, helper T cells, B cells, cytokotic T lymphocytes, natural killer cells, and bacterial proliferation/dissemination were predictive of data from experimental mouse models of tularemia. **Conclusions:** This in silico model can be used to study questions that are difficult to answer using traditional animal model approaches, such as quantifying the risk posed by a potential human exposure scenario, or simulating the enhanced virulence of genetically altered pathogens. Through the incorporation of prophylactic and therapeutic agents into the model, this research will help identify novel biodefense initiatives to counter pathogen exposure. Ultimately, this work will provide useful decision-making tools for difficult operational military questions.

**104 (J)**

**Killing of Staphylococcus haemolyticus on Metallic Copper Surfaces**

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**Background:** Copper (Cu) in its metallic form has regained interest for its antimicrobial properties. Use of metallic Cu surfaces in worldwide hospital trials resulted in remarkable reductions in surface contaminations. Yet, our understanding of why microbes are killed upon contact to the metal is limited. This knowledge is crucial for sustained use of such surfaces in hospitals and other hygiene-sensitive areas. Here we report on the molecular mechanisms by which *S. haemolyticus*, as a Gram-positive model, is inactivated by metallic Cu. **Methods:** *S. haemolyticus* cells were exposed to Cu or stainless steel control coupons and plated. Mutagenicity of Cu was assayed with cycloserine selecting for random cycA mutants. ICP-MS and fluorescent Cu(II) binding dye Cu-sensor-1 were used to quantify intracellular Cu(II) after contact to Cu. Live/Dead staining was used to quantify cells with damaged membranes on Cu or steel. **Results:** *S. haemolyticus* was inactivated within minutes on Cu but not steel demonstrating the antimicrobial efficacy of metallic Cu. ICP-MS analysis and in vivo staining with Cu-sensor-1 indicated that Cu ions contribute to lethal damage in metallic Cu-exposed cells. Cells accumulated large amounts of Cu ions from metallic surfaces. Mutation rates of Cu or steel-exposed cells were similar. However, DNA in cells from Cu surfaces was degraded post mortem. Live/Dead staining indicated cell membrane damage in Cu- but not steel-exposed cells. **Conclusions:** This study improves our knowledge of the cellular targets of metallic Cu-toxicity in bacteria. Mutagenicity assays and staining of *S. haemolyticus* suggested metallic Cu is not genotoxic and does not kill via DNA-damage. In contrast, membranes constitute the Achilles’ heel of Cu surface-exposed cells. We predict that DNA (including resistance-plasmids) cannot be transmitted easily from cell to cell on Cu because in killed cells DNA becomes damaged.

**105 (J)**

**Advanced Management of Biological Threats (AMBIT): Raising Awareness Towards Biological Incidents in Primary Care and Public Health**

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Severe biological incidents, may they occur deliberately as a bioterroristic (BT) event or accidently, represent a great challenge to physicians and staff in primary care institutions and public health. Besides the highly infectious nature of such agents, the broad inexperience in handling situations and diseases related to BT-relevant substances poses the biggest challenge for first responders. Additionally, early detection of such infections can be life saving but is vastly complicated as symptoms are typically not specific. Also, a biological incidence of especially deliberate significance will lead to an accelerated exhaustion of resources and manpower. Considering these circumstances we have developed a training concept that addresses these issues with these main curricula:

- Sensitizing by raising the awareness for a BT-event among primary care and public health institutions.
- Biological threat-management regarding primary care and public health aspects.
- Generation of easy to recall algorithms in biological crisis-situations.

Approximately 70% of the courses have practical features. Large parts of the training consist of scenario-plots that simulate severe biological incidents, proper use and knowledge of Personal Protective Equipment, and media response training.

The courses have been very successful regarding the evaluation results and response. In a short amount of time the courses became established and renowned entities nationally and internationally up to G8 settings. The numerous resonances towards these courses indicate the urgent need for professional training of primary care and public health institutions with regard to BT-events. Further, experiences from these courses have also proven to be applicable to naturally occurring highly infectious diseases, epidemics, and pandemics.

**106 (J)**

**Legal Framework for Decontamination and Containment of Biological Weapons in the Former Soviet Union Countries: Case Study of Defense Threat Reduction Agency’s Anthrax Decontamination of Voz. Island, Uzbekistan**

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In 2002, the Defense Threat Reduction Agency (DTRA) and Republic of Uzbekistan decontaminated a former-Soviet-biological-weapons-testing facility in Voz. Island, Aral Sea. DTRA decontaminated approximately 100-200 tons of weaponized anthrax in three months. Instead of formalizing the operation under international agreements, DTRA and Uzbekistan executed an agreement upon an ad hoc basis. A reason for an ad hoc agreement is partly due to a lack of an underlying implementation mechanism for Article VII of the Biological Weapons Convention (BWC) and United Nations Resolution 1540, which provide nonproliferation assistance to signatory countries. Therefore, our research focuses on a legal framework that allowed for DTRA’s decontamination operation that could be used for drafting an underlying mechanism for BWC and Resolution 1540. First, we have analyzed publicly available information. Second, we have requested DTRA’s records of its operation. We expect that our analysis will provide policymakers with clear insights and criteria to apply when drafting a proposal for review by international bodies.
108 (K)

**Toward a Better Referee: Tracking Simulant Disseminations Using Molecular Dyes**

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**Background:** There is a need for the development of better detection methods to protect both war-fighters and the civilian population from the ever present threat of a biological attack. In acquisition of new technologies by the DoD, efficacy of candidate systems must be rigorously evaluated. As such, the challenge exists to identify a suitable referee having the necessary fidelity to properly assess technologies of the newest detection systems. Current bio-aerosol detectors use particle sizing and the intrinsic fluorescent properties of ubiquitous biomolecules to detect bioaerosols. Thus it becomes a challenge to distinguish the test simulant from the native microflora of the test environment. Molecular dyes have been used for decades to visualize the molecular characteristics of cells. In this work, molecular dyes were used to stain microorganisms to increase the detection sensitivity and specificity of simulant aerosols.

**Methods:** Four different molecular dyes were used to stain either the DNA or lipid components of Gram positive spores and Gram negative cells. Fluorescence microscopy was used to detect the emission spectra from the stained spores and cells. Finally real time PCR was performed to check for potential fluorescent cross-talk among the fluorophores and the real-time probe as well as any PCR inhibition due to the nucleic acid binding properties of the dyes used.

**Results:** All dyes were shown to successfully stain the cells without cross reactivity. Substantial reduction of fluorescence of all dyes was observed after 84 hours exposure to direct sunlight enabling the use of the same dyes on future tests. Furthermore, the dyes did not interfere with the real-time probe and no PCR inhibition was observed.

**Conclusions:** Molecular dyes are a viable option for increasing the detection sensitivity and specificity of referee systems, and offer the opportunity to significantly enhance our capability to resolve test materials from background contamination during field tests.

109 (K)

**Accelerating R&D with Target Product Profiles**


**Background:** The University of South Florida Center for Biological Defense (CBD) is a multidisciplinary network that enhances public health preparedness for bioterrorism. The overall goal of the USF CBD is to support and promote homeland security through laboratory research and education programs. Our collaborative effort with partners such as USF Draper Labs and Claro Scientific supports the full spectrum of research activity.

**Methods:** The cornerstone of this highly productive and innovative effort is through communication of DTRA/RDECOM unmet needs and developing dual use countermeasures for the military and civilian communities. Special emphasis has been placed on enhancing R&D productivity to accelerate our product development process. **Results:** Essential to our ability to “translate” technologies to the war fighter/first responder is maintaining and establishing a critical path for product development. This process requires constant development and refinement of product development plans which include Target Product Profiles (TPPs) unique for specific interventions. Modeled after the FDA Critical Path Initiative (CPI) for therapeutics, TPPs represent unique roadmaps, which summarize the typical processes that encompass a diversity of efforts. **Conclusions:** As globalization is having major impacts on product development for detection and diagnostic technologies, we must proactively identify and validate these enabling technologies into the critical path of product development and achieve product realization. Equally important, is to “begin with the end in mind” with focused implementation strategies which address the end-user. Early integration of regulatory and implementation requirements into TPPs is equally essential to accelerating the R&D process. Our Center along with our partners are poised with vision and purpose to protect as well as to prepare for and react to the big next event.
Clinical and Epidemiologic Characteristics of Cutaneous Anthrax Infection in Georgia

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Background: Cutaneous anthrax is endemic in Georgia, providing the opportunity for insight into this infection with prospective observation. Methods: Subjects from two regional hospitals enrolled and assessed with a clinical and epidemiologic questionnaire on presentation and multiple time points up to 1 year after enrollment. Results: To date, 27 subjects have been enrolled, with subjects withdrawn due to alternative diagnosis. The most common occupation was farmers (52%) and most common exposure risk was contact with animals or animal products (87%). Two clusters were identified (three and four cases each). Many patients had systemic symptoms, such as regional lymphadenopathy (87%), fever (78%), and headache (48%). Surprisingly, a significant portion reported pain (52%) at the lesion site. Penicillin and ciprofloxacin were the most frequently prescribed antibiotics. Intravenous steroids (16/24) and oral H1-blockers (14/24) were used on hospitalized subjects. All patients have recovered without significant complications with the exception of one case with scarring-restricting hand motion. Conclusions: Unique observations about cutaneous anthrax include the high frequency of systemic symptoms and pain at the lesion site. The recovery of patients and lack of long-term complications suggests the effectiveness of the prescribed treatment although the impact of steroid use is unknown. Studying variations of epidemiological and clinical parameters of anthrax yield benefit for both the biodefense and international medical community.

Development and Validation of Animal Models to Support Testing of Vaccines and Therapeutics

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Background: Development of vaccines and therapeutics against biological organisms typically includes testing in an animal model. These studies investigate both safety/toxicity as well as efficacy using an animal challenge model. MRI has developed and validated a number of animal models to study a variety of biological agents, including influenza viruses, F. tularensis (Ft), encephalitic viruses, and prions. These development and testing programs are conducted under GLP in accord with FDA and WHO guidelines. Methods: Animal species selected for challenge studies with each pathogen were based on previous experience of MRI and others, and similarity of response in humans. Species included: mice and ferrets, influenza viruses; rabbits and mice, Ft; mice, encephalitic viruses; and hamsters for prion bioassay studies. Vaccine/therapeutic was typically administered via oral gavage or IM injection and pathogen challenge ranged from direct injection to aerosol exposure. Prior to conducting the animal studies, each pathogen is characterized in the relevant vehicle. Results: Animal challenge models, as noted above, have been successfully developed and validated. In addition, a recent safety/toxicology study was conducted for a candidate H1N1 influenza vaccine using a rabbit model to support the Phase I clinical trial. The challenge dose range-finding studies conducted prior to the vaccine/therapeutic efficacy studies all demonstrated a dose-response relationship between administered dose and clinical response. The LD50 calculated from these studies ranged from 29 CFU (colony forming units) for Ft in the rabbit aerosol model to 1.7 x 10^4 infectious virus particles (TCID50 in MDCK cells) in the mouse intranasal study with highly pathogenic avian influenza. Conclusions: The animal models noted above have been successfully employed to support development of a variety of vaccines and therapeutics. This presentation will describe study designs and detail technical approaches for animal model development and testing.
**016. Tuesday Poster Session**

**Genetic and Phenotypic Changes Associated with Passage of Eastern Equine Encephalitis Virus and Japanese Encephalitis Virus Under Selective Pressures**


**Background:** Eastern equine encephalitis virus (EEEV) and Japanese encephalitis virus (JEV) share many common characteristics including a positive-sense single-stranded RNA genome, an enveloped icosahedral nucleocapsid, and the ability to cause encephalitis in humans with significant morbidity and mortality. Despite their many similarities EEEV and JEV can be differentiated by genomic organization. **Methods:** In this study, the effects of genome organization on the replication of EEEV and JEV is compared along with the accumulation of genetic and phenotypic changes following passage in mice, in olfactory bulb neurons, and in Vero cells under conditions for rapid growth, rapid penetration, and antibody escape. These passage conditions were chosen in order to challenge commonly held assumptions regarding the effect of serial passage on viral phenotype. **Results:** EEEV replicated to greater titers in less time and accumulated fewer genetic changes than JEV in all systems tested. The mortality rates decreased and survival probabilities increased in most cases for mice challenged with JEV following passage, whereas the mortality rate and survival probabilities associated with EEEV remained unchanged and was dependant on the route of exposure. Following olfactory bulb passage of EEEV, the mortality rate in mice was unchanged however the survival probability was significantly decreased following subcutaneous exposure. The change in phenotype was confirmed in a 50% lethal dose analysis and could be traced to a single amino acid change in the E2 glycoprotein. **Conclusions:** The differences observed between EEEV and JEV can be attributed to the manner in which viral proteins are expressed and how genome replication is controlled.

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**Hematopoietic Progenitor Cells in Dengue Virus Infection**

G. Perng, S. Noisakran, H. Hsiao, K. B. Clark, F. Villinger, A. Ansari; Emory Univ., Atlanta, GA.

**Background:** Dengue is an important mosquito borne viral disease. Currently, there is neither a vaccine nor a chemo-therapeutic modality for the treatment of Dengue. Severe disease is particularly common in school-aged children for reasons that remain poorly understood. Age-related changes are common in many tissues and organs. For example the bone marrow (BM) is initially dominated by hematopoietic cells (HPC) but starting at 5 years of age, white cell frequency increases, reaching maximum levels at adulthood. BM suppression, especially of megakaryocytes, in dengue patients has been noted but the significance of such suppression to clinical disease remains ill-defined. We hypothesize that differences in the cellular composition of the BM that occurs as a function of age may account for differences in susceptibility to dengue virus (DV). **Methods:** BM samples from monkeys of different ages were compared for the outcome of DV infection ex vivo to assess if the pattern of response is age dependant. Freshly BM aspirates were infected with a low dose of dengue virus at an MOI = 0.1. Cell smears were performed and supernatant fluids were collected daily for 10 days. Quantitative real-time RT-PCR was used to measure the viral titers in the supernatant fluids and IHC staining with antibodies for cell surface markers and dengue viral antigen (DVA). Results. BM from i) young monkeys (under 5 years) were highly permissive to infection and able to support dengue virus replication with viral titers peaking at 2-3 days post infection (PI); ii) older monkeys (over 5 years) either showed the same pattern as younger monkeys or showed viral titers that steadily declined. HPC expressing CD41 were positive for DVA early (1-3 days) PI and shedding of virus containing platelets/vesicles was observed. These DVA-containing platelets/vesicles were engulfed by cells positive for the BDCa marker expressed by dendritic cells. Binding assays also showed that DV predominantly bound to CD41+CD34+ cells. Conclusions. Permissiveness of dengue virus infection in BM is age-dependent.

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**Development of Differential Molecular and Immuno-Assays for Identification and Characterization of Filovirus Sub-Types**


**Background:** In recent years more frequent and larger outbreaks of filovirus hemorrhagic fevers in central Africa have increased international public health concerns. As the Ugandan 2007 outbreak has demonstrated, new species of filovirus continue to be identified. These events, combined with the threat of bio-terrorism have resulted in the need to quickly and accurately identify filovirus infection. Our aim is to develop strategies that address the molecular and serological aspects of these viruses, with the goal of developing pan and species-specific detection/diagnostic assays. **Methods:** Filoviruses were propagated in Vero E6 cells. qRT-PCR RNA was prepared and qRT-PCR was performed targeting the nucleoprotein gene. Antigenic Peptides Bioinformatics was used to predicted antigenic regions of filovirus glycoproteins (GP). Virus-Like Particles Species-specific virus-like particles (VLP) were produced using recombinant baculoviruses (rBV) containing GP/VP40. Monoclonal Antibodies Mice were immunized with peptide antigens, or VLPs. Monoclonal hybridoma populations were assayed for antigen specificity and monoclonal antibody (mAb) stability by ELISA. **Results:** The qRT-PCR assays showed a high degree of specificity to the species intended, as the no-observed cross-amplification was observed. Analyses of different VLP species confirmed the presence of GP/VP40 and particle morphology. Both predicted linear epitopes and VLPs were shown to produce immune responses in mice and generate mAbs; positive clones were banked and cryopreserved. **Conclusions:** We have described different strategies for developing pan- and species-specific filovirus detection assays. Having successfully developed qRT-PCR diagnostic assays, we continue to develop others. We have also shown that antigens for mAb production can be effectively produced using in vitro and in silico methods, for an array of filoviruses.
leng. Some studies also were done in a ferret Influenza A model (H3N2). Oseltamivir served as the positive control and also was used in combination with EV-075-2. Endpoints for the models were lung viral titers, lung consolidation (LC), clinical disease scores (CDS) and weight loss. EV-075-2 was tested in lethal Marburg and Ebola Zaire mouse models, administered i.p., BID, starting on day 1. Endpoints were survival, weight loss and CDS. Liver and spleen viral titers were additional endpoints for Ebola. Results: Influenza: Prophylactic mouse studies demonstrated that EV-075-2 reduced CDS (50%), similar to Oseltamivir. However, EV-075-2 was more efficacious than Oseltamivir in reducing LC (20 vs. 30%) and viral titers (75 vs. 250 pfu/lung) and CDS (50%), when treatment started 1 day post challenge. The reduction in LC in the ferret model was more pronounced than for Oseltamivir. The combination of Oseltamivir with EV-075-2 provided added benefits over each treatment alone on all parameters tested. Hemorrhagic fevers: EV-075-2 significantly lowered viral titers and increased survival of mice infected with Ebola (70-80%) and Marburg (30%). Conclusions: EV-075-2 has a host-based broad spectrum mode of action. It is superior to Oseltamivir when administered on day 1 post Influenza infection. A combination with Oseltamivir appears to accelerate clearance of the virus and reduce clinical recovery time.

153 (A)

**Dengue Virus Infection and Modulation of Human Endothelial Cells**

A. NARAYANAN, E. R. MACKOW; Stony Brook Univ., Stony Brook, NY.

**Background:** Dengue hemorrhagic fever and dengue shock syndrome result from fluid leakage of the vascular endothelium. Immune responses contribute to increased capillary permeability by impacting the primary fluid barrier formed by endothelial cells (ECs). Dengue virus (DV) infection of ECs also alters normal transcriptional responses and microRNA regulation, suggesting additional means by which DV modifies barrier function and contributes to immune enhanced pathogenesis. Elucidation of these key steps may also provide new avenues for mitigating DV pathogenesis.

**Methods:** We analyzed DV (serotype 4) interactions with primary human EC receptors. Blocking antibodies and potential inhibitory compounds were used to define EC receptors required for DV infection. Additionally, microRNA levels within DV infected and uninfected ECs were analyzed using arrays. Changes in microRNA levels between uninfected and DV infected ECs were determined at both 24 and 48 hours after infection. Results: Approximately 70% of ECs were infected with DV 24 hours after infection. Although many antibodies and ligands failed to block DV infection of ECs, addition of heparin or heparan sulfate during DV infection or pretreating ECs with heparinase III resulted in a 60-80% reduction in infected ECs. In addition, DV infection of ECs significantly induced (5-fold) several EC specific microRNAs with known and unknown mRNA targets. Conclusions: Our findings indicate that DV efficiently infects ECs and that DV infection is mediated by heparan sulfate-containing EC receptors. Furthermore, DV infection leads to unique changes in the microRNA profile of ECs that may alter permeability responses of the endothelium that contribute to DV disease. Since ECs are highly susceptible targets of DV infection, targeting EC receptors or tissue specific microRNAs may also provide means of regulating DV pathogenesis.

154 (A)

**NFκB Mediated Modulation of Host Response Following Infection by Rift Valley Fever Virus**

A. NARAYANAN, K. KEHN-HALL, C. BAILEY, F. KASHANCHI; George Mason Univ., Manassas, VA.

Rift Valley Fever Virus (RVFV), the causative agent of Rift Valley Fever (RVF) is a RNA virus belonging to the genus *Phlebovirus*, family *Bunyaviridae*. It is classified as a potential bioterrorism agent and an emerging infectious pathogen. RVFV infects both humans and livestock. Earlier reports of fatality in humans were very low (1%), while current reports have escalated sharply to >40%. In livestock, there is an enormous economic burden associated with the health burden as the infection results in 100% abortion in adult animals and high fatality rates in newborns. The growing disease susceptibility and fatality rates is partly due to a lack of deeper understanding of the host-pathogen interactions and partly due to lack of FDA approved targeted therapeutics to counter the infection. We are particularly interested in the early host responses to RVFV infection so that we can utilize that information towards development of novel diagnostic tools and intelligent drug design. We analyzed the host response to RVFV infection using Reverse Phase protein MicroArray (RPMA) and identified multiple host signaling events as been altered during infection. Our initial studies carried out with ZH501 strain infected cells revealed that the p65 subunit of NF-κB is phosphorylated at Ser 536 in response to the exposure. We carried out more detailed analysis using the vaccine strain MP-12 and found that the phosphorylation of p65 was a very early host cell response that happens as early as 30 minutes following exposure. Our on-going studies include use of NF-κB inhibitors and evaluation of their influence on viral replication. Additional studies are directed at understanding the relevance of p65 phosphorylation to cytokine expression following viral exposure. Such studies will result in the identification of therapeutics that not only control viral multiplication, but also regulate aberrant host responses.
156 (A)

Influence of Influenza on Adenoviral Infection Detection in Basic Military Trainees at Lackland Air Force Base

J. M. MCDONALD; L. LOTTO; H. YUN; D. NIEMEYER; Eagle Applied Sci., San Antonio, TX; 2Brooke Army Med. Ctr., Ft. Sam Houston, TX; 3Chief Scientist Office, Lackland AFB, TX.

Background: Basic Military Trainees (BMTs) are at increased risk of febrile respiratory illness (FRI). Indoor crowding and stress during training facilitates transmission of respiratory pathogens. Up to 50% of BMTs develop FRI during basic training. Adenovirus is the major respiratory pathogen encountered, accounting for up to 70% of FRI cases. Characterizing respiratory infections in BMT populations so the epidemiology of respiratory outbreaks is identified allows for targeted preventive medicine counter measures. The presence of concurrent pathogens can make characterization challenging. Therefore, the Advanced Diagnostic Laboratory (ADL) at Lackland AFB identifies respiratory pathogens of military significance. Methods: Volunteers with FRI symptoms submitted nasal washes and throat swabs and completed standardized questionnaires. Specimens were tested with a universal Human Adenovirus assay plus assays for Human Influenza A and B, and Human Influenza hemagglutinin 1 and 3. Results: Between 2007-May-18 and 2010-August-17, 69% were positive for Human Adenovirus (1223 of 1772 enrollments). In contrast, only 44% (41 of 94) were positive for adenovirus during September of 2009. During that month, 63% (59 of 94) of samples were positive for Human Influenza virus A, H1N1. Six samples were observed to be positive for both adenovirus and influenza virus. Conclusions: The predominant respiratory pathogen causing FRI in BMTs at LAFB is adenovirus. Adenovirus can be superseded by other pathogens, as observed during the H1N1 pandemic of 2009. “The opinions expressed on this document are solely those of the author(s) and do not represent an endorsement by or the views of the United States Air Force, the Department of Defense, or the United States Government.”

157(A)

Cowpox Virus as a Model of Human Hemorrhagic Smallpox


Background: The threat of intentional release of variola virus has led to interest in improved animal models of orthopoxvirus infection to identify countermeasures and define disease pathogenesis. Although hemorrhagic smallpox reportedly accounted for less than 5% of smallpox cases, the case fatality rate was nearly 100%. Methods: Here we describe intravenous inoculation of Cowpox Brighton in cynomolgus macaques which resulted in disease with similar presentation as hemorrhagic smallpox. Results: IV inoculation resulted in a lethal disease across all doses attempted (increasing 10 fold doses of 5x105 to 5x107 PFU) within 12 days post inoculation. Serial samplings for hematology and clinical observations, as well as necropsy and histopathology findings support hemorrhagic disease. Specifically, petechial rash, hemorrhagic lesions in skin and gut, hematuria, anemia, thrombocytopenia, increased APTT times, and increased fibrin degradation products and D-dimers were consistently observed. Virus replicated to high levels in blood (8.0-9.0 log10 gene copies/ml) and tissues with wide organ system distribution. Conclusion: This unique model of hemorrhagic orthopoxvirus infection provides a more accessible, alternate means to study hemorrhagic orthopoxvirus infections and identify treatment strategies.

158 (B)

Determination of Inactivation of C. botulinum Neurotoxin via In Vitro Activity Assay

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Background: Current methods to validate inactivation of HHS and USDA Select Toxins involve the use of the mouse bioassay. This assay requires numerous animals, is expensive, and necessitates sending the toxin/toxoid sample via mail service to organizations outside of DOD which is restricted by regulation and adds additional cost. As a result of these issues, an alternate assay is needed based on toxin activity to validate inactivation of toxoid material. Methods: Currently, we have adapted a previously published procedure for an in vitro C. botulinum neurotoxin activity assay (Bagrayman et al. PLoS ONE 3(6):e2041. doi: 10.1371/journal.pone.0002041) to be used to validate inactivation of toxin. Results: Here we show that the assay is as sensitive as, or more so, than that the mouse bioassay. We demonstrate that the assay is effective with crude toxoid preparations and that the assay detects residual activity of toxoid that has been validated nontoxic via the mouse bioassay. Conclusions: This assay appears to be a good candidate to replace the mouse bioassay validation of inactivated of C. botulinum neurotoxin. Further testing is needed to validate the assay against the mouse bioassay.

159 (B)

Neurotoxicity and Pathogenesis of 6 B. anthracis Related Environmentally Isolated Bacteria Members of the B. cereus Group

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Background: Members of the Bacillus cereus group can produce several toxins such as neurotoxins, hemolysin, emetic toxins, in addition to enterotoxins. The objective of this work is comparing the toxigenic ability of environmentally isolated members of this group. Methods: 4 Bacillus cereus group members that were isolated from the soil by Lake Lassen in addition to B. cereus and samanii were grown on TSB & plasma, brain-Heart infusion and TSA & 5% Sheep blood. They were incubated at 37C and 30C. The biomass collected, cells were lysed or extracted depending if they were from solid media or broth. Toxicity bioassay was studied using in some cases the effect of toxins on nerve cells. Other methods such as Western blot, SDS-PAGE, PCR and ELISA were used to determine toxins type & the producing genes. Results: All the 6 study bacteria produced toxins at varying levels. B. samanii produced the highest levels of neurotoxins and had more damaging effect on nerve cells compared to the other 5 bacteria in the study. The amount of toxins produced was higher in all of the study bacteria at 37 C compared to 30C. Bacillus cereus did not produce any neurotoxins at 30C and very small amount at 37C. Incubation temperature affected their ability to produce certain toxins. They all showed resemblance that of B. cereus hemolysin BL. Neurotoxins effects were determined by the inhibition of growth & cell lysis of never cells. Conclusions: All the study bacteria have some degree of virulence and are potential pathogens. This is a property shared by all the members of the group. The toxicology study does indicate a common genetic virulence make up.
Production of 70 MAbs Directed against One or Several Strains of Salmonella

P. LAMOURETTE, C. FERAUDET-TARISSE, C. CRÉMINON, H. VOLLAND; CEA, Gif sur Yvette, France.

Background: Salmonella is a large genus of bacteria containing over 2500 species, among those Salmonella enteritidis and Salmonella typhimurium are responsible for the majority of salmonellosis cases in humans. These bacteria, considered as a food safety threat, are classified as a Category B agent by the CDC. To improve the laborious and time-consuming standard procedure for the detection of Salmonella, various rapid methods based on immunological principles have been reported. Those tests rely on the availability of specific monoclonal antibodies (mAbs). The aim of our study was a large-scale production of mAbs against each of the two main specific strains (Salmonella enteritidis and typhimurium) and against a range of Salmonella species. Methods: MAbs were raised by using 3 types of immunogens: i) heat-killed Salmonella typhimurium, ii) heat-killed Salmonella enteritidis and iii) alternance of heat-killed Salmonella typhimurium and Salmonella enteritidis. Three different kinds of microtiter plates coated with live bacteria (either S. typhimurium or S. enteritidis or E. coli) were used for antibody-binding assays (hybridoma screening) to investigate antibody specificity. Results: The strategies using immunisation with Salmonella typhimurium or Salmonella enteritidis yielded 25 mAbs and 9 mAbs respectively, specifically directed against the corresponding Salmonella strain. Interestingly, after immunisation of mice with an alternation of these two strains, we obtained 16 mAbs directed specifically against Salmonella typhimurium, 6 mAbs directed specifically against Salmonella enteritidis and 14 mAbs recognising both strains, and sometimes others strains of Salmonella. Conclusions: When immunizing mouse with a mix of different strains of Salmonella, we can isolate and produce mAbs directed against each specific strain and mAbs recognising a wider range of Salmonella strains. We report the production of 70 mAbs that can be useful in the development of immunological assays (see Abstract by Féraudet-Tarisse).

Characterization of the Natural History of Inhalational Melioidosis in Non-Human Primate Models


Background: Infection with Burkholderia pseudomallei can occur by either natural or deliberate means. In either scenario, inhalation is a potential route of exposure. The aim of the present study was to develop non-human primate models of inhalational melioidosis for use in therapeutic testing to satisfy the FDA’s animal efficacy rule. Methods: African green monkeys and rhesus macaques were exposed to aerosolized B pseudomallei in a well-characterized head-only exposure chamber. Average inhaled doses for both species ranged from 10 to 100 CFU. Results: Many characteristics of the disease in both species resembled the disease in humans. In animals that succumbed to disease, an increase in body temperature and alteration of the diurnal pattern occurred. The dominant pathologic changes observed are abscess in the lungs composed of necrosuppurative inflammation with numerous degenerate neutrophils fewer macrophages, lymphocytes and plasma cells with necrotic debris. Lesions were restricted to the lungs in the most acute cases. Survival animals in this study ranged from no significant lesions to mild edema in the lungs. The most pronounced immunological findings was leukocytosis and neutrophilia. Bacteremia was not a consistent indicator of outcome in either species. However, PCR on whole blood was able to detect the presence of bacteria in all non-human primates by 48 h post exposure. Conclusion: Additional analyses to determine cytokine profiles in plasma and the presence of bacteria in throat and rectal swabs collected post exposure are ongoing. These preliminary data suggest that both species represent accurate models of the human disease.

Proficiency Testing for Biological Agents

J. L. GRAY, W. D. RAWLINSON 2; 1RCPA QAP, Sydney, Australia, 2The Prince of Wales Hosp., Sydney, Australia.

Background: The RCPA BioSecurity QAP has been established by a request from the Australian Department of Health and Ageing (DoHA) to provide a proficiency testing program for Biological Agents. Commencing in 2009 the focus has been on the preparation of surveys for Bacillus anthracis. This bacterium has been used in bioterrorism incidents and is an organism that laboratory may not encounter in the normal course of their work. The surveys are available to laboratories within Australia that have facilities for handling biological agents.

Program purpose is to:

• Confirm or rule out B.anthracis
• Characterize the different strains of B.anthracis to assist forensic investigations
• Establish antibiotic susceptibility of B.anthracis to assist in establishing treatments

Performing these functions quickly and accurately would result in a significant reduction in loss of life.

The program is unique in that the scenarios and clinical histories are well researched to reflect current information, research and trends in bioterrorism/biosecurity, associated technologies and clinical research. Methods: Complies with ISO/IEC 17043:2010 Conformity assessment - General requirements for proficiency testing.

Specimens are cultures (pure or mixed), blood, cerebral spinal fluid, swabs, white powders, environmental specimens, virtual microscopic images or genetic material. Results: The first surveys indicated that participants were not always aware of the unique characteristics of B.anthracis e.g. morphology and tenacity of colonies. They varied in their ability to deliver accurate results for those tests that they knew were indicative of B.anthracis e.g. motility, haemolysis and penicillin sensitivities. Conclusions: Through performing these surveys the participants have become increasingly consistent and confident in their ability to confirm or rule out B.anthracis in a timely manner.

Modules for 2011 include Yersinia pestis, Ricin and Botulism toxin.
belted rabbits with either 0.67 mg/kg LF and 0.24 mg/kg PA (low dose) or 0.98 mg/kg LF and 1.95 mg/kg PA (high dose). The animals' heart rate and mean arterial pressure were continuously monitored via telemetry until 72 hours post-challenge. Additional animals were used for cardiac troponin I quantitation and cardiac histopathology. Results: Low-dose LeTx depressed heart rate without affecting mean arterial pressure, and it also induced mild cardiac pathology which was confirmed by the increase in cardiac troponin I. High-dose LeTx caused cardiac pathology as well, however to a much greater extent, and the increase in cardiac troponin I was more dramatic. Conclusion: Our study demonstrates the depressive, pathological effects that LeTx has on the heart, and it provides evidence for the toxin's direct impact on the heart of higher organisms.

**164 (B)**

**Impairment of Macrophage Bioenergetics by *Bacillus anthracis* Derived Nitric Oxide**

M. CHUNG, T. G. POPOVA, A. NARAYANAN, C. L. BAILEY, S. G. POPOV; George Mason Univ., Manassas, VA.

*Bacillus anthracis*, a causative agent of anthrax, is able to germinate and survive within macrophages. A recent report suggested that *B. anthracis*-derived nitric oxide (nNOS) is a key bacterial defense protecting bacterial DNA against the oxidative burst in the macrophages. Here, we report that in addition to this protective effect, nNOS induces macrophage killing by S-nitrosylation of bioenergetic proteins within mitochondria. Nitroso-proteomic analysis with a biotin-switch technique demonstrated that mitochondrial proteins were S-nitrosylated during *B. anthracis* Sterne spore infection in RAW264.7. Mass spectrometry and Western blot confirmed that enzymes involved in oxidative phosphorylation, energy supply, and antioxidation were predominantly S-nitrosylated. Non-toxicogen deltaSterne challenge decreased proteins S-nitrosylation in macrophages. For each target enzyme tested (aconitase 2, complex I, complex III and complex IV), Sterne challenge caused enzyme inhibition. N-Nitro-L-arginine methyl ester, an NOS synthase (NOS) inhibitor, reduced S-nitrosylation and partially restored ATP depletion and cytotoxicity in macrophages. Biochemical and microscopic analysis demonstrate that NO for S-nitrosylation of mitochondrial proteins originated from NOS of *B. anthracis*. Together, our data suggest that nNOS leads to energy depletion driven by impaired mitochondrial bioenergetic machinery, and ultimately contributes to macrophage death.

**166 (B)**

**Zeptamole Detection of Anthrax Edema Factor Activity by LC-ESI-MS/MS**

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Background: Anthrax is caused by the pathogenic bacterium *Bacillus anthracis*. It secretes two major exotoxins: Lethal factor (LF) and edema factor (EF) combine with protective antigen (PA) to form lethal toxin (LTx) and edema toxin (ETx) respectively. LF inactivates proteins centrally involved in host defense, affecting a wide range of immune cells. EF is a highly active calmodulin-dependent adenyl cyclase (AC) which converts ATP to cyclic AMP (cAMP) causing edema and immune dysfunction. ETx and LTx synergize to reduce inflammation and immunity and allow the bacterium to proliferate unchallenged. We have developed a high sensitivity AC activity-based method for quantitative measurement of EF. *Bordetella pertussis* AC (PAC) was evaluated to determine specificity of the EF method. Methods: Anti-EF monoclonal antibodies (mAbs), EF ligand PA63 and anti-PA mAbs alone and combined were immobilized on magnetic protein G or Tosyl-activated beads, and used to purify EF. Purified EF was incubated in an optimized reaction buffer using protocols designed by JMP statistical software. ATP and cAMP were analyzed by LC-ESI-MS/MS and quantified. EF levels were measured in serum from 5 rhesus macaques with inhalation anthrax and compared to LF levels. Results: The EF method is specific, exquisitely sensitive and can be performed in ≤4h. A limit of detection of 15 fg/ml (170 zmoles/ml) was obtained for 200 μl sample purified with EF mAb and ligand PA63 combined. Recovery approached 100% for a large dynamic range. Additional capture combinations were optimized for late stage rhesus infection samples. Conclusions: This method for EF detection is the most sensitive reported for any anthrax toxin. Combined with our MALDI-TOF-MS method for LF, the EF method expands our capability to detect and measure both toxins early and throughout infection.

**165 (B)**

**Microbial Forensic Investigation of a *Yersinia pestis* Outbreak in Afghanistan**

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Background: An outbreak of severe disease occurred in the Nimroz Province of southern Afghanistan in December of 2007. The symptoms include fever, impeded mitochondrial bioenergetic machinery, and ultimately contribute to macrophage death.

**167 (B)**

**Molecular Typing of *Yersinia pestis* Outbreak Strains from the West Nile Region of Uganda**

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Background: *Yersinia pestis* is a Category A bacterial pathogen and is the etiological agent of plague, a highly pathogenic zoonotic disease most commonly transmitted to humans through exposure to fleas and their mammalian hosts. Plague foci are found throughout the Americas, Africa and Asia. Human cases of plague are most often sporadic, with outbreaks relatively rare. In 2008-2009, an outbreak of plague occurred in the districts of Arua and Nebbi, Uganda (an area of app 50 sq km), with >50 human plague cases culture-confirmed in a 5 month period. This study used pulsed-field gel electo-
Identification of SNPs Responsible for Antibiotic Resistance in Biothreat Agents by DNA Microarrays and Illumina Sequencing

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**Background:** Antibiotic resistant bacteria are a growing concern in the areas of public health and national security. The ability to effectively identify the modifications needed to confer this resistance is of critical importance. In this study, we grew avirulent strains of bacterial biothreat agents under various antibiotics and selected resistant isolates. Genetic variations and mutations in the resistant isolates were identified by both DNA microarrays and Illumina sequencing. **Methods:** We grew avirulent strains of *B. anthracis*, *Y. pestis*, and *F. tularensis* in multiple rounds of increasing concentrations of ciprofloxacin and doxycycline. *B. thailandensis* (a surrogate for *B. pseudomallei*) was grown similarly in TMP/SMX and cefazidime. Genomic DNA from antibiotic resistant mutants for each agent-ab combination was sequenced by Illumina sequencing and also applied to an LLNL developed genomic tiling array specific for each bacterial strain. Single nucleotide polymorphisms (SNPs) identified by these methods were confirmed and tested in mutants in all growing rounds by Sanger sequencing. **Results:** We were able to obtain highly resistant mutants for most agent-ab combinations. We identified mutations in known antibiotic resistant mechanisms as well as novel mechanisms for resistance. Illumina sequencing and the DNA microarrays showed concurrence on SNP identification. With Sanger sequencing we were able to confirm the order by which the mutations occurred as well as which were implicated in generating the higher levels of resistance. **Conclusion:** In this study, Illumina sequencing and DNA microarrays were used to identify mutations in biothreat organisms that are implicated in conferring a high level of resistance to antibiotics. The identification of these mutations will provide better understanding of antibiotic resistant mechanisms of biothreat agents and prove useful to developing assays to quickly screen antibiotic resistant isolates in the future.

Susceptibilities of Novel Antimicrobial Compounds against Multiple Bioterrorism Agents

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**Introduction:** Our ability to rely upon antibiotics is severely threatened due to antimicrobial resistance. As a consequence, a concerted effort is needed to ensure the continuous availability of effective drugs to treat bacteria that cause human disease. This work investigated the effectiveness of novel compounds sourced from multiple companies against bacterial type strains and clinical isolates of potential bioterrorism agents. **Methods:** Bacterial Isolates of *Bacillus anthracis*, *Burkholderia pseudomallei*, *Burkholderia mallei*, *Francisella tularensis* and *Yersinia pestis* were obtained from the National Collection of Type Cultures (NCTC), HPA, UK and Biodefense and Emerging Infections (BEI) Resources Repository, USA. Clinical isolates of the agents were also obtained from numerous sources. Novel compounds These were obtained from companies around the world and tested against the above agents. Our ability to rely upon antibiotics is severely threatened due to antimicrobial resistance. As a consequence, a concerted effort is needed to ensure the continuous availability of effective drugs to treat bacteria that cause human disease. This work investigated the effectiveness of novel compounds sourced from multiple companies against bacterial type strains and clinical isolates of potential bioterrorism agents.

**Results:** Initial minimum inhibitory concentration (MIC) results show that 17 of the 66 novel compounds exhibited antimicrobial activity against one or more of the bacterial agents. These compounds were effective at 4µg/mL or less. Of these 17 compounds, several gave MIC results of 0.12µg/mL or less. Six compounds showed significant activity against *F. tularensis*, five against *B. anthracis*, two against *Y. pestis* and one against *B. mallei*. **Conclusions:** This study has identified a number of potential novel compounds that could be of clinical importance. These compounds will be further investigated to ultimately elucidate their modes of action.

Pneumonic Tularemia in Rabbits: Utility of X-Rays and Laboratory Diagnostics in Predicting Outcome

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**Background:** Francisella tularensis is the causative agent of tularemia. Because *F. tularensis* can cause major morbidity and mortality at low doses when inhaled, it is a category A select agent. The current study evaluated the New Zealand white rabbit as a relevant model of the human disease caused by inhalation of *F. tularensis*. **Methods:** Rabbits were exposed to aerosolized *F. tularensis* SCHU S4 using a nose-only exposure chamber (CH Technologies, Westwood, NJ) controlled by the AeroMP exposure system (Biara Technologies, Hagerstown, MD) with real-time plethysmography (Buxco Research Systems, Wilmington, NC). Rabbits were monitored for changes in body temperature, weight, physical activity, food & water intake, pulse, blood oxygen levels, clinical chemistries, and erythrocyte sedimentation rate (ESR). **Results:** Fever and weight loss were observed within 3 days of aerosol infection. On day 4, ESRs were elevated in 5 of 6 rabbits, while food and water intake declined to near zero. X-rays indicated development of pneumonia in rabbits 4 days after infection. Regardless of *F. tularensis* dose, all unvaccinated rabbits were moribund by 5 days after infection and were euthanized. Gross pathological changes were noted in the lung, spleen, and liver, and *F. tularensis* was detected in all three organs. Surviving rabbits appeared normal by 14 days post-infection including body temperature, food/water intake and reduced ESRs but x-rays indicated slow resolution of pneumonia. **Conclusions:** Rabbits are susceptible to low respiratory doses of *F. tularensis*, and the disease resembles what has been reported for humans. Food and
water intake were the parameters most associated with outcome. ESR levels, along with body temperature and food & water intake, may predict survival in vaccinated rabbits. Together, these data indicate that rabbits are a useful model for evaluating efficacy of candidate vaccines or therapeutics against pneumonic tularemia.

171 (B)

Susceptibility of Bacillus anthracis Isolates from a UK Outbreak in Intravenous Drug Users against a Range of Antimicrobial Agents and a Novel Compound

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Introduction: Bacillus anthracis is the causative agent of the bacterial disease anthrax, which is widely distributed around the world. Anthrax in the UK is an uncommon disease with only 20 cases being reported from 1981-2008 under the Public Health Act. However, since December 2009, an outbreak of the infection has been observed amongst intravenous drug users. The infection commonly presents as cutaneous, inhalational or gastrointestinal; however, in the case of the intravenous drug users, it has been observed as injectional allowing the bacteria direct access to the blood stream resulting in vast numbers of deaths. The susceptibility of 32 B. anthracis isolates from the outbreak have been tested against ciprofloxacin, clindamycin, gentamicin, meropenem, penicillin and a novel compound supplied by a pharmaceutical company. Methods: 32 isolates of B. anthracis were obtained from the Special Pathogen Reference Unit (SPRU), HPA, UK. B. anthracis Ames was used as a control and was obtained from Biodefense and Emerging Infections (BEI) Resources Repository, USA. The minimum inhibitory concentration (MIC) of each drug was determined using the broth microdilution method described in the guidelines published by the Clinical and Laboratory Standards Institute. 100 µl bacterial suspension was added to each well of a 96-well microtiter plate containing 100 µl antibiotic dilution. Plates were incubated for 24 hours at 35°C after which the optical density was measured at 620nm.

Results: All strains were susceptible to the five drugs tested, with the MIC values falling within the CLSI or published recommended ranges where available. The novel compound was active at an MIC range of 0.25-4 µg/ml with an MIC 50/90 of 2 µg/ml. Conclusions: This data expands the spectrum of antimicrobial agents that could be used to treat B. anthracis. The novel compound results have been sent back to the source company to be further investigated.

172 (B)

Characterization and Aerosol Uniformity of a Whole-Body Exposure System

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Background: A well-characterized aerosol exposure chamber is necessary to ensure spatial uniformity of generated aerosols. Therefore, the aim of the present study was to verify spatial concentration uniformity within a whole body exposure chamber (WBEC) used for small animal challenges. Methods: Bovine serum albumin (BSA) and Bacillus anthracis were separately generated within the WBEC using a 3-jet Collison nebulizer. Filter samplers were randomly placed within the WBEC. Samplers were compared within runs to identify aerosol mixing characteristics and between runs to verify day-to-day consistency. Each measured aerosol residence time was also compared with theoretical to confirm a sufficient mixing chamber. Plastic bottles were placed inside two animal cages within the WBEC to simulate the volume occupied by small rodents. Results: Chamber leak rate was found to be within nominal range of those described in previously published studies. The average BSA and B. anthracis concentrations on filters were 122 ± 14 µg/ml and 0.58 ± 0.11 cfu/ml, respectively. The average sampler within-run coefficients of variation were 11% for BSA and 18 % for B. anthracis, which are similar to previously published studies. ANOVA statistical analysis confirmed no significant difference in concentration between filter locations. Measured residence times of aerosols within the WBEC were comparable to theoretical residence time. Conclusion: This study confirms that BSA or B. anthracis aerosols generated within a WBEC are uniform throughout the chamber. Thus, each animal or sampler placed within the chamber would be exposed to the same aerosol concentration regardless of location, resulting in uniform dosing.

173 (B)

An In Silico Model of Endotoxic Shock

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Background: Biologically-based in silico models of pathogen-host response interactions were designed to leverage experimental animal studies to predict human responses to infection. However, animals vary in response and susceptibility: sensitivity to E. coli endotoxin is guinea pig > hamster > mouse (McCuskey 1984). To establish a sound basis for interspecies extrapolation, a model of host response leading to pathogenesis and shock processes is needed. Macrophages demonstrate various responses to LPS, including the release of potent lipid autacoids, which are involved in a cascade of events leading to endotoxic shock. Platelet-activating factor (PAF), in particular, is a key mediator of the inflammatory response and participates in anaphylactic shock and subsequent death. In guinea pigs, PAF (2 ug/kg IV) causes bronchoconstriction and hypotension in seconds and lethality within 25 minutes. In rats, however, doses as high as 3 ug/kg scarcely changed the heart rate (Tanaka 1983). These results suggest species differences in the biomodulatory effects. Methods: A dynamic model for PAF and its antagonist ginkgolide B (GB) was developed to link a pathogen’s kinetics and host response. The kinetic models include lung, heart, and rapidly and slowly perfused tissues with IV and inhalation exposure and routes for binding and elimination of PAF. Kinetic parameters (acyethylhydrolase activity, receptor densities, binding affinities) were obtained for several species. Results: These data reveal potential explanations for species differences in sensitivity to increasing concentrations of PAF. The model was used to simulate experimental exposure data sets to PAF and GB, and to generate internal dose metrics (receptor occupancy) and response profiles (cardiac output). Dose metrics were correlated with observed signs of infection and lethality in an attempt to identify the most appropriate metrics for predicting adverse effects. Conclusion: This model of pathogen kinetics and these dose metrics help to elucidate mechanisms of host response dynamics and improve cross-species extrapolation of response data.

174 (B)

Attenuation and Protective Efficacy of a Type A Strain of Francisella tularensis Complemented for Loss of O-Antigen

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Background: Francisella tularensis causes tularemia and is a potential biowarfare agent. An approved vaccine for tularemia is not currently available. Type A mutants were screened on Congo Red agar to identify an isolate with the same phenotype as F. novicida (non-pathogenic for humans), in the hope of identifying an attenuated, protective isolate. Methods: Type A mutants were screened on Congo Red agar to identify an isolate with the same phenotype as F. novicida (non-pathogenic for humans), in the hope of identifying an attenuated, protective isolate. Methods: Mutants of type A strain T10902 were selected on supplemented Congo Red agar. The mutation was identified by sequencing, complemented with the normal gene in expression vector pFNLT6, and recombinants characterized by Western blotting, serum resistance, macrophage survival, and virulence in mice. Results: A mutant with the same red phenotype on Congo Red agar as F. novicida was isolated, and determined to have a base substitution in wbtK
175 (B)  
**Francisella novicida** QseC and Novel Motility Phenotype Is Inhibited by Norepinephrine  
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We have recently shown that *F. novicida* forms biofilms, which are at least partially regulated by the QseBC two-component quorum sensing system. QseC is the sensor histidine kinase that senses autoinducers. QseB is the response regulator that is phosphorylated by QseC and controls gene expression. The QseBC system of *E. coli* O157:H7 has been shown to control motility and biofilm formation. The QseBC two-component system of *E. coli* responds to autoinducer 3 (Ai-3), and also to host-produced epinephrine and norepinephrine (NE). It has also been shown that the *F. novicida* QseC and *E. coli* QseC are functionally interchangeable, suggesting that *F. novicida* QseC may also respond to NE. NE affects the motility phenotypes of *Salmonella* enterica serovar Typhimurium and *E. coli* O157:H7. *F. tularensis* express type IV pili (Tfp) Tfp on its surface and pilE4, pilF, and pilT mutants do not express Tfp. PilE4 is the major pilin subunit for *F. novicida*. Tfp have been found to be important in colonization of the host for many bacterial species, including *Francisella* LVS, Tfp have been shown to be important in the biofilm formation and motility of *Pseudomonas aeruginosa* and other bacteria. Swimming and swarming depend on flagella, but *F. tularensis* does not have flagella. Tfp control twitching motility and twitching takes place on wet surfaces. We have shown for the first time that *F. novicida* demonstrates a phenotype of halo formation on 0.3% agar, although the exact nature of this motility has not been fully elucidated. It has been shown that Tfp are completely absent on the surfaces of pilE4, pilF, and pilT mutants, and we have examined the motility phenotype of these mutants in detail, including agar plates, motility stab assays, etc. We also show that NE has an effect on *F. novicida* motility, and that this may be due to NE signaling through QseC. The motility of qseC mutants is not affected by NE. Current studies are underway to determine the molecular regulation of this phenotype.

176 (B)  
**Evaluation of Real-Time PCR Assays for the Detection of Brucella Spp. in Human Clinical Samples in Georgia**  

Background: *Brucella spp.* is the most common zoonosis distributed worldwide and remains a major cause of morbidity in humans in Georgia. The conventional diagnostic methods have significant limitations. Although PCR platforms for Brucella have been developed, the performance of these assays on clinical samples needs further assessment. We evaluated various PCR tests to develop reliable conditions for identification of Brucella species in human clinical samples. **Methods:** To date, blood, serum and peripheral blood mononuclear cells (PBMC) obtained from 10 patients with a diagnosis of brucellosis, confirmed by culture and serology, have been tested by real-time PCR using Brucella Target Kit 1 kit (Idaho Technology Inc) or B4/B5 and IS711 primers in combination with SYBR Green. **Results:** IS711 primers were able to detect as small as one Brucella genome per reaction whereas B4/B5 primers were less sensitive, when using Brucella melitensis DNA. In contrast, no reliable amplification was obtained with DNA isolated directly from human clinical samples from any of the tested platforms. **Conclusions:** Results of our ongoing study suggest that further extensive work to evaluate and validate PCR assays suitable to routine laboratory diagnostic testing for detection of Brucella, is needed.

177 (D)  
**Fast and Reliable Identification of Bacteria Based Biological Warfare Agents by the MALDI Biotyper**  
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Background: Fast and reliable identification of microorganisms is a requirement not only for clinical routine diagnostics or food control but also for mobile and stationary labs in terms of identification of potential biological warfare agents. Cultivation independent detection methods, e.g. real-time PCR and fluorescence in-situ hybridisation are fast. However, they are limited by their primer or probe sequences, respectively, MALDI-TOF mass spectrometry fingerprinting combined with a dedicated pattern matching algorithm using a library of reference spectra has been found to be excellent for robust identification of bacteria on the genus and mostly on the species level. **Methods:** For sample preparation the biological material was processed according to short inactivation/extraction protocols using trifluoroacetic acid. Mass spectra were acquired with a benchtop MALDI-TOF MS in a mass range from 2000 to 20000 Dalton. Based on the acquired profile spectra reference data sets were created containing species- and subspecies-related information as well as a regular database (> 3200 entries) was used for identification. **Results:** Potential agents of bioterrorism (e.g. *Francisella tularensis* or spores of *Bacillus anthracis*) are often difficult to distinguish from closely related species or subspecies, which are less virulent. In case of *Francisella* even subspecies of blind-coded samples could be correctly identified using the MALDI Biotyper. Furthermore, discrimination of *B. anthracis* spores from closely related *B. cereus*, *B. thuringiensis* and *B. mycoides* could be shown. However some “white powders” which have been reported to be potentially used as fakes in a terrorist attack showed no similarities to the spore spectra. **Conclusion:** The identification of different bacteria based biological war-
Development of an Internal Positive Control Molecular Inversion Probe Assay

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Background: Molecular inversion probes (MIPs) are a technology that can specifically amplify multiple targets in a single PCR reaction as well as potentially overcome problems associated with high-order multiplex PCR. Successful MIP assays depend on samples free of inhibitors. For this reason, a positive control MIP was developed based on USAMRIID’s patented PCR internal positive control (IPC) assay (U.S. Patent 7,005,267). Methods: A novel MIP-IPC target template was constructed by PCR mutagenesis, resulting in a 125bp product which was then cloned. The MIP design incorporated specific sequences necessary for each step of the assay, ligation, excision, and PCR amplification. On the 5’ and 3’ ends of the MIP are complementary regions to the target DNA. The MIP anneals to the target DNA, creating double stranded DNA. A thermostable DNA ligase is then used to ligate the MIP into a circularized structure. The internal sequence of the probe contains several specific sequences, including three UTP residues and incorrectly oriented primer sites. A Uracil DNA Excision Mix is used to cleave the probe at the UTP site and results in an inversion and linearization of the MIP. After this cleavage, the primers are oriented correctly for amplification to proceed. Results: An initial MIP assay for an internal control DNA target was developed. In the optimized assay 100pM MIP was used with a limit of detection of 125ag of template DNA. This demonstrated the feasibility of MIPs for sensitive recognition of target DNA and detection of amplified probes. Conclusions: Establishment of an initial MIP assay can serve as a basis for development of a multiplex assay for biowarfare agents that will speed agent identification and thus improve treatment and treatment outcomes of exposed personnel.

Association of Early Lethal Factor Levels with Survival Time and a Point of No Return in Rhesus Macaques and NZW Rabbits with Inhalation Anthrax

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Background: During the 2001 anthrax letter attacks the fatality rate was 45% despite the use of antimicrobials and aggressive supportive care, suggesting that a point of no return (PNR) exists after which antibiotic rescue fails. Mass spectrometry-based measurement of anthrax lethal factor (LF) showed that the kinetics of toxinemia in rhesus macaques is triphasic and phase-2 declines might represent the PNR. Expanded studies revealed that the PNR is toxin-rather than time-dependent. Two studies with different treatments in two animal models of inhalation anthrax show that toxin levels are associated with survival times in both animals and suggest a PNR in rhesus. Methods: Studies in rhesus macaques included naïve and ciprofloxacin treated animals receiving 16 mg/kg commencing at Phase-1 (48h) and Phase-2 (72h). NZW rabbits included naïve, anthrax immune globulin intravenous (AlGIV) treated pre-challenge (5, 10, and 20 mg/kg), and IgIV Fiebogamma treated animals. Results: Among rhesus, there was one untreated survivor and 2 survivors from each treatment group. Phase-1 LF levels (48h) were elevated in rhesus with shorter survival (<72 h) and lower in animals with longer survival (96h) (p=0.011). PNR for LF was identified between 40-60 ng/ml, above which untreated animals died early or died despite early treatment. All rabbits receiving 20 and 10 mg/kg AlGIV survived and 7 of 12 survived with 5 mg/kg. LF levels were ≤0.39 ng/ml in treated survivors and were ≤3.9 ng/ml in treated non-survivors. Early Phase-1 LF levels were higher in rabbits that died early (Day 3 = 8.34±0.70ng/ml) compared to those that died later (Day 5 = 0.76±0.60ng/ml). Conclusions: Elevated LF levels are associated with reduced survival time and suggest an LF-dependent PNR in both rhesus macaques and NZW rabbits. LF levels provide a measure for advanced infection and therapeutic efficacy.
Lethal Toxin Complex and Total Lethal Factor Levels in Rhesus Macaques and NZW Rabbits with Inhalation Anthrax


Background: Bacillus anthracis produces two binary toxins. Protective antigen (PA) binds to lethal factor (LF), a zinc endopeptidase, and edema factor, an adenylyl cyclase, forming lethal toxin (LTx) and edema toxin respectively. LF hydrolyzes and inactivates proteins central to immune activation. We previously described a triphasic kinetics of anthrax toxemia using methods to quantify total LF (free LF + LTx). A method for measuring LTx alone allowed determination of the ratios of LTx to total LF associated with the three phases of infection in two animal models. Methods: Plasma from 17 rhesus macaques and 17 NZW rabbits exposed by aerosol to Bacillus anthracis Ames spores were analyzed for LTx and total LF. Anti-LF monoclonal antibodies (mAbs) on magnetic beads captured the total LF and anti-PA mAbs captured LTx; captured LF and/or LTx were incubated with a MAPKK peptide that was hydrolyzed by LF. LF-cleaved peptide products were detected and LF activity was quantified by MALDI-TOF MS. Results: Total LF in rhesus macaques increased during phase 1 (12-48 h) to a geometric mean concentration ± standard error (GMC± SE) of 144.5 ± 0.70 ng/ml, declined at 72 h (phase 2) to 22.7 ± 0.25, and increased during phase-3 (96-120 h) to 376.2 ± 0.80. Total LF for NZW rabbits increased during phase-1 (12-30 h) to 4.661 ± 0.41 ng/ml, leveled during phase 2 (42 h) to 4.061 ± 0.62, and increased during phase-3 (72 h) to 105.24 ± 0.82. Total LF levels were lower for the rabbits during the three phases and the triphasic period was shorter. For both animal models, the ratio of LTx/total LF usually exceeded 20% near death. Conclusions: A triphasic kinetics for total LF and LTx was observed in both rhesus macaques and NZW rabbits with 20% LTx/total LF characterizing phase-3. The triphasic timepoints and LF levels are lower in rhesus macaques which reach phase-3 at 72 h when rhesus macaques are in phase-2.

Development of Monoclonal Antibodies Against Plasminogen Activator Pla of Yersinia pestis


Background: The plasminogen activator of Yersinia pestis is an outer membrane protein considered as a central virulence factor in plague. In contrast to the F1 antigen (classically used for diagnosis), which is synthesized only at 37°C, Pla is produced both at 20°C and 37°C. This makes it a more suitable target for environmental detection of Y. pestis by immuno-methods. Methods: To produce monoclonal antibodies (mAbs), mice were immunized with purified and refolded recombinant Pla. Hybridomas were screened with an ELISA test, using E. coli expressing Pla (E. coli/pla) as coating antigen. 26 Pla-specific antibodies (with no cross-reaction with host E. coli antigens) were selected and further cloned. To develop a sandwich immunoassay, a combinatorial analysis of all the possible pairs of mAbs (one used for capture and the other labeled as a tracer) using E. coli expressing Pla (E. coli/pla) as coating antigen. The best pairs gave a sensitivity of 10^5 cfu/ml, i.e. 5.10^4 cfu/well. The test efficiently detected Y. pestis of all three biovar strains (Antiqua, Medievalis and Orientalis) grown at room temperature. Moreover, this assay exhibited a very good specificity, since none of the Gram− bacteria possessing proteins sharing homologies with Pla, such as Yersinia pseudotuberculosis, Yersinia enterocolitica, Salmonella typhimurium, Erwinia pyrifoliae and E. coli was detected.
Immunostrips were subsequently developed, reaching a sensitivity of 10^7 cfu/ml (10^6 cfu/strip). **Conclusions:** Our study confirms the feasibility of rapid tests either for laboratory application (15 hours) or for field application (20 min), even if the sensitivity of our tests remains to be improved. Pla is a valuable target for *Y. pestis* detection in environmental samples.

### 186 (D)

**Development of Inhibitor Resistant Real-Time PCR Methods for Biothreat Agent Detection**  
**A. TROMBLEY, A. ZOVANYI, T. MINOGUE; USAMRIID, Frederick, MD.**

**Background:** Sample processing typically removes inhibitory factors inherent to most complex clinical sample matrices and concentrates the target for analysis. Current processing technologies are not 100% efficient resulting in less than the original target input. Direct assessment of clinical samples is a potential solution for this issue as well as eliminating significant amounts of time required to fully process the sample. This research tested 10 commercially available buffers and polymerases to determine their efficacy for removing inhibition causes factor by various matrices. **Methods:** Chemistries were tested in combination and independently according to the manufacture’s specifications. Duplicate 5 and 1 µl aliquots of purified *Francisella tularensis* SCHU S4 genomic DNA concentrations were characterized for Ct and EPF values by real-time PCR in the presence and absence of various inhibitory matrix dilutions used straight, 1:10 and 1:100. **Results:** In whole blood, Phire™ Hot Start DNA Polymerase, Phusion® Blood Direct PCR Kit, KAPA Blood PCR Kit, and Ampdirect® buffer combined with Phire™ Hot Start DNA Polymerase showed promising results with detection in a 1:10 dilution when compared to F. tularensis master mix. STRboost™ combined with Phire™ Hot Start DNA Polymerase displayed textbook logarithmic change in EPF and detected 50 fg in 5 µl and 20 fg in a 1 µl sample of template. These five chemistries were further evaluated for inhibition relief in stool, sputum, sand, and soil. In sputum and soil, all master mixes performed similarly with detection in sputum at a 1:10 dilution and a 1:100 dilution in soil. In stool and sand most showed target detection at a 1:10 dilution. **Conclusion:** This direct organism detection shows that application of inhibitor resistant chemistries can serve as a mechanism to remove the requisite for sample processing in PCR diagnostics. Elimination of sample processing will greatly enhance turnaround time of sample detection allowing for quicker diagnoses and enhanced protection for the warfighter.

### 187 (D)

**Real-Time Assays for hms and ipr2 in a High Pathogenicity Island Required for Virulence in *Yersinia***  

**Background:** Virulent isolates of *Yersinia pestis* harbor a 102 Kb chromosomal High Pathogenicity Island (HPI). The pgm region of this HPI, which conveys the pigmented phenotype on Congo red agar at 28°C, has long been associated with *Y. pestis* virulence. The *ipr2* gene needed for synthesis of yersiniabactin, a siderophore required for *Y. pestis* virulence, is located in the HPI but outside the pgm region. Many virulent *Y. pseudotuberculosis* and *Y. enterocolitica* strains also harbor a very similar HPI. **Methods:** We developed four TaqMan® Real-Time PCR assays to test for the presence of iron regulating proteins, hmsF, hmsH, and hmsR in the pgm region and for the presence of the *ipr2* gene. The *Y. pestis* 102 Kb unstable region (GenBank Accession AL031866) and the GenScript real-time PCR (TaqMan) primer design tool (www.genscript.com) were used to design putative primers and probes. Empirical down selection was made, followed by inclusivity and exclusivity testing using DNA extracted from 75 *Yersinia* species and 103 threat and near neighbor organisms. All tests were performed on the R.A.P.I.D.® or the LC 2.0 systems. Results were compared to those obtained with two existing standard PCR assays for the hms region and one for *ipr2*. **Results:** Real-time hms assays for 61/75 *Yersinia* species were in agreement with the corresponding standard PCR assays. Agreement was best with *Y. pestis* (22/24) and *Y. pseudotuberculosis* (18/21). Real-time *ipr2* assay for 72/75 *Yersinia* species were in agreement with the *ipr2* standard PCR assay. **Conclusions:** Since complete loss of the HPI is an attenuation event resulting in exempt status for a *Y. pestis* strain, these assays can be used to provide a rapid demonstration of the HPI status of a nominally exempt strain before using it outside of Biosafety Level -3 containment. Besides utility in characterization of various *Yersinia* species these assays have the potential to provide a rapid indication of the virulence status of a suspected *Y. pestis* isolate.

### 188 (D)

**Multiplexed Diagnostic Assays Applied to the Detection of Bio-Threat Agents in Complex Food Matrices**  
**A. CARRILLO, J. OLIVAS, A. ROSA, P. NARAGHI ARANI; Lawrence Livermore Natl. Lab., Livermore, CA.**

Public facilities and infrastructure, including the food and water supplies, are under constant threat from natural, accidental, and intentional contamination. The threat of intentional contamination by introducing a bio-threat agent endangers the safety and security of the nation’s food supply. The availability of rapid, rigorously tested diagnostic assays capable of detecting multiple bio-threat agents is critical towards meeting the challenge of detecting and confirming that such an introduction has occurred. Methods currently used for diagnostics generally detect only a single agent and can be time-consuming, labor-intensive, and difficult to scale up. Multiplexed PCR detection capabilities provide many advantages over conventional single agent. Because of these advantages, coupled with their inherent adaptability and multi-agent utility, multiplexed PCR assays are ideal for use in detection of bio-threat agents in the food system. Lawrence Livermore National Laboratory (LLNL), in collaboration with the Biosearch Corporation and the Food and Drug Administration’s Center for Food Safety and Nutrition [FDA-CFSAN], has developed candidate multiplexed assays that simultaneously test samples for 3 high-priority bacterial agents (*Francisella tularensis*, *Yersinia pestis*, and *Bacillus anthracis*) with a minimum of 3 signatures per assay to enable multiloci detection of 12 genomic signatures. To determine the selectivity of each signature, the assay will be tested against DNA and RNA samples from up to three different food and environmental matrices. Subsequently, the performance of each signature will be will be assessed by using a panel of 5 target and 5 near-neighbor bacterial agents. The performance of the multiplex assay will be evaluated with respect to selectivity and sensitivity in terms of limit of detection. This work represents the initial step toward the development of multiplexed diagnostics acceptable for routine use in the detection of bio-threat agents in the food system.

### 189 (E)

**Multiplex Detection of Category A and B Biothreat Pathogens**  
**M. JONES, A. TAYLOR, C. OSWALD, R. CRISP; Idaho Techn., Salt Lake City, UT.**

The FilmArray® is a molecular detection device that features minimal sample handling, integrated sample preparation, and a multiplexed PCR output. This fully automated system is capable of detecting many PCR targets from a single specimen in less than one hour. The FilmArray Biothreat Pathogen Detection System was designed to meet the critical need for reliably detecting biothreat pathogens from an unknown threat sample. The following pathogens are detected: *B. anthracis*, *Y. pestis*, *F. tularensis*, *C. burnetii*, Rick- ettsia spp., *Brucella spp.*, *Burbholderia spp.*, *Ebolavirus*, *Marburgvirus*, *equine encephalitis viruses* (Eastern, Western, Venezuelan), *Variole major*, *Orthopoxvirus*,
190 (E)  
Development and Characterization of Novel Monoclonal Antibodies to Coxiella burnetii  
S. HAYWARD, J. RANCHES, E. FULTON; Defence Res. and Dev. Canada - Suffield, Medicine Hat, Canada.  
Background: Coxiella burnetii (Cb) is easy to manufacture and is environmentally stable; it causes disease (Q fever), which is difficult to distinguish from natural outbreaks of other diseases; and its degree of infectivity rivals that of anthrax. Efficient treatment requires rapid diagnosis followed by antibiotic treatment. Unfortunately, Cb does not grow on standard media and in most cases Cb is detected via immunohistochemistry or ELISA. Such methods rely on sensitive and specific reagents and in this study we sought to produce and characterize novel monoclonal antibodies (mAbs) to Cb that are sensitive and specific. Methods: Anti-Cb hybridomas were generated by fusion of splenocytes, from mice immunized with inactivated Cb, with P3X63Ag8.653 cells. After subcloning, four clones were selected, expanded, and their mAbs purified and tested for their sensitivity and specificity to Cb by ELISA in both buffer and biological fluids. Results: The four anti-Cb mAbs tested were found to be specific to Cb and not reactive with other biothreat agents or common environmental organisms; in addition, the mAbs were not reactive with ovalbumin, a common cross-reactant of Cb. All four Abs appeared to be functional as both capture and detector reagents. All were also functional in the presence of biological fluids (serum, blood, and urine) and did not show significant false-positives with the battlefield contaminants, soils, and powders tested; one false-negative was observed in the presence of skim milk powder. Conclusions: By their nature, mAbs are useful tools for specifically detecting bacteria and all four mAbs have the potential to be useful detection and diagnostic reagents for Cb. Unfortunately, ELISAs can be extremely time-consuming with many steps and therefore are not well suited for use in the field; future studies will include testing this panel of anti-Cb mAbs for their effectiveness in fieldable and/or handheld platforms, in a format more feasible for use by the Canadian Forces or first responders.

191 (E)  
Field Based Real-Time PCR Detection of Biothreat Pathogens without Sample Extraction or Purification  
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Background: First responders require reliable, sensitive, and easy to use field based biothreat pathogen detection to facilitate proper incident management. Current detection methods have low sensitivity, high false positives or extensive sample preparation not conducive to field use. Idaho Technology developed a ten target pathogen detection system for the field based RAZOR™ EX platform using “dilute and go” sample preparation. Third party testing by Signature Science demonstrates the system’s ability to detect relevant organism levels without sample purification. Methods: Sig. Sci. determined and confirmed detection limits for 8 organisms. Organisms tested were: B. anthracis spores, B. melitensis, C. botulinum, E. coli O157:H7, F. tularensis, R. communis, Salmonella and Y. pestis. Synthetic Variola major template was also prepared and tested (organism not available). The organism procedures used small scale production from stock cultures enumerated via viable plate counts, total protein quantification or ELISA. Agent was diluted in water to a test range, and select concentrations analyzed on the RAZOR EX using the 100® DNA Pathogen Screen to confirm. Production purity of bacterial agents was verified via Gram stain, microscopy and colony morphology. Results: Most organisms were detected at or below 3,000 cfu/ml. The RAZOR 10® Pouch assay detected ricin DNA in a crude preparation with 1 µg total protein. B. melitensis was detected at 30,000 cfu/ml; this result is consistent with other work performed with this assay. S. enterica was detected at 30,000 cfu/ml but testing with a different strain showed detection at 325 cfu/ml. Variola detection was 250 copies/mL. Conclusions: Real-Time PCR can detect vegetative or sporulated biothreat organisms without sample extraction or purification at relevant environmental monitoring levels.

192 (E)  
Development of a Real-Time Quantitative PCR Assay to Detect Cryptosporidium parvum Oocysts in Soil  
Background: The risk of Cryptosporidium parvum contamination is a serious issue with respect to drinking water. Soil contaminated with C. parvum can serve as an important source and pathway from which oocysts can be transported into water sources. Rapid and sensitive techniques are required for the timely and efficient detection of C. parvum in soil; however, most of the available detection methods were developed for clinical samples and thus their applications on soil samples are questionable. The objective was to develop Real time quantitative PCR (qRT-PCR) assays for detecting C. parvum in soils. Methods: The efficiency of two DNA extraction kits were evaluated for the removal of DNA from soil spiked with C. parvum. Primers and probe targeting C. parvum 18S rRNA gene were designed and qRT-PCR analyses were conducted on an ABI 7500 sequence detection system (Applied Biosystems, Carlsbad, CA) using Taqman quantitative assay to determine the detection limit(s). Bovine serum albumin (BSA) and Polyvinylpyrrolidone (PVP) were also tested to relieve the inhibition on RT-PCR by the material co-extracted from soil. Results: PowerSoil DNA isolation kit (MoBio, Carlsbad, CA) was more effective in extracting high quality DNA than other kits tested. BSA efficiently improved the performance of RT-PCR in the detection of oocyst spiked soil samples, while PVP did not enhance the efficiency of RT-PCR. Preliminary data showed that this qRT-PCR method has a detection limit of 10 oocysts per gram of soil. Conclusions: Those results demonstrate that this
qRT-PCR procedure is rapid and straightforward and provides a sensitive and economic means for the detection of *C. parvum* in soil.

193 (E)

**Evaluation of the PLEX-ID Biothreat Kit for Use with Environmental Air Samples**

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**Background:** Midwest Research Institute, an independent not-for-profit organization, has collaborated with Ibis Biosciences, Inc., a subsidiary of Abbott Molecular, to evaluate the PLEX-ID Biothreat Assay Kit. The basis of the PLEX-ID approach is, first, the use of primers to amplify PCR products from broad groupings of organisms, rather than single organisms; second, the use of mass spectrometry to analyze the products; and third, the use of nucleic acid base composition to identify the organisms present. While most methods can only answer the specific question: “Is organism ‘X’ present in my sample?”, the PLEX-ID provides an answer to the broader question “What organisms are present in my sample?”

**Methods:** For biodefense applications it is critical for a method to detect and identify threat organisms present in complex matrices and differentiate these from non-threat near neighbors. The evaluation data presented here was performed to determine sensitivity and specificity of the Biothreat assay kit when challenged with the complex milieu of an environmental air collection. Sixteen different bacterial and viral bioterror agents were used for testing and validation.

**Results:** The kit provides a method capable of rapidly detecting biological threat agents in environmental air samples. The validation measurements were determined for sixteen different species in the presence of environmental background. Presented are the Limits of Detection, breadth of coverage of threat and near neighbors, False Negative Rates and False Positive Rates for each organism used to challenge the assay.

**Conclusions:** The PLEX-ID and the Biothreat Assay kit provide sensitive and specific method for threat detection for use in environmental air sample analysis. The approach would also allow for detection of emerging or engineered threats.

194 (E)

**An In Vitro Comparison of Two Types of Viral Throat Swabs for Sampling Orthopoxviruses**


**Background:** Disease diagnosis often relies on the detection of a causative organism, which in virology is often localised to a cell type or specific tissue. Although rayon swabs have been used on-site at HPA-Porton for *in vitro* studies in the past, their use has been linked to poor sample recovery. This in vitro study was designed to compare the efficiency of sample collection and virus recovery by comparing traditional rayon swabs with a potentially more efficient flocked alternative. Method: The efficiency of two swabbing systems for Monkeypox virus recovery were compared by plaque assay on Vero E6 cells, and by real time quantitative PCR amplifying the Orthopox virus Haemagglutinin (HA) gene. Copan #147C Virus Transport Swab is non-flocked and contains a carrier sponge soaked in virus transport medium in a transport tube. Copan #359C consists of a flocked swab with a moulded breakpoint, and a transport tube containing 1ml of Universal transport medium (UTM).

**Results:** The results indicate that the flocked swab (Copan #359C) has a better retention volume (27% greater) than the rayon swab (Copan 147C). Subsequent plaque assays yielded a much greater titre with the Copan #359C (over 200% improvement) indicating that virus viability is also improved with a flocked swab. Likewise, DNA recovery from qPCR saw a 65% increase in copies/ml when swabbing with the flocked system (Copan #359C). These increases are in excess of those caused by the increased retention volume.

**Conclusions:** Copan #359C swabs retain a significantly larger volume of sample and improve viable virus recovery as well as improving DNA yields, resulting in increased sensitivity for *in vitro* analyses. These, therefore, represent an improvement over the currently used #147C system.

195 (F)

**A Combined Computational/Experimental Approach for Specificity Profiling of Lead Therapeutic Agents**

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**Background:** Improved prediction and control of specificity of binding between small molecule ligands and protein targets is a primary goal of cheminformatics. Control of specificity across a broad range of targets and off-target receptors is essential when developing broad-spectrum drugs for bioterror agents, active against families of threats (eg. *flaviviridae*) without binding off-target host proteins. Conversely, host-based therapies require narrow specificity to target only the desired host protein and not related ones. The focus of our work is studying the determinants and limits of specificity across a target protein set, and designing the best candidate ligands. By combining cheminformatics, bioinformatics, statistical, and experimental approaches we have implemented an unsupervised pipeline to classify binding interactions across large sets of proteins and ligands and identify Specificity Determining Features (SDFs) at molecular interaction interfaces. SDFs are constellations of hydrogen-bonding interactions, hydrophobics, etc that provide intuitive, predictive models for understanding and tuning binding specificity of compounds. Methods & Results: Our pipeline was developed and tested using experimental data from Karaman et al, a matrix of interactions between 338 human kinases and 38 inhibitors. Human kinases are an important family of > 500 signaling proteins with > 30% identity. Members have many therapeutic applications including host-based therapies (eg Abi kinase inhibitors block hiv fusion). Clustering of proteins and ligands by binding patterns, followed by docking and structural bioinformatics analysis, identified SDFs driving each cluster. We validated our approach with cross-validation and by showing that the SDFs were predictive for cluster affinities of ligands outside our original dataset. Conclusion: We developed a general approach for specificity profiling, and showed that SDFs are predictive and useful for tuning specificity.

196 (F)

**Tool for Identifying Sequence Variations that Correlate with Virus Phenotypic Characteristics**

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**Background:** The increased number of sequencing efforts for pathogenic viruses has provided many whole genome sequences with associated information about the characteristics of each isolate. Studying the variation within these sequences has illuminated the evolution of these viruses over time; however, by integrating the metadata associated with these virus isolates into any analysis, we can better understand how the virus spreads and causes disease in its host. As the virus-centric, NIH-funded, freely-available Bioinformatics Resource Center, the Virus Pathogen Database and Analysis Resource (Vipr, www.viprbrc.org) has developed a Metadata-driven Comparative Genomics Analysis Tool (meta-CGAT) to assist researchers in using this breadth of sequence data along with the associated metadata.

**Methods:** This tool allows users to: select multiple sequences at the genome, gene, or protein level; divide those sequences into multiple groups based on

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any metadata type, perform a chi-square statistical test at each aligned position, and identify all residues that significantly differ between the user-specified groups. Results: The results from this tool have been validated using a previously published sequence set divided into two groups based on phylogenetic topology. In this study we divided all Dengue type 3 whole genomes according to the geographic location of isolation (eastern or western hemisphere) and identified over 70 amino acid positions that significantly differed between the two groups. Conclusions: These divergent residues likely represent regions of the viral genome that are capable of variation in response to external evolutionary pressure while maintaining infectivity.

197 (G)

Assessing the Therapeutic Efficacy of Intravenous Levofloxacin for the Treatment of Inhalational Anthrax in New Zealand White Rabbits

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Background: Recently, we characterized a detailed natural history for New Zealand white (NZW) rabbits exposed to aerosolized Bacillus anthracis (Ames strain) and identified antigenemia (i.e., protective antigen in the blood) as an early biomarker of infection. To verify NZW rabbits as a therapeutic model for inhalational anthrax, antigenemic rabbits were intravenously treated with levofloxacin (LEV), a fluoroquinolone antibiotic approved by the FDA for inhalational anthrax postexposure prophylaxis in adults. Methods: Accordingly, 60 NZW rabbits were exposed to aerosolized B. anthracis spores (Ames; ~150 LD50). Blood samples collected at various time points postchallenge were assayed for antigenemia (via electrochemiluminescence assay) and bacteremia. After antigenemia detection or shortly thereafter, rabbits were treated with LEV (range, 2.5 to 25 mg/kg) for up to 5 days. Study end was 28 days after challenge. Results: All animals were antigenemic and later confirmed bacteremic before treatment initiation. A LEV dosing regimen as low as 6.25 mg/kg proved to be therapeutically efficacious (100% survival at study end) for B. anthracis-challenged NZW rabbits, eliminating bacterial load after the first treatment day. The peak for a single daily dose of 6.25 mg/kg LEV was approximately equivalent to the Cmax for a 500 mg/kg daily LEV dose in humans, where as the peak for 6.25 mg/kg given at half-daily doses was approximately one-half the Cmax. Trough concentrations for both LEV dosing regimens were below the LEV minimum inhibitory concentration. Decreased survival occurred with increasing treatment delay after antigenemia. Conclusions: Taken together, these results demonstrate the potential of NZW rabbits as a therapeutic model and indicate that intravenous LEV is an effective treatment for inhalational anthrax.

198 (G)

Comparison of Gentamicin Dose Schedule for Treatment of Infection from Aerosolized Yersinia pestis

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Objective: Yersinia pestis (Y. pestis) is a Gram-negative organism causing both pneumatic and bubonic forms of plague. Our objective was to establish the LD50 for an aerosol Y. pestis exposure and explore the effects of a 5 day gentamicin therapy administered 4 hours or 24 hours post-exposure. Methods: For each study, a 100ml culture of Y. pestis CO92 was grown at 28°C to late log-phase (~2.5x108 CFU/mL). The IITRI bioaerosol system was utilized for administering aerosolized Y. pestis to Swiss Webster mice. The LD50 inhale dose was determined over an inhaled dose range of 4.8 x 107 to 4.9 x 108 CFU. Additionally, mean time to death (MTD) and percent survival were determined. For determination of the effect of gentamicin therapy, mice were administered a nose-only exposure to 50 LD50 of Y. pestis. Gentamicin at 48 mg/kg was administered subcutaneously once per day for 5 days with administration beginning either 4 hr or 24 hrs after challenge exposure. All study animals were monitored twice daily for 14 days post-challenge. Infected control animals did not receive any gentamicin dose. Results: The LD50 inhale dose was determined to be 2.31x107 CFU. When exposed to a 50 LD50 challenge dose, 100% of the Swiss Webster mice survived with 48 mg/kg gentamicin administered beginning 4 hrs after challenge. When administration of 48 mg/kg gentamicin was delayed 24 hrs, 80% of the challenged Swiss Webster mice survived with a MTD of 3.5 days. Control animals which did not receive any gentamicin did not survive over the 14 day observation period and had a MTD of 3.3 days. Conclusions: Using the IITRI bioaerosol system, the LD50 for Y. pestis CO92 in Swiss-Webster mice was calculated to be 2.31x107 CFU inhale dose. While protection from Y. pestis was evident from both post-exposure administration strategies, this study demonstrates that full protection occurs with rapid administration of gentamicin.

199 (G)

Efficacy Evaluation of DEF201 in the Mouse Model of Venezuelan Equine Encephalitis Virus Infection

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Objective: DEF201 consists of a proprietary replication deficient adenovirus type 5 (Ad5) vector expressing IFN-α that is under development as a broad spectrum antiviral. Herein, we evaluated the prophylactic and therapeutic efficacy of DEF201 against lethal intranasal challenge with VEEV TC83 strain in the C3H/HeN mouse model. Methods: Eight groups of female C3H/HeN mice (n=10) were treated with a single intranasal dose of 1x107 pfu of DEF201 on Day 21, 14, 7, or 1 prior to challenge. One group was similarly treated 4 hours after challenge. Untreated mice and mice treated daily with a single intraperitoneal injection of 2x107 IU/kg IFNα recombinant protein served as negative and positive controls, respectively. An additional control group received a single intranasal dose of an Ad5 vector without IFNα gene. On Day 0, all mice were challenged intranasally with a lethal dose (3x108 PFU) of VEEV TC83 strain. All mice were monitored daily for clinical signs of disease during the 14 day post-challenge period. Results: Treatment of mice with DEF201 provided complete protection against otherwise lethal challenge with VEEV TC83 when dosed on Day 21, 14, 7 or 1 day prior to challenge. Moreover, when treatment was given 4 hours post-challenge 9 mice of 10 survived the VEEV TC83 challenge. Protected mice demonstrated no significant change in body weight with little to no clinical signs of VEEV infection which typically include ruffled hair, hunched posture, lethargy and ataxia. Conclusion: DEF201 exhibited high antiviral activity by inducing rapid and long-lasting protection against lethal VEEV TC83 infection of mice both prophylactically and as an early post-exposure treatment. Efforts are currently underway to investigate the potential broad-spectrum application of DEF201 as a prophylactic and/or therapeutic for bioterror agents and emerging pathogens. This work was funded by NIAID contract N01-AI-30063.
200 (G)

Efficacy of a Single Intravenous Administration of Anthrax Immune Globulin (AIG) in New Zealand White Rabbits

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Background: Bacillus anthracis is a Category A priority biological threat agent. Anthrax Immune Globulin (AIG) is a medical countermeasure that has been deposited in the Strategic National Stockpile for emergency or compassionate use. This study establishes the efficacy of a single infusion of AIG and provides proof of concept that AIG can be used as an inhalation anthrax prophylactic.

Methods: 72 New Zealand White (NZW) rabbits were randomized into 2 control groups receiving Flebogamma (Fig: 625mg/kg total protein dose; Grps 1 and 2 ), 3 intravenous (IV) treatment groups receiving 5, 10, or 20 mg/kg AIG (Grps 3, 4 and 5) and 1 untreated control group (Grp 6). Groups 2-6 were aerosol exposed to an average dose of 200 LD50 equivalents of B. anthracis Ames spores 24 ± 5 hours post AIG or Fig treatment. Following challenge, each rabbit was observed for survival, time to death, circulating human anti-PA IgG and emergence of a host anti-PA IgG immune response.

Results: Untreated control rabbits (Grp 6) and Fig treated rabbits (Grp 2) had a geometric mean time to death (GMTTD) of 3.91 and 4.88 days, respectively. Group 3 rabbits (5mg/kg AIG) had a GMTTD of 5.72 days whereas Groups 4 and 5 (10 and 20 mg/kg AIG, respectively) had 100% survival. Human anti-PA IgG analyses of serum samples showed decreased levels in Groups 3 to 5. Surviving animals in Groups 3 and 4 demonstrated resurgent anti-PA IgG levels by day 14 compared to day 10. Conclusions: AIG administered 25 hrs prior to a lethal aerosol exposure to B. anthracis Ames protects NZW rabbits compared to controls. AIG given at 5mg/kg protects 58% of the rabbits, whereas 10 and 20 mg/kg of AIG protect 100% of the animals. There are indications that high level exposures to A. anthracis can stimulate an acquired immune reponse in NZW rabbits passively protected with human AIG.

201 (G)

Immune Augmentation of Chemotherapy against Tularemia

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Infections with Francisella tularensis, the causative agent of tularemia, are treatable by antibiotics, but relapse and treatment failure do occur. Therefore, treatment regimens that augment standard antibiotic therapies and reduce disease relapse would enhance current biomedical counter measures used to treat infections by this Category A pathogen. To assess whether immune augmentation would enhance antibiotic treatment, a suboptimal 10 day regimen of gentamicin (10 mg/kg, starting 24 hrs post-infection) was established in a murine model of tularemia using an F. tularensis Schu4 infection. The immune augmentation applied along with suboptimal chemotherapy was an i.p. post-exposure (day 1 and day 4) vaccination with the F. tularensis LVS membrane protein fraction (MPF) mixed with a cationic liposome-DNA complex adjuvant (CLDC). The suboptimal gentamicin treatment alone resulted in all animals succumbing to infection by day 7 as compared with death by day 4 in untreated controls. Post-exposure vaccination with MPF-CLDC resulted in 100% survival to day 25 when given along with the daily suboptimal gentamicin regimen. Additionally, it was found that MPF was the critical component of the post-exposure vaccine. Measurement of bacterial burdens demonstrated that MPF post-exposure vaccination significantly reduced bacterial loads in the liver and spleen by day 7 as compared to animals receiving gentamicin alone. The evaluation of F. tularensis specific antibody responses demonstrated that IgM and IgG production began at day 4 post-infection in the vaccinated animals. Additionally, the depletion of NK cells using the anti-asialo GM1 antibody resulted in a loss of immunotherapy augmentation, but did not alter the ability of mice to generate a rapid IgM and IgG response. These studies demonstrated that post-exposure vaccination with MPF successfully augments suboptimal gentamicin treatment and that protection was dependent on effective NK cell activity.

202 (G)

Liposomal Botulinum Toxin Heavy Chain Targets Neuronal Cells via the Ganglioside Receptor


Background: Botulinum toxin (BoNT), consisting of a heavy chain (HC) that binds to neuronal cells and a toxic light chain, acts at peripheral neuromuscular junctions, resulting in muscular paralysis, impaired vision, and respiratory failure. Potential therapeutic inhibitors of BoNT have been developed, but require targeting to the neuronal cells either for efficacy or because they are toxic to other cells. The goal of this study is a liposomal delivery system using membrane-linked BoNT type B HC to specifically target therapeutics to neuronal cells. Methods: Unilamellar liposomes (∼200 nm) containing egg phosphatidylcholine, sphingomyelin, cholesterol, and cholest-5-en-3β-dithiopyridine (PDS-Chol) were prepared by extrusion. PDS-Chol, a thiol-reactive lipid, was used to couple BoNT/B HC to the liposomes. Coupling of BoNT/B HC to the liposomal surface was verified by flow cytometry (FC) using liposomes containing Rhodamine-labeled phospholipid (N-Rh-PE) and FITC-labeled HC. FC was used to determine binding specificity of liposomes with or without coupled BoNT-HC to SH-SYSY (neuronal cells) or PC12 cells (control cells lacking BoNT/B receptors). N-Rh-PE-liposomes with or without coupled FITC-HC were incubated with cells and cell-associated fluorescence determined. Results: Liposomes alone did not bind to either cell line until >65 min of incubation. BoNT/B HC, free or coupled to liposomes, bound to the SH-SYSY cells, but not to control PC-12 cells except when the PC-12 cells were pre-loaded with the BoNT/B ganglioside receptor GT1b. Conclusions: Our liposomal BoNT/B HC construct targeted neuronal cells. Since liposomes are known to deliver encapsulated drugs to cells, these BoNT/B HC-coupled liposomes form the basis for delivery of BoNT therapeutics to intoxicated neuronal cells. The opinions or assertions are the private views of the authors, and not official views of the Department of the Army or the Department of Defense. Supported by DTRA 3.10032_08_WR_B.

203 (G)

Antibiotic Delivery Platform Enables Increased Intracellular Delivery and Killing of Virulent Brucella abortus


Polyanhydride microspheres and nanospheres (PAParticles) elicit unique cellular responses from immune cells that stimulate internalization, direct intracellular trafficking and degrade slowly within the cells. Antimicrobial compounds can be encapsulated into the PAParticles that will be released as the particle slowly degrades. Varying the chemistry of the particle affects particle degradation and alters the fate of the particle within cells. Microbial pathogens survive within host tissues by protecting themselves against immune defenses. Intracellular Brucella abortus survives and replicates within macrophages that protect the bacteria from high concentrations of circulating antibiotics. Encapsulating of doxycycline increased the intracellular killing of B. abortus by 40% over the soluble antibiotic. The highly effective targeting of the intracellular environment greatly reduces the amount of antibiotic needed to treat such an infection and also provided delayed release.
Good Laboratory Practices (GLP) in High Biocontainment Laboratories: Challenges and Solutions


Select agents and toxins are dangerous exotic agents capable of causing disease or life-threatening illnesses. Barrig an outbreak, it is not feasible to assess the efficacy of medical countermeasures, including vaccines and therapeutic drugs and biologics for use with select agent organisms and toxins. It is also unethical to expose humans to select agent organisms and toxins in clinical trials to determine if treatments are effective. However, efficacy data for select agents can be obtained via the animal rule in biosafety level 3 and 4 (BSL-3 & 4) laboratories, when the studies are conducted in compliance with 21 CFR Part 58. Implementing Good Laboratory Practice (GLP) requirements in biocontainment laboratories poses many challenges due to the enhanced facilities, precautions, safety, and security measures required. USAMRIID has identified the many challenges of implementing GLPs in biocontainment laboratories as well as solutions to meet the requirements of 21 CFR Part 58 at BSL-3 & BSL-4. GLP-compliant studies need to be performed in BSL-4 laboratories to further develop countermeasures against select agents. USAMRIID has successfully performed GLP-compliant studies in its BSL-3 laboratories and is prepared to perform GLP-compliant studies in support of the animal rule in BSL-4 laboratories. USAMRIID’s mission is to conduct basic and applied research on biological threats resulting in medical solutions to protect the warfighter by conducting cutting-edge medical research against biological threats. These medical advancements also protect civilians. USAMRIID is prepared to perform GLP compliant studies in BSL-4 laboratories while maintaining safety and security in the laboratory, producing quality data in support of the animal rule.

Silver Nanoparticles Inhibit Vaccinia Virus Infection by Preventing Viral Entry

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Previously our laboratory has demonstrated that silver nanoparticles (AgNPs) are capable of inhibiting Vaccinia virus infection. The current study expands on these results by exploring the hypothesis that AgNPs prevent Vaccinia virus infection by preventing viral entry into the host cell. Utilizing an FDA β-galactosidase assay for vaccine efficacy previously adapted by our lab to measure viral entry, we found AgNP inhibitory concentrations of 50% (IC50) at 48 μg mL-1 and 84 μg mL-1 for virus treated with AgNPs prior to infection and cells treated with AgNPs prior to infection, respectively. Statistically significant inhibition of viral entry was found at 32 μg mL-1 (p<0.010). This inhibition of entry was qualitatively verified via confocal microscopy utilizing a GFP-core protein genetically modified virus in combination with a cytoplasmic dye. Using the same technique, it was observed that Vaccinia virus binding to the host cell was similarly affected by AgNP treatment. The inhibitory effects of viral entry were found to extend throughout the viral replication cycle as determined by plaque reduction assay. At the previously mentioned concentration of 32 μg mL-1, there were virucidal and cytoprotective effects that correlated to a 4-log reduction in viral titer. Similar reductions in viral titer were found during post-infection treatments as low as 16 μg mL-1. These results demonstrate that AgNPs are capable of binding to Vaccinia virus causing virucidal and cytoprotective effects through inhibition of viral entry.

Cyclodextrin Derivatives as Novel Antimicrobial Agents against Francisella

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Background: Dr. Karginov and his co-workers have used beta-cyclodextrin (CD), a sevenfold symmetrical cyclic molecule, with symmetry and size like the PA pore, and it was found that this compound bound the pore and protected mice in vivo. These results serve as the basis for a structure-based drug discovery program with the eventual goal of finding new drug candidates for treatment of bioterror infections such as anthrax. More generally, this can be considered as a general approach for the discovery of new drugs by blockage of pores with molecules having the same dimensions and symmetry as the pores. Current Study: Members of the genus Francisella are the causative agent of tularemia, and are considered to be Category A threat agents due to historical use as biological weapons and high infectivity via aerosol. In this study, we tested novel cyclodextrin derivatives with various symmetry for activity against Francisella. In these studies, we have demonstrated the activity of the panel of 20 novel cyclodextrin derivatives against F. novicida, and have determined the MIC and EC50 for the effective compounds. We identified 3 cyclodextrin compounds that were active against bacteria. These compounds are further tested against intracellular Francisella bacteria, in J774A.1 murine macrophages. Finally, the ability of CDs to potentiate the activity of common antibiotics such as ciprofloxacin was tested, and 2 derivatives were found which potentiated ciprofloxacin activity against Francisella bacteria in vitro. We are currently testing the effectiveness of our lead compounds, with and without antibiotics, in the wax moth caterpillar, G. mellonella, an in vivo model for Francisella infection. The target of these cyclodextrin molecules is not yet known, but may include the multi-drug efflux pumps such as ToLC, which has recently been demonstrated to play a role in Francisella pathogenesis.

Longevity of Vaccine Protection Against Pneumonic Plague in BALB/c Mice


Background: Yersinia pestis is the etiological agent of bubonic and pneumonic plague. Subunit vaccine approaches comprising recombinant forms of the fraction 1 (F1) and V (virulence) proteins have demonstrated protection against pneumonic plague in mice. At last year’s meeting, we reported on protection conferred by rYP vaccine at Day 49 post-vaccination and that F1 and V antibody levels correlated with protection against challenge with Y. pestis CO92. Here, using a similar vaccination regimen we have extended our studies to investigate the longevity of protection induced by rYP vaccine. Methods: On day 0 and 21, three sets of Balb/c mice (N=110/set) in Groups 1-6, were vaccinated with 2.50, 0.63, 0.16, 0.04, 0.01 and 0µg vaccine plus Alhydrogel, respectively. Blood collected on various times post-vaccination was analyzed for F1 and V antibodies by ELISA. On day 79, 109 and 139 all mice were exposed to 1.1x10^10 CFU/L Y. pestis CO92 via nose-only inhalation exposure and monitored for 14 days. Y. pestis CO92 bacterial counts were performed on terminal blood samples using selective agar plates. Results: On Day 21, mean F1 and V antibody titers in Groups 1-5 were 2.58, 2.42, 2.22, 2.13, 1.05 and 1.08, 1.06, 1.01, 1.04, 1.03 log10 assay units, respectively. F1 and V antibodies continued to rise in Groups 1-5 following the 2nd vaccination on Day 21. Notably, V-specific antibodies were significantly boosted in Groups 1-4 by Day 29 (p<0.01). As expected, F1 and V antibodies were not detected in vaccinated mice.
in Group 6. Following challenge with *Y. pestis* CO92 (mean inhaled dose, 6.7x10^7 CFU) on Day 79, survival rates in Groups 1-6 were 95, 90, 80, 40, 0 and 0%, respectively. **Conclusions:** These findings demonstrate that F1 and V antibodies induced by an F1/V-based vaccine correlated with protection against pneumonic plague.

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208 (H)

**Comparative Efficacy of Recombinant Anthrax Vaccine against Intramuscular Challenge with Anthrax Spore**


Recombinant protective antigen (rPA) is the active pharmaceutical ingredient as a second generation anthrax vaccine undergoing pre-clinical evaluation. By using the rPA produced from *Bacillus brevis* 47-5Q pN212 expression system, the serological immune response to anthrax protection efficacy was analyzed in an *in vivo* animal model. To determine that effective immune interval of recombinant anthrax vaccine, we compared immune effects after immunization with vaccine during 6 months. We have immunized to SPF rabbit in two or three intramuscular injections by anthrax vaccine at 2 or 4 weeks intervals. Rabbit immunized with 2-week interval, anti-rPA IgG ELISA (p=0.0378) and lethal toxin neutralization assay (TNA) titer (p=0.0134) in 4 weeks after first dose were higher than immunized with 4 weeks interval. However, antibody titer (p=0.0141-0.0488) of group of 4 weeks interval in 6 weeks was higher than 2-week weeks interval that lasted for 10 weeks (p=0.0348-0.0407). At 26 weeks after the primary injection was no difference between the immune groups. Also in three doses at 2 weeks, no significant difference between two dose and was maintained for 26 weeks that anti-rPA IgG ELISA titer (p=0.0260) and TNA assay titer (p=0.0390) at an equal level. In 4 weeks interval three dose of anthrax vaccine higher than the 2 week interval groups between 8 to 14 weeks. This study shows that primary immune in the immunization schedule of recombinant anthrax vaccine were appropriated to two doses with 4 weeks interval better than at 2 weeks interval or three doses.

209 (H)

**F1/V Plague Vaccine Protects Cynomolgus Macaques but not African Green Monkeys from Death after Lethal Aerosol Plague Challenge Despite Inducing a Humoral Response**


**Background:** *Yersinia pestis* is the causative agent of plague and can manifest as pneumonic or bubonic forms. **Methods:** Established models of pneumonic plague in cynomolgus macaques (CM) and African green monkeys (AGM) were used to test the efficacy of a recombinant F1/V vaccine. NHPs were vaccinated intramuscularly with 10 μg vaccine on Days 0 and 30. Controls were given adjuvant only (aluminum hydroxide). All animals were aerosol challenged with 50 LD50 *Y. pestis* CO92 70 days after initial vaccination. **Results:** Controls succumbed to *Y. pestis* within 7 days. *Y. pestis* was cultured from control CM and AGM blood, lungs, spleens, livers, brains, and lymph nodes. Vaccinated CM uniformly survived *Y. pestis* challenge and had no detectable bacteremia or tissue burden. Survival correlated with increased serum IgG anti-F1 and -V levels measured by Protein G ELISA as compared to controls. All but one vaccinated AGM succumbed to disease in the same time frame as controls despite mounting a humoral response to vaccination. The sole survivor had levels of anti-F1 and -V that were comparable those in CM and higher than those found in the other vaccinated AGM on Day 46. Clinical signs and parameters including pathology underscored differences in disease severity between these species. Higher lymphocyte/monocyte counts were seen in the vaccinated CM and the sole surviving AGM post challenge.

**Conclusions:** These data suggest that vaccine-induced levels of IgG anti-F1 and -V correlate with protection from death by pneumonic plague post vaccination in CM, but not in AGM compared to controls. Possible differences that lead to this result were failure of most AGM to achieve anti-F1 and -V levels similar to CM on Day 46, and/or limited lymphocyte/monocyte responses post challenge. This project was funded in whole with Federal funds from NIAID, NIH, DHHS, Contract No. HHSN266200400095I.

210 (H)

**Development and Pre-Clinical Evaluation of a Recombinant Ricin Vaccine (RVEC)**

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Ricin is an extremely potent toxin extracted from the castor bean that is a potential biowarfare agent due to its heat stability and its worldwide availability. Ricin toxin is synthesized as a single ~65-kDa glycoprotein that is post-translationally processed into two polypeptide chains (A-chain and B-chain), which are bound by a single disulfide bond. The toxic consequences of ricin are attributed to the biologic activity of the A-chain, whereas the function of the B-chain is to bind the toxin to cell surface receptors, thereby facilitating internalization of the toxin into the cell. USAMRIID has developed a Recombinant Ricin Toxin A-Chain Vaccine 1-33/44-198 (RTA 1-33/44-198) (RVEC™) produced in *Escherichia coli*. (Olson et al-2004). Based on pre-clinical studies, including a pivotal repeated-dose toxicity study conducted under GLP, this product was determined to have a reasonable safety profile for use in human studies; no detectable Ribosome Inactivating Protein (RIP) activity or Vascular Leak Syndrome (VLS) symptoms, no change in body weight, serum chemistry profile, or hematology, and no noticeable irritation at the injection site were observed in mice, rabbits, and nonhuman primates vaccinated. A Phase I first in human escalating, multiple-dose, single-center (USAMRIID) study to evaluate the safety and immunogenicity of RVEC is in the final stages of development with planned launch - December 2010.

211 (H)

**Update of an Attenuated Smallpox Vaccine LC16m8 Research**

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**Introduction:** LC16m8 is an attenuated smallpox vaccine and was licensed in 1975 in Japan. However, due to the cessation of routine vaccination against smallpox in 1976, the LC16m8 vaccine had not been placed on the market at that time. Currently, in response to the increasing threat of smallpox bioterrorism, the LC16m8 vaccine is being stockpiled in Japan as a countermeasure against bioterrorism with smallpox. As a practical issue, the information filed to the Authorities and that is included the package insert was prepared based on the results obtained in clinical researches conducted in children 30 years ago. There are no clear grounds to apply this vaccine to adults. To address this issue, we conduct a PMS study based on discussion with the Agency in Japan.

**Methods:** The PMS study was conducted through 2005 to the beginning of 2010 in Japan. The study included total 268 healthy adults (196 primary vaccinees and 71 revaccinees) vaccinated with the single dose of LC16m8 for the safety and efficacy evaluation. Regarding the safety evaluation, all individuals were examined. As for efficacy evaluation, "Take" rate was calculated with results from 268 individuals. PRNT was conducted with 100 individuals after excluding 18 individuals with no PRNT data. Basically, the analyses were conducted in two separate groups: a primary vaccination group and a revaccination group. **Results:** There was no report on the severe adverse events or deaths caused by the LC16m8 vaccination. Among 268 individuals, adverse reactions were observed in 58 individuals, that is 21.6% (58/268) of frequency of reaction adverse reactions. Major adverse reactions included 52 cases
(19.4%) of lymphadenopathy, 14 cases (5.2%) of erythema at the injection site, and 4 cases (1.5%) of fever. Meanwhile, “Take” rate was evaluated with results from all 268 individuals. Overall take rate resulted in 94.4%. **Conclusion:** The results of PMS study indicated that LC16m8 has a high safety and immunogenicity profile for both vaccinia-naive and -experienced subjects. Based on the results, we will update the description of the package insert.

**212 (H)**

**Evaluation of *F. tularensis* Type A Derived Live Attenuated Vaccines in the Rabbit Model**

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Francisella tularensis is a category A select agent and the causative agent of tularemia. The development of vaccines for *F. tularensis* is a biodefense priority. Two *F. tularensis* subspecies cause the most disease in humans: the highly virulent subspecies *tularensis* (type A) and the less virulent subspecies *holoarctica* (type B). A live attenuated type B strain, LVS, can confer partial protection against aerosol challenge with a type A strain. Our hypothesis is that a live attenuated vaccine derived from a type A strain will provide better protection against a type A challenge. We constructed a series of live attenuated vaccine candidates derived from the Schu S4 strain containing specific deletion mutants in target genes. Initial evaluation in the mouse model demonstrated attenuation of virulence in 5/9 strains. Furthermore, 2 of the strains were able to protect mice from a lethal wild type challenge. A subset of candidates was chosen for evaluation in the rabbit model. Except for a transient increase in temperature, no adverse effects were observed following vaccination by scarification. Rabbits were challenged 30 days after vaccination with aerosolized Schu S4 at doses ranging from 1,000 to 20,000 CFU. All rabbits developed fever and clinical signs of disease within 3 days of challenge; the severity of the febrile response was greatest in unimmunized rabbits. Unimmunized control rabbits succumbed to infection within 3 days of challenge. Unimmunized control rabbits succumbed to infection within 3 days of challenge. Survivors had reduced erythrocyte sedimentation rates relative to nonsurvivors. This is the first report of protection against aerosol type A challenge by a type A vaccine strain in the rabbit model and supports the use of this model for the evaluation of safety and efficacy of *F. tularensis* vaccines.

**213 (I)**

**Growth Media Affects *Francisella tularensis* SCHU S4 Virulence in Aerosol Challenged LVS-Vaccinated *Cynomolgus macaques***


**Background:** *F. tularensis*, the causative agent of tularemia, is a highly virulent, Gram-negative bacterium capable of infecting and proliferating within host macrophages and other cell types. Human infection can occur via several routes; strain type and route of infection govern the severity of tularemia. Vaccination with viable *F. tularensis* Live Vaccine Strain (LVS) in nonhuman primates (NHPs) was investigated to develop a positive control for future vaccine efficacy studies. Two growth media (Chamberlain’s broth [CB] and Mueller Hinton broth [MHB]) commonly used for propagation of *F. tularensis* were tested.

**Methods:** Twenty NHPs were aerosol challenged with SCHU S4 35 days after vaccination. Six animals served as sham vaccinated controls (Ct) and 14 were vaccinated (V) subcutaneously with at least 1 x 10^5 viable LVS. Half of the control and vaccinated animals were challenged with a target presented aerosol dose of 1000 CFU of *F. tularensis* SchuS4 (SchuS4) grown in CB and the remainder with a target presented dose of 1000 CFU of SchuS4 grown in MHB. **Results:** All Ct animals succumbed to tularemia infection 5 - 7 days post-infection (PI). Two V animals challenged with CB grown SCHU S4 also succumbed on Days 6 and 7 PI. No V NHPs challenged with MHB grown SCHU S4 died. Blood CRP levels, respiratory rates, development of hypothermia and lung inflammation appeared to correlate with severity of disease and disease progression. Tissue burdens were high in animals succumbing to infection and were decreased in surviving V animals. **Conclusions:** LVS vaccination enhanced survival in NHPs infected with aerosolized SCHU S4. In this study, disease caused by CB grown SCHU S4 appeared to be clinically more severe in V NHPs than those infected with MHB grown SCHU S4. This project was funded in whole or in part from federal funds from NIH, NI, DHHS, Contract No. HHSN266200500040C.

**214 (I)**

**Flow Cytometric Quantification of Lung Natural Killer Cell Activity Associated with TLR-3 Signalling Pathway Activation**


**Background:** Natural killer (NK) cells represent the major component of innate immunity against viruses and tumors due to their potent cytotoxic activity and rapid production of cytokines. Poly ICLC, a synthetic double-stranded RNA, is a broad-spectrum antiviral agent which activates TLR-3 signaling pathway. The objective of this study was to evaluate NK activation as a potential immunological biomarker for antiviral protection provided by pre-treatment with liposome-encapsulated (LE) Poly ICLC and free Poly ICLC. **Methods:** Lungs of mice intranasally treated with LE Poly ICLC or free Poly ICLC were harvested at various time points post drug treatment and NK cells were isolated and used as effectors in a cytotoxicity assay. A dual-fluorescent dye flow cytometric assay was developed to examine the duration of lung-associated NK cytotoxic activity in mice. Two methods of flow cytometric analysis were employed: one based on 7-AAD viability dye incorporation and the other combining 7-AAD incorporation with FSC parameters. **Results:** LE Poly ICLC and Poly ICLC stimulated a virtually identical lung NK activity at 7 days post treatment (p > 0.05). However, LE Poly ICLC-augmented cytotoxicity remained elevated through 14 days, significantly higher than that by Poly ICLC (p < 0.05). **Conclusions:** Our results demonstrated that LE Poly ICLC induced a potent augmentation of lung-associated NK activity for 14 days post intranasal treatment. The correlation between the duration of NK activation and the previously reported window of antiviral protection suggests that NK activation may be a good predictive biomarker for antiviral protection by LE Poly ICLC.
the systemic antibody levels post-vaccination and challenge are presented. **Methods:** Vaccination ACAM2000™ was administered to the mid scapular area by scarification using a bifurcated needle (2.5x10^5-1.25x10^6 PFU). Imvamune® was administered via the s.c. route to the mid scapular area (1.0x10^8 TCID_50).

Aerosol challenge Monkeypox Zaire 79 (NR2324, BEI) was aerosolised using the AeroMP-Henderson apparatus and delivered to 24 cynomolgus macaques (1.0x10^6 PFU). Primate respiration was monitored throughout; each was exposed to 3.0L accumulated volume.

ELISA Serum samples were assayed for the presence of Orthopox-specific IgG antibody using a psoralen UV-inactivated Vaccinia IgG ELISA. **Results:** Orthopox-specific IgG antibody levels were found to be lower post-vaccination in animals receiving a single dose of Imvamune® when compared with ACAM2000™. Highest levels were found in animals receiving a prime-boost of Imvamune®. Post challenge IgG levels peaked at day 9 for both vaccines. 4/6 animals survived after a single dose of Imvamune®; all animals receiving ACAM2000™ or a prime-boost of Imvamune® survived. **Conclusions:** This study showed that Day 9 post-challenge is the peak time for IgG antibody levels and animals receiving Imvamune® had higher levels of antibody compared to ACAM2000™; with those animals in the prime-boost group being fully protected.

**216 (I)**

5 Colour Immunophenotyping to Characterize the Immune Response after Smallpox Vaccination and Aerosol Challenge with Monkeypox

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**Background:** The potential of Smallpox as a bioterrorist weapon, the emergence of Monkeypox as a zoonoses and the contraindication of vaccination for many people has prompted development of new, safer smallpox vaccines. Using aerosolised Monkeypox virus in cynomolgus macaques as a model for Smallpox in humans, we conducted a protection study to assess the efficacy of the MVA based vaccine Imvamune®. This study had a negative control Tris/HCl buffer. Highest levels were found in animals receiving a prime-boost of Imvamune®. Post challenge IgG levels peaked at day 9 for both vaccines. 4/6 animals survived after a single dose of Imvamune®; all animals receiving ACAM2000™ or a prime-boost of Imvamune® survived. Im vamune® was administered via the s.c. route to the mid scapular area (1x10^8 TCID_50). 24 macaques were challenged with Monkeypox virus Zaire 79 via the aerosol route (1x10^6 PFU). Immunophenotyping Peripheral whole blood was stained with anti-CD3 PE Cy7, anti-CD4 APC, anti-CD8 PE TR, anti-CD16 FITC and anti-CD20 PE antibodies (BD Bioscience). Samples were acquired on a BC FC 500 flow cytometer and results were analysed using CXP and Microsoft Excel software. **Results:** Vaccination with Imvamune® was seen to induce earlier increases in CD4+ and CD8+ T cells than ACAM2000™. After aerosol infection with Monkeypox, very large increases in lymphocytes, NK cells and CD8+ T cells were observed before euthanasia. Unlike other populations, circulating CD4+ T cell levels were comparable to vaccinated animals post-challenge. **Conclusions:** These results indicate that Imvamune® elicits an earlier proliferative response than ACAM2000™ directly after vaccination. After aerosol challenge with Monkeypox, lymphocyte proliferation was reduced in vaccinated survivors in compared to TSB immunised macaques.

**217 (J)**

Emphasis on Impact: An Analysis of BSL-4 National Laboratory Biosafety Measures in Environmental Impact Statements

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**Background:** Since the implementation of the Project BioShield Act of 2004, millions of dollars have been directed to a multitude of agencies with one main purpose: build laboratories to research deadly pathogens. Laboratories funded by NIH, Homeland Security, and the Army have popped up across the U.S. with varying public opinions, ranging from accommodating to out-right hostility. In order to meet the requirements of the National Environmental Policy Act (NEPA), each laboratory completed an Environmental Impact Statement (EIS) addressing many issues, including biosafety, biosecurity, and worse case scenarios. Litigation in one laboratory stemmed directly from perceived gaps in the EIS. **Methods:** This analysis included cross-referencing the biosafety and biosecurity measures, including the worst case scenario from the environmental impact statements of the following BSL-4 laboratories: National Biodefense Analysis and Countermeasures Center, Fort Detrick, MD; Galveston National Laboratory, Galveston, TX; National Emerging Infectious Diseases Laboratory, Boston, MA; National Interagency Biodefense Campus, Fort Detrick, MD; and National Bio and Agro-Defense Facility, Manhattan, KS. **Results:** Similarities stretched across the statements, but some laboratories provided greater details of security measures and governmental regulations. **Conclusions:** The EIS plays a key role in meeting NEPA requirements and in providing a guide to concerned members of the public. Future BSL-4 laboratories should craft their EIS from laboratories that included a thorough and comprehensive biosafety and biosecurity explanation.

**218 (J)**

Quantification of Bacillus anthracis Spores on Complex Surfaces

**J. J. Calomiris; U.S. Army, USANCA, Fort Belvoir, VA.**

Development and evaluation of technologies for detection or control of biological agents on surfaces requires methods for accurate microbial quantification. Enumeration of microorganisms on complex surfaces poses challenges since organism recovery from surfaces can be inefficient and variable. In addition, treatments can promote release or retention of surface organisms and yield inaccurate results. To overcome confounding variables typically associated with surface testing, a system employing both direct microscopic counts and plate counts was developed to quantify B. anthracis spore inactivation on a variety of surfaces (military fabric and plumbing). The system involves (1) delivering spores to antimicrobial and control surface samples, (2) exposing samples to defined conditions, (3) terminating antimicrobial exposure by chemical neutralization, (4) recovering spores by physical agitation with elution buffer, and (5) enumerating recovered spores for viability by plate count and total number by microscopic count (Petroff-Haussser chamber). Inefficient spore recovery from surface samples was demonstrated by significant portions of spores remaining affixed to fabric following elution with physical agitation. In addition, the impact of treatment on spore recovery was illustrated by significantly reduced elution of spores from antimicrobial-treated fabric as compared with control fabric. However, viable-count to total-count ratio (VC/TC) provided accurate assessment of spore inactivation on the complex surfaces. VC/TC values allowed derivation of inactivation rates for B. anthracis spores on fabrics amended with a quaternary ammonium compound and a hypochlorite-generating formulation. Inactivation rates based on VC/TC values for B. anthracis spores on pipe surfaces and in bulk waters of a model wastewater distribution system provided the basis to understand the fate of the pathogen in chlorinated drinking water systems. Combination of viable counts and total counts can improve testing accuracy of strategies aimed to assess microbial inactivation on complex surfaces.
**BiGRUDI: Mobile Diagnostics for Multiplex Detection of Biothreat Agents**

H. ELLERBROK; Robert Koch Inst., Berlin, Germany.

**Background:** Agents that can be used in a bioterrorist attack comprise bacteria, viruses, and toxins. While bacteria and viruses can be detected simply through amplification of specific genome sequences by PCR toxins can be harmful in the absence of the producing organisms and therefore cannot be detected by PCR. These particular requirements for diagnosis make it a challenge to develop an integrated diagnostic approach for parallel detection and identification of all three types of agents. Methods, Results and Conclusions: BiGRUDI (Risk Assessment, Rapid Detection and Identification of Agents with relevance to Bioterrorists in Biological Threat Situations) is a network project funded by the German Ministry of Research and Technology (BMFT) comprising partners from academia, industries and governmental organisations and that develops an integrated approach for fast, sensitive and reliable mobile BT-diagnosis covering the entire process from development of scenarios over a first rapid matrix-based risk assessment using portable Raman spectroscopy to mobile analysis of agents in the field and a final risk evaluation. Since quality of a detection system depends on the quality of the reagents that are available to capture and to specifically detect an agent the project centres on generation and secured supply of highly specific and sensitive reagents and diagnostic tools. For this purpose polyclonal and monoclonal antibodies and specifically binding aptamers have been selected using B. anthracis, F. tularensis, Orthopox viruses, Filoviruses, and Ricin and Botulinum toxins A and B as model agents. Optimized combinations of these reagents are implemented into diagnostic platforms that can be used for rapid and specific mobile detection of samples. First reagents have been characterized and integrated into the immune filtration-based Abicap system and the array-based Zentplex system from BiGRUDI partners FZMB and Zenters and tested together with first responders. These platforms allow rapid mobile multiplex detection of different biothreat reagents.

**Preparing Biodefense Professionals: MS in Biotechnology Concentration in Biodefense and Certificate in National Security Studies**

K. OBOM,1 A. I. ROTH1; P. J. CUMMINGS1; Johns Hopkins Univ., North Potomac, MD, 2Johns Hopkins Univ., Washington, DC, 3Johns Hopkins Univ., Baltimore, MD.

To respond effectively to a biological threat, the nation will need a well trained workforce. Currently, there is a shortage of biodefense professionals, particularly individuals who have a strong background in the science and policy related to biodefense issues. To prepare professionals who have this expertise, students will be able to pursue a Certificate in National Security Studies and a Master of Science in Biotechnology with a concentration in Biodefense at Johns Hopkins University (JHU). This unique partnership between JHU’s National Security Studies and Advanced Biotechnology Studies programs and the United States Army Medical and Research Institute of Infectious Disease (USAMRIID) will provide students with a curriculum that includes graduate course offerings in Bioinformatics; Bioterror Response and Microbial Forensics; Science, Medicine & Policy in Biodefense; Vaccinology; Radiation Biology; and Biodefense Lab Methods, as well as Preserving American Security; Crisis Management and The Art and Practice of Intelligence. As part of the curriculum, USAMRIID offers a competitive fellowship program for up to five students each year to work at USAMRIID, while completing graduate courses at JHU’s campuses in Baltimore or Rockville, MD and Washington DC. Graduates of the degree program and the certificate will have an in-depth knowledge of the policy and practical laboratory skills in the field of biodefense and will fill a growing need for trained professionals skilled in this essential discipline within the science community and for homeland security.

**The Bundeswehr Rapidly Deployable Biolab: High-Tech Microbiological Diagnostics for Biodefense Operations Abroad**

R. WÖLFEL; Bundeswehr Inst. of Microbiol., Munich, Germany.

The Bundeswehr Institute of Microbiology has developed a set of lab equipment capable of rapid deployment to operations abroad. It allows reliable detection of biological warfare agents as well as other dangerous pathogens, even in low-resource environments and has been successfully used several times for diagnostic support missions in the Kosovo and Germany. As modern microbiological methods place high demands on the infrastructure of a lab - particularly when employed for bioforensic or medical purposes - a core capability of the rapidly deployable biolab consists in utilizing even very basic facilities for modern diagnostic investigations. By virtue of the system’s modular design it is possible to take along only the equipment actually needed to fulfill a mission. The equipment can be deployed within 72 hours by air transportation as passenger luggage and is operational in less than 6 hours after arrival. The space typically required for the lab in the field is around 20 square meters. To protect the personnel, the preparation of unknown samples can be carried out in a mobile isolator until possible pathogens have been safely inactivated. The deployable biolab allows the identification of bacteria, viruses, certain toxins and parasites with the aid of conventional and real-time PCR, immunological tests, as well as light and immunofluorescence microscopy. Laboratory methods and documentation techniques conform to pertinent NATO requirements regarding handling and confirmed identification of biological warfare agents. At present, the diagnostic spectrum of the deployable biolab encompasses more than twenty diseases, among them anthrax, plague, tularemia, Q-fever, brucellosis, viral hemorrhagic fevers, smallpox, influenza, and malaria. Modular accessory equipment allows broadening both the diagnostic spectrum and the sustainability of the deployable biolab. Further immunological methods (e.g. ELISA), extra lab equipment, as well as additional personnel (to allow shift work in theatre) can be incorporated.

**Collaborative Efforts Between the Oklahoma Public Health Laboratory and Emergency Responders in Ensuring Public Safety**

N. ZITTERKOPF-KHOURY, M. MCDERMOTT, J. MURRAY; Oklahoma State Dept. Hlth., Oklahoma City, OK.

**Background:** Effective communication between state and local public safety and health departments is critical in responding to public health threats. Public health labs (PHL) and Chemical, Biological, Radiation, and Nuclear response teams (CBRNE Hazmat teams) must work together to establish standardized threat response protocols to respond to “white powder” emergencies. Method: Potentially suspicious letters and packages continue to be reported to law enforcement and emergency response agencies in Oklahoma. Standard protocols were developed for credible threat assessment, collection and documentation. Receipt and testing protocols were developed for samples received during business hours and after business hours. Protocols for 24/7 on-call laboratory staff were established for triaging calls from responders and for establishing efficiency in the laboratory. Training programs for collecting and documenting “white powders” were produced by the PHL and implemented with the 17 CBRNE Hazmat teams in Oklahoma. Results: Reduced laboratory cost associated with testing, shorter response times, improved sample integrity, and a reduction in risk of cross-contamination were results of implemented training programs, protocols and improved labora-
Communicating the needs for samples to be field screened and properly packaged prior to transport has reduced the potential for occupational and facility exposure. Improved notification of collection status has allowed the PHL to more efficiently prepare for testing. **Conclusion:** Collaborative efforts have resulted in safer and more efficient responses to biological threats. Opportunities for improvement relevant to PHLs nationwide include inconsistency in field screening and threat assessments, and the lack of standardization in collecting and documenting specimens.

**223 (K)**

**Information Concerns for Biosecurity Labs**

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**Background:** The Center for Biodefense, Law & Public Policy at Texas Tech University School of Law established a toll-free hotline in October 2008 for biological safety officers, biodefense researchers, and attorneys, to provide guidance select agent rule compliance questions. This WRCE hotline is supported by Grant # U54 AI057156 from NAID/NIH. The goal of the hotline is to help identify questions that arise under the current Select Agent Program. The hotline is open to callers 24 hours a day, all year round. Since the hotline's inception, the Center has received many questions about securing electronic lab information. This poster addresses the most common questions of information security in labs, including the following: Do emails fall under the umbrella of information security? Yes. Statutory law applies information security to computer systems. As a result, email must also be included. As a result, information security applies to email. The CDC considers all information provided to Select Agent Programs Sensitive but Unclassified (SBU). Publication of SBU information could compromise the security of the regulated community. Any records or information systems that could allow access to select agent or toxin data must be safeguarded to prevent release. Can records be scanned and kept on file? Yes. Labs may scan and keep records on file, but the lab must be able to verify accuracy and authenticity of the records under a restricted access system. Access to scanned records can be easily restricted if the files are in a stand-alone computer or storage system with controlled access.
Efficacy of IMVAMUNE: A Third Generation Smallpox Vaccine Candidate in a Lethal Monkeypox Virus Nonhuman Primate Inhalation Model

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Background: First and second generation smallpox (SPX) vaccines composed of replicating vaccinia are associated with adverse health risks. Because of this, testing of new, safer vaccines is desirable. IMVAMUNE®, a formulation of live Modified Vaccinia Ankara, is a promising candidate due to its inability to replicate in mammalian cells. The objective of this study was to evaluate the efficacy of IMVAMUNE® versus a second generation vaccine, ACAM2000, in a lethal aerosol monkeypox virus (MPX) nonhuman primate (NHP) challenge model. Methods: NHPs were vaccinated with ACAM2000, IMVAMUNE® (prime or prime-boost) or vehicle beginning on Study Day -60. MPX neutralizing antibody (Ab) production was assessed bimonthly until euthanization. Clinical observations were performed daily for up to 30 days post-challenge. Blood and pharyngeal swab analysis, lesion counts, and clinical pathology were conducted every third day and upon euthanization. Upon necropsy, tissues from each animal were collected and analyzed for MPX burden and histopathological changes. Results: Increases in neutralizing Ab titers were measured in all non-control animals following vaccination. Alterations, predominantly associated with respiratory distress, were noted in all groups post-exposure; the highest severities were seen in controls, all of which succumbed to disease. Death in these animals was attributed to severe fibronecrotic pneumonia. All ACAM2000- and prime-boost IMVAMUNE®-vaccinated NHP survived MPX challenge with minimal disease. Among the prime IMVAMUNE®-vaccinated animals, one (17%) survived challenge. Non-survivors from this group presented similarly to controls, though results, including minimized lung pathology and viremia, suggested that partial protection was conferred. Conclusions: A prime-boost regimen of IMVAMUNE® demonstrated complete protection in a NHP lethal aerosol MPX challenge model thereby indicating it as a safer alternative to traditional SPX vaccines.

Evaluation of the Nasal and Sublingual Mucosa as Sites for Rapid Induction of Immunity to Ebola

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Background: A primary goal is to develop novel delivery methods that confer potent immune responses. Intranasal (IN) delivery of an adenovirus-based vaccine induces strong T and B cell responses against Ebola Zaire glycoprotein (ZGP) and confers protection from challenge. Methods. Virus was modified with poly(ethylene) glycol (PEG-Ad) by standard methods and incorporated into poly(lactic-co-glycolic) acid (PLGA-Ad) polymer by double emulsion. Results. IN delivery of PEG-Ad increased anti-ZGP IFN-γ+ T cells (37.5 + 14.9 vs. 53.7 + 47.1 SFCs) and reduced expansion of effector/effector memory CD8+ T-cells three-fold. IN delivery of PLGA-Ad induced a similar effector/effector memory response (3.0 ± 0.7 vs. 2.2 ± 0.8%). SL delivery induced higher levels of ZGP-specific Th1 and Th2 type responses than intramuscular (IM) injection (p<0.01) and conferred 100% protection against challenge. A secondary goal is to evaluate the type of immune response elicited after prior exposure to the vaccine carrier. When pre-existing immunity (PEI) was established by IM injection (5 x 10⁹ infectious virus particles (ipv)), 100% of mice dosed IN and SL survived. Only 20% of the IM group survived. The anti-ZGP antibody response was unaltered by PEI (IN and SL). When PEI was established by the IN route, 88% of mice dosed IM and SL survived. An increase in effector/effector memory CD8+ T-cells was noted after SL delivery (1.53 ± 0.3, vs. 2.31 ± 0.6%). A decrease in systemic anti-ZGP IgG1 was also found (SL and IM). Only 63% of mice vaccinated IN survived and a significant reduction in Th1 type responses noted (p<0.01). PLGA-Ad given IN improved survival (78%). Effector/effector memory CD8+ T-cells were not affected by PEI (2.2 ± 0.3, vs. 1.9 ± 0.5%). Conclusions. These delivery methods are effective and would radically improve compliance. These results are also useful for immunization strategies that employ vaccine carriers that are ubiquitous pathogens (adenovirus, influenza).

Pre-Existing Immunity to Vaccinia: Does It Affect the Efficacy of Recombinant MVA Vectored Vaccines?

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Background: Modified vaccinia Ankara (MVA) has been shown to be safe and an effective alternative vaccine for small pox. Its safety profile has made it an attractive live viral vector for many vaccine candidates. However, concerns about the efficacy of MVA in the face of pre-existing immunity to vaccinia in numerous adults over the age of 30 years still remains. We report on efficacy...
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**Cellular Immune Responses to Anthrax, Plague and Tularemia Vaccines**

J. J. THOMPSON, E. DYSON, M. STOKES, N. BAILEY, J. PRIOR, A. SIMPSON; Dstl, Salisbury, United Kingdom.

**Background:** Understanding cell mediated immune (CMI) responses to vaccines is crucial in determining correlates of protection. In this study we assessed CMI responses to antigenic components of tularemia (LVS), plague (CSL) and anthrax (AVP) vaccines. Method: Single blood samples (40ml) were taken from naïve and vaccinated healthy volunteers. Intervals since last vaccination varied: 14-20 years for tularemia, 5-10 years for plague, 0.5-4 years for anthrax vaccines. There were two groups of AVP recipients: (1) up-to-date with current annual booster schedule, and (2) last booster dose > 3 years previously. PBMCs were stimulated with vaccine-specific antigens and responses assessed using IFNg and IL-5 ELISPOT assays. **Results:** Strong IFNg CMI responses were observed for volunteers who had previously been vaccinated against tularemia and plague, with a weaker IFNg response from volunteers vaccinated against anthrax. No significant IL-5 responses were observed. **Conclusions:** All three vaccines generated antigen-specific CMI responses with an IFNg bias, indicating induction of a Th1-type profile. Tularemia (live attenuated) and plague (killed cell) vaccines generated larger responses than the anthrax (acellular) vaccine, especially in individuals boosts > 3 years previously. Both tularemia and plague vaccines had been given > 5 years previously, suggesting they induce a long lasting CMI response. © Crown copyright Dstl 2010.

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009. Vaccines Bacteria

Monday, February 7, 2011 | 3:00 PM – 4:00 PM

**Passive Transfer of Serum Collected from Humans and Animals Vaccinated with a Recombinant Plague Vaccine (rf1V) Protects Mice from Aerosol Challenge with *Yersinia pestis***


**Objective:** These studies evaluated the ability of antibodies generated in rf1V-vaccinated humans, cynomolgus macaques and mice to protect naïve Swiss Webster mice against pneumonic plague. In addition, immune rabbit serum was evaluated as an assay control. **Methods:** rf1V immune serum was pooled for each species and 1 mL was transferred into naïve Swiss Webster mice via the intraperitoneal route on Day 0. Blood was collected by the retro-orbital sinus approximately 4 hours following administration of the sera to determine the circulating levels of donor anti-rf1V antibodies in the recipient mice. Approximately 2 hours later, when mice recovered from blood collection, they were exposed to a mouse-only aerosol challenge of 25 LD90 (50,000 cfu) *Yersinia pestis* CO92 and survival monitored for 14 days. **Results:** Passive transfer studies demonstrated that mouse survival was positively associated with anti-rf1V antibodies in the recipient mice. **Conclusion:** Antibody levels in the donor sera and the circulating levels of these antibodies in the recipient mice. **Conclusion:** Antibody levels in the donor sera and the circulating levels of these antibodies in the recipient mice. **Conclusions:** Antibody levels in the donor sera and the circulating levels of these antibodies in the recipient mice. © Crown copyright Dstl 2010.
Francisella novicida Outer Membrane Vesicles: Novel Vaccine and Possible Role in Virulence

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Outer membrane vesicles (OMVs) are 50 to 250 nm spherical structures produced by gram-negative bacteria. OMVs contain normal constituents of the bacteria from which they originate. We have recently identified OMVs from Francisella novicida. We hypothesize that OMVs may represent a novel Francisella vaccine with many favorable properties, and without the drawbacks of live vaccine approaches. F. novicida OMVs have biochemical activity in vitro, such as acid phosphatase, PLC, and hemolytic activity, and are cytotoxic to host cells. Francisella OMVs are 43-125 nm in size, observed by TEM and AFM. LC-MS/MS analysis identified 416 F. novicida proteins, including well-known virulence factors and membrane proteins, confirmed through Western Blot and biochemical assay. These results suggest that OMVs may represent a novel mechanism by which Francisella can alter its extracellular environment. OMV production may be significant with respect to Francisella pathogenesis, suggesting a new mechanism by which the bacterium can affect the surrounding host tissue. Finally, we demonstrated that an I.N. OMV vaccine can protect mice against I.N. exposure to Francisella. We are currently translating this approach to the fully virulent Francisella SchuS4 strain. We propose that OMVs are a novel and useful vaccine approach for Francisella. Acknowledgement: This work was partially supported by a grant to MVH from Virginia Academy of Science.

A Live Attenuated Strain of Yersinia pestis KIM5+ as a Vaccine against Plague

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Yersinia pestis, the causative agent of plague, is a potential weapon of bioterrorism. Y. pestis evades the innate immune system by synthesizing tetra-acylated lipid A with poor Toll-like receptor 4 (TLR4)-stimulating activity at 37°C, whereas hexa-acylated lipid A, a potent TLR4 agonist, is made at lower temperatures. Synthesis of Escherichia coli LpxL, which transfers the secondary laurate chain to the 2'-position of lipid A, in Y. pestis results in production of hexa-acylated lipid A at 37°C, leading to significant attenuation of virulence. Previously, we described a Y. pestis vaccine strain in which crp expression is under the control of the arabinoise-regulated araC Pbad promoter, resulting in a 4-5 log reduction in virulence. To reduce the virulence of the crp promoter mutant further, we introduced E. coli lpxL into the Y. pestis chromosome. The ΔlpxP32::P(lpxL)ΔPcrP22::TT araC Pbad crp construct likewise produced hexa-acylated lipid A at 37°C and was significantly more attenuated than strains harboring each individual mutation. The LD50 of the mutant in mice, when administered subcutaneously or intranasally was >1011-times and >104-times greater than wild type, respectively. Mice immunized subcutaneously with a single dose of the mutant were completely protected against a subcutaneous challenge of 3.6 x 105 wild-type Y. pestis and significantly protected (80% survival) against a pulmonary challenge of 1.2 x 105 live cells. Intranasal immunization also provided significant protection against challenges by both routes. This mutant is an immunogenic, highly attenuated live Y. pestis construct that merits further development as a vaccine candidate.

PCR-Based Techniques for Rapid Diagnosis and Improved Surveillance of Tularemia: A French Experience

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Background: The incidence and the geographic spread of tularemia have recently increased in France. However, human infections remain difficult to diagnose because they are rare and clinically unspecific, serological diagnosis is often delayed and F. tularensis culture lacks sensitivity. We evaluated the usefulness of PCR-based assays to confirm tularemia cases and improve biothreat surveillance. Methods: Clinical, epidemiological and diagnostic features in 39 PCR-confirmed tularemia cases were reviewed. Specific antibodies were detected by microagglutination (MAT) and immunofluorescence (IF; IgM and IgG separately). F. tularensis was detected by culture (chocolate agar, BioMérieux, France) or real-time PCR tests (IFflu2 or tul4 gene targets). Subspecies identification of the involved F. tularensis strain was obtained by PCR amplification and sequencing of the 16S-23S rDNA intergenic spacer region. Results: Patients had a mean age of 50.1 years, with a male/female ratio of 0.69. The clinical forms were ulceroglandular (11 cases), glandular (12 cases), ocuolugal (2 cases), pharyngeal (13 cases) and pneumonic (1 case). Most patients were infected by contact/ingestion of lagomorphs, or after tick bites. Antibody titers > 80 were found in 89.6% of cases for MAT, 86.2% for IF-IgM and 100% for IF-IgG. F. tularensis DNA was detected in lymph nodes (28 cases), skin ulcers (3 cases), pharyngeal swabs (5 cases), a conjunctival discharge (1 case) and serum samples (2 cases). In 9 patients, real-time PCR tests were positive before seroconversion. Culture was positive in 5/29 cases (17.2%). F. tularensis subsp. holarctica was identified in 9 patients (5 strains and 4 clinical samples). Conclusions: The PCR-based tests now allow rapid and accurate diagnosis of tularemia, especially at an early stage of the disease. Moreover, rapid molecular identification of the subspecies involved represents a major advantage in the context of biothreat surveillance.

Anthrax and Plague Diagnostic Identification, and Antibiotic Susceptibility Testing Using Bioluminescent Reporter Phage

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Yersinia pestis and Bacillus anthracis are the etiologic agents of the plague and anthrax, respectively. Both diseases have a rapid clinical course, high mortality rate, and produce clinical symptoms shared by many common diseases. Therefore, prompt culture identification and administration of appropriate antibiotics are vital for a positive prognosis. Bioluminescent reporter phages hold promise for the rapid, specific, and sensitive detection of B. anthracis and Y. pestis. Since the bioluminescent response is dependent on bacterial fitness, it may be possible to detect the presence of these microbes and perform antimicrobial susceptibility testing simultaneously. Recombinant “light-tagged” reporter phages were generated by integrating the bacterial luxAB reporter genes into a non-essential region of the phages genome through homologous recombination. The resulting reporter phages were able to confer a bioluminescent phenotype to Y. pestis or B. anthracis within 15-20 min of infection. The sensitivity limits of detection for Y. pestis and B. anthracis were 104 and 105 CFU/mL, respectively. Non-antiarachis Bacillus species
and non-pestis Yersinia species did not generate a signal, or produced significantly reduced responses upon incubation with the reporter phage. Serum neither quenched bioluminescence nor prevented the ability of the phage to transduce a bioluminescent signal response to recipient cells. The reporter phages were unable to produce a bioluminescent signal in the presence of inhibitory antibiotic concentrations. In addition, the light response in the presence of varying antibiotic concentrations produced profiles within 20 min that were comparable to the standard CLSI method for determining antibiotic susceptibility. The bioluminescent reporter phages display promise for the specific detection and antimicrobial susceptibility testing of Y. pestis or B. anthracis.

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Universal Pathogen Detection Employing High Throughput Sequencing

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Background: Pathogens in samples that also contain high quantities of cellular nucleic acids are less amenable to detection using non-specific PCR-based techniques, even when paired with high throughput sequencing. High non-specific PCR sensitivity is critically important for such samples. Method: DOP-PCR combines physical and biochemical methods to purify host material away from pathogens. We extract DNA and RNA from the same sample, which is not only time efficient, it also allows working with small sample volumes. We perform a truly generic, highly degenerate PCR that has been optimized for sensitivity, followed by high-throughput sequencing (454 Roche). Use of barcode-including primers permits increased throughput and inclusion of critical controls in every reaction. Results: Our assay detects members from varying virus families such as retroviridae, coronaviridae, caliciviridae, flaviviridae, picornaviridae, orthomyxoviridae, paroviridae, adenoviridae, circoviridae, and polyomaviridae. It also differentiates and identifies different strains of Burkholderia. Multiple pathogens can be identified within a single sample. Discussion: High throughput sequencing allows deeper analyses of complex samples and further improves the detection limit. The ability of the DOP-PCR to reproducibly and sensitively amplify parts of viral genomes without any prior knowledge of those sequences makes this a very promising assay not only for detection and discovery of clinically relevant viruses but also in bioterrorism preparedness and response.

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Old World Hantaviruses in New Orleans, Louisiana: From Surveillance to Virology of Local Isolates


Background: Hantavirus infections in humans are associated with acute diseases that include Hemorrhagic fever with Renal Syndrome (HFRS) and Hantavirus pulmonary syndrome. Old World Hantaviruses (OWHV) are associated with HFRS and can be found globally, likely due to the spread of its reservoir, Rattus norvengicus, by maritime trade. As a result, there may be elevated risk for exposure to rodents harboring these viruses for those living in port cities. New Orleans, Louisiana is an international shipping port in a sub-tropical environment that is extremely suitable for the support and maintenance of Rattus species and hence, any diseases they may harbor. Methods: This work is the product of an ongoing survey for OWHV in Rattus species in New Orleans. Rodents were sampled by convenience based on trouble calls to city sanitary authorities over 24 months. Prevalence was based on RT-PCR and sequence analysis of positive samples. Isolates were confirmed by immunohistochemistry. Vascular leak, a hallmark of viral hemorrhagic fever infection, was modeled by use of trans-endothelial electrical resistance (TEER) assay to measure endothelial barrier integrity. Results: To date, 11% the 150 rodent samples are RT-PCR positive and 3% have high sequence homology to Tchoupitoulas virus, a Seoul-variant first isolated in New Orleans in 1985. We also report a significant decrease in TEER by both survey isolates and Andes Virus, but not in controls beginning at 48 hours post-infection. Conclusions: By providing evidence of the persistence of a Seoul-like virus over the last 25 years, this work demonstrates the usefulness of a rodent-borne viral disease surveillance system in New Orleans, LA. It also presents novel means for vascular leak modeling in Hantaviruses utilizing the TEER system.
Development of Pneumonic Plague Is Dependent on the Immunomodulating Effects of the *Yersinia pestis* Yersiniabactin Siderophore

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*Yersinia pestis* is a gram-negative bacterium responsible for the disease commonly known as plague. As common in other pathogenic bacteria, *Y. pestis* possesses multiple iron acquisition systems to acquire iron from the mammalian host to allow for bacterial survival and growth. The yersiniabactin (Ybt) system is the most studied iron acquisition system and thus far has been proven to play a large role in the pathogenesis of bubonic and pneumonic plague. We found that intranasal infection of BALB/c mice with *Y. pestis* strains with specific deletions in biosynthetic Ybt system genes, therefore eliminating Ybt production, resulted in significant loss of virulence as well as the inability to induce pneumonic disease. Further characterization of the animal disease model revealed that the loss of virulence was associated with the Ybt-deficient strain’s inability to suppress development of acute inflammation, a hallmark of wild type *Y. pestis* pneumonic infection. With consideration of the importance of iron status in the function of mammalian immunological processes, we explored the effects of a high affinity siderophore such as Ybt on the pulmonary innate immune system through its interactions with mammalian transferrin and lactoferrin as well as its effects on inflammatory cell function due to sequestration of bioavailable iron from the host. Our findings lead us to propose a model in which Ybt is acting to both increase bacterial fitness as well as decrease host ability to mount an effective defense against the pathogen. Further characterization of this mechanism by which *Y. pestis* exerts the acute immunomodulating effects necessary for development of severe pneumonic plague disease may lead to more options towards development of preventative and therapeutic measures.

Effect of LVS Vaccination and Growth Media on *Francisella tularensis* SCHU S4 Aerosol-Induced Serum Cytokine Response in Non-Human Primates

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Background: The objective of this study was to investigate cytokine levels in serum of *F. tularensis* live vaccine strain (LVS) vaccinated cynomolgus macaques, aerosol exposed on Day 35 post-vaccination to virulent *F. tularensis* strain SCHU S4 grown in either Chamberlain’s broth (CB) or Mueller-Hinton broth (MH). Methods: Blood was drawn on the day of challenge, every 2-4 days until Study Day 49, then weekly until study termination and tested for serum cytokine levels. Results: All non-vaccinated controls (CTL) succumbed 5-7 days post exposure. Two vaccinated (V) animals exposed to SCHU S4 grown in CB (n=7) succumbed, whereas all V animals exposed to SCHU S4 grown in MH (n=7) survived. Of 15 cytokines measured, interferon-gamma (IFN-γ) was of special interest as it is presumed necessary, though insufficient, for LVS mediated protection. With the exception of IL-8, all cytokines measured post SCHU S4 exposure were stable in surviving V animals, in contrast to succumbing animals, which showed increasing levels of several cytokines. CTL animals exposed to CB-propagated SCHU S4 (n=3) had significantly higher serum levels of IFN-γ on Day 40 (p<0.05) compared to animals exposed to SCHU S4 grown in MH (n=3). Growing SCHU S4 in CB also induced significantly higher levels of G-CSF on Day 38 and Day 40 (p<0.05) compared to MH broth, in CTL animals. Conclusions: A rise in serum cytokines preceded *F. tularensis*-induced death and appeared unrelated to protection. We speculate that growth media used to expand *F. tularensis* may modulate epitope expression, and thereby host response, and influence the protective capacity of live vaccines. This project was funded in whole or in part with Federal funds from NIAID, NIH, DHHS, Contract No. HHSN266200500040C.

Cellular Immune Responses to Three Licensed Anthrax Vaccines

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Background: Understanding immune responses to anthrax vaccination remains a priority in biodefense countermeasure development. We compared cell-mediated immune (CMI) responses in recipients of Live Attenuated Anthrax Vaccine (LAAV), the licensed UK (AVP) and US (AVA) anthrax vaccines, and unvaccinated controls. Methods: Blood samples were obtained from 56 volunteers, 16 LAAV, 16 AVP, 11 AVA recipients and 13 controls with no history of anthrax infection or vaccination. CMI responses to protective antigen (PA), edema factor (EF) and lethal factor (LF) were assessed using IFN-γ ELISPOT assays. Results: The number of vaccine doses received ranged from 2-14 and time since last dose from one month to 19 years, respectively. CMI responses to PA and LF were demonstrated in recipients of all 3 vaccines. Clear responses to EF were seen in recipients of LAAV and AVP, but not AVA, compared to controls. There were no obvious relationships between CMI responses to anthrax antigens and either the number of vaccine doses received or time since last dose. Responses were demonstrable in some vaccinees several years after their last vaccine dose. Conclusions: Each of the 3 licensed anthrax vaccine elicited demonstrable CMI responses. This study, involving individuals with a wide range of doses and intervals since vaccination, suggests there may be previously unrecognized differences between these vaccines (EF content). Further studies of CMI responses are indicated, particularly to compare responses shortly after booster doses.

Biology of *Francisella tularensis* Subspecies *Holarctica* in Tick Vectors

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Background: *Francisella tularensis* (Ft) causes tick-transmitted tularemia epizootics in rodent and rabbit hosts and incidental infections in humans. The objective of this study was to develop a Ft tick colonization model for elucidating the salient features of its biology in tick vectors. Methods: Colony-reared larvae, nymph and adult Dermacentor variabilis (Dv) and Amblyomma americanum (Aa) ticks were fed 10°CFU/ml Ft strain LVS via capillary tubes (CF). Post feeding (PF), level of colonization was determined by CFU determinations of tick midgut. Results: Transmission of Ft from larvae to nymph was seen in both tick species, but only Aa nymphs maintained Ft for longer periods of time (18 weeks PF). Transstadial transmission from nymph to adult was also demonstrated in both the tick species, but only Dv ticks maintained...
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Stability of Virulent Coxiella burnetii Strains Grown in Host Cell-Free Media
G. J. KERSH, L. D. OLIVER, K. A. FITZPATRICK, J. S. SELF, R. F. MASSUNG; CDC, Atlanta, GA.
Coxiella burnetii is an obligate intracellular bacterium that causes the zoonotic disease Q fever. The requirement to be grown in tissue culture cells, chicken eggs, or animal hosts has made it difficult to isolate strains and perform genetic studies on C. burnetii. However, it was recently demonstrated that the attenuated Nine Mile Phase 2 C. burnetii strain will grow axenically in an Acidified Citrate Cysteine Medium (ACCM) in a 2.5% oxygen environment. The current study was undertaken to determine whether more virulent C. burnetii strains could be grown in ACCM, and whether they would maintain virulence after passage. The ACCM media supported an approximately 3-log expansion of Nine Mile Phase 1, Nine Mile Phase 2, M44, and Henzelering strains of C. burnetii, whereas the Priscilla (Q177) strain grew somewhat less well (100-fold), and the K strain (Q154) grew poorly in ACCM. To determine if passage in ACCM would maintain the virulence of C. burnetii, the Nine Mile Phase 1 strain was grown for up to 26 weekly passages in ACCM. C. burnetii maintained in ACCM for 5 or 8 passages maintained full virulence in a mouse model, but Nine Mile Phase 1 passages for 23 or 26 times was somewhat attenuated compared to Nine Mile Phase 1 purified from embryonated eggs. These data demonstrate that virulent strains of C. burnetii can be successfully passaged in ACCM, but that they can lose virulence after extended passage. The loss of virulence in axenic culture was associated with some truncation of lipopolysaccharide chains, suggesting a possible mechanism for attenuation.

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Evolution of Viral Quasispecies During Interspecies Transmission Events
Background: The high mutation rate of RNA viruses increases the ability of these viruses to adapt to diverse hosts (interspecies transmission events) and cause new human and zoonotic diseases. However, very little is known about the particular mutations that enable interspecies transmission events to occur. Methods: The objective of this project is to define the genetic mutations associated with interspecies transmission for two different RNA viruses, rabies virus and bovine coronavirus. In each case ultra-deep genome sequencing is combined with advanced computational analysis to characterize genomic changes of host-switching events, focused particularly on identifying the role of changes in the viral quasispecies. Two different sequencing platforms, 454 and Illumina are being tested for performance, reliability, and ease of use. Results: Initially Illumina was used on three naturally-infected samples (2 rabies samples and 1 bovine coronavirus sam-
Evaluation of the Innate Immune Response of Human Brain Endothelial Cells to Henipavirus Infection

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Nipah (NiV) and Hendra (HeV) viruses are highly pathogenic paramyxoviruses (genus henipavirus). Both viruses have the ability to infect humans, causing severe acute encephalitis or respiratory symptoms. Vascular endothelial cells (EC) are the predominant target cells. Despite the fact that NiV and HeV have a 70-85% nucleotide homology within the coding regions, HeV infections result in less neurologic pathology. To better understand the molecular mechanisms of the host responses to henipavirus infection, we have analyzed the global host gene and immune response of human brain ECs over time in response to NiV and HeV infection. Our results have shown that NiV-infected ECs developed syncytia as early as 18 hours post infection (hpi), as well as a high degree of cytopathic effect at later time points (24-36hpi). This observation was not made in HeV-infected cells within the same time frame. Analysis of the patterns of the global gene expression of ECs at 24 hpi using cDNA microarrays indicate that in NiV-infected cells a total of approximately 10-times more genes were differentially regulated than in HeV-infected cells. A large number of these genes could be assigned to pathways related to the immune response and apoptosis. Further, cell culture supernatants of infected ECs were collected at regular intervals and the gene and protein expression levels of cytokines mediating vascular inflammation and an up-regulation of cytokines/chemokines were analyzed. Our results have shown an early up-regulation of cytokines mediating vascular inflammation and an up-regulation of cytokines/chemokines involved in leukocyte and macrophage recruitment at later stages of the infection. These data offer evidence that brain ECs are capable of promoting an active but different immune response against NiV and HeV. This observation, also reflected in the higher number of differently regulated genes, may provide a likely explanation for the different degree of neurologic pathology observed in NiV and HeV infections.

Cell Specific Innate Immune Recognition of Rift Valley Fever Virus

M. ERMLER, E. YERUKHIM, K. FITZGERALD, C. H. KING, A. G. HISE; 1 Case Western Reserve Univ., Cleveland, OH, 2Univ. of Massachusetts, Worcester, MA.

Background: Rift Valley fever virus (RVFV) is a zoonotic pathogen endemic to regions of Africa and the Arabian Peninsula. In the most severe cases, RVFV infection can cause retinitis, encephalitis, hemorrhagic fever, or death. The innate immune response to RVFV is suspected to be important in viral clearance but is still poorly defined. Type I interferon (IFN) has been shown to be protective in humans and animal models. Members of the Toll-like receptor (TLR) and RIGI-like receptor (RLR) families recognize viral patterns and stimulate type I interferon responses. These studies seek to elucidate which innate receptors are important for immune response against RVFV. Methods: In this study, bone marrow-derived dendritic cells (DCs) and macrophages (macs) were generated from wild-type and knockout mice and were stimulated with virus and TLR/RLR ligands. Commercial FLT3L was used to generate mixed plasmacytoid (pDCs) and conventional (cDCs) dendritic cells. J558L cell supernatant containing GMCSF was used to generate a pure population of cDCs. L292 supernatant containing MCSF was used to generate macs. Results: In macs and cDCs, TLR3 did not contribute to downstream IFNα or NFκB mediated cytokine production. However, functional RLR adaptor MAVS was necessary for IFNα production. NFκB mediated cytokine production, such as IL-6, was not dependent upon MAVS, although this pathway contributed to overall IL-6 production. Conclusions: RLRs play a major role in innate immune cell production of type I IFN in response to RVFV. A yet unidentified pathway contributes to NFκB mediated cytokine production, most likely through an endosomal TLR such as TLR7.

Identification of Host Factors that Interact with Bunyavirus Nucleoproteins


Background: Simple RNA viruses such as bunyaviruses must rely heavily on host cell networks for their replication. Thus, by dissecting protein interactions within complexes of viral and cellular proteins, we expect that much can be learned about how these viruses exploit and reprogram host cells to replicate. This work is focused on four highly pathogenic viruses in the family Bunyaviridae: Crimean-Congo hemorrhagic fever virus (CCHFV), Rift Valley fever virus (RVFV), and Hantaan virus and Andes virus (HTNV, ANDV). Methods: In this study, we transiently expressed 3xFLAG-tagged N proteins of ANDV, HTNV, CCHFV, and RVFV in 293T cells and then isolated bunyavirus-host protein complexes by co-immunoprecipitation with anti-FLAG magnetic beads. Once virus-host protein complexes were isolated, the constituent proteins were separated by SDS-PAGE, subjected to in-gel trypsin digestion, and then identified by reversed-phase liquid chromatography-tandem mass spectrometry. Results: Using co-immunoprecipitation and mass spectrometry, we identified numerous host proteins that may interact with the N proteins of ANDV (30), HTNV (64), CCHFV (33), and RVFV (43). In total, 98 unique host proteins were identified, with 17 of these being common interactions for at least three of the four N proteins. Host proteins overrepresented in these data include those involved in RNA binding, processing, and stability; stress response proteins; and components of the cytoskeletal network. We are currently attempting to validate these interactions by secondary assays and functional screens. Conclusions: This simple and robust approach has demonstrated its potential for identifying novel virus-host interactions. In addition, the host factors identified here may serve as novel targets for therapeutic development against bunyaviruses and other biothreat agents.
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PANACEA Broad-Spectrum Antiviral Therapeutics

Background: Currently there are relatively few therapeutics for viruses on the category A-C priority pathogen lists, and most which do exist are highly virus-specific or have undesirable side effects or other disadvantages. We have developed a radically new and very broad-spectrum antiviral therapeutic with the potential to revolutionize the treatment of category A-C as well as common clinical viruses. Our Double-stranded RNA (dsRNA) Activated Caspase Oligomerizer (DRACO) selectively induces apoptosis in cells containing any viral dsRNA, rapidly killing infected cells without harming uninfected cells.

Methods: We have produced preliminary DRACO designs and evaluated their toxicity, antiviral efficacy, and other properties in quantitative cell assays and in mouse trials. Results: We have demonstrated that DRACOs are nontoxic in all 11 cell types tested thus far, including human, monkey, and mouse cells representing numerous tissue types. Using these cells, we have shown that DRACOs are effective against all 15 viruses tested thus far, including DNA and RNA viruses, enveloped and nonenveloped viruses, human and rodent viruses, and viruses that use a variety of receptors. Among the viruses against which DRACOs have proven effective in vitro are dengue hemorrhagic fever virus, arenaviruses, and bunyaviruses. In mice, we have demonstrated that DRACOs rapidly penetrate into all organs tested, persist for over 24 hours after each dose, and are nontoxic. We have shown that DRACOs rescue mice from a lethal H1N1 influenza challenge, and we are conducting efficacy trials against dengue in mice. Conclusions: We have demonstrated that DRACOs are nontoxic and effective against a broad spectrum of viruses. We hope to optimize our DRACO designs and demonstrate them against additional viruses and in additional animal models. This work should greatly advance DRACOs toward ultimate utility as safe, broad-spectrum therapeutics for priority and clinical viral pathogens, filling a large gap in existing therapeutics.

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Therapeutically Targeting Hantavirus Induced Endothelial Cell Permeability
E. R. MACKOW, E. GORBUNOVA, T. PEPINI, I. GAVRIFOVSKAYA; Stony Brook Univ., Stony Brook, NY.

Background: Hantaviruses nonlytically infect endothelial cells (ECs) and cause 2 vascular permeability based diseases, HFRS and HPS. ECs form the primary fluid barrier of capillaries and pathogenic hantaviruses enhance EC permeability responses that alter capillary integrity. These findings suggest that receptor and pathway specific inhibitors may be used to therapeutically enhance EC integrity following infection by pathogenic hantaviruses. Methods: Our studies analyzed inhibitors of ANDV and HTNV induced EC permeability and target responses altered within the infected endothelium that enhance the integrity of EC adherence junctions. We evaluated the role of siRNAs, EC specific lipids, growth factors and small molecule inhibitors for their ability to block the paracellular permeability of ECs infected by pathogenic ANDV and HTNV. Results: We found that siRNAs to Src family kinases and downstream regulators of actin directed VE-cadherin internalization blocked ANDV and HTNV induced EC permeability. In addition, the platelet derived lipid S1P and its analog FTY720, as well as an EC specific growth factor Ang-1 reduced ANDV and HTNV induced EC permeability. Pathway specific small molecule inhibitors also blocked hantavirus induced EC permeability 50-70% at nanomolar concentrations. Conclusions: Our findings indicate that there are several means for reducing the paracellular permeability of hantavirus infected ECs. Antagonistic Tie-2 and Edg-1 receptors as well as VEGFR2 pathway inhibitors are potential targets for therapeutically reducing ANDV and HTNV induced EC permeability and as a result capillary permeability during HPS and HFRS. Since the function of these inhibitors are already well defined and FDA approved for clinical use, these findings rationalize their therapeutic evaluation for efficacy in reducing hantavirus disease.

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Zaire Ebolavirus Infection Requires Access to and is Blocked by Drugs Targeting Late Endosome-Golgi Trafficking Pathway
M. F. SAEED, R. A. DAVEY; Univ. of Texas Med. Branch, Galveston, TX.

Zaire ebolavirus (ZEBOV) causes highly fatal hemorrhagic fever in humans, posing public health, ecological and bioterrorism threats. While good vaccine candidates exist, none are yet licensed for use and broad-spectrum drugs like ribavirin are ineffective. Conventional drug development has focused on targeting virus factors. However, high virus mutation rates mean that virus escape is inevitable. Instead, targeting host cell processes for which a virus is dependent may offer a feasible alternative and provide a way to design broad-spectrum antiviral agents that are not so easily overcome. Cell signaling molecules and other cellular factors offer potentially useful targets as many of them play important roles in various steps of virus replication. In recent work, we defined the requirements for ZEBOV entry into cells in terms of PI3K activation and viral induction of macrophagocytosis that allows access to the endocytic pathway of the cell. Some of the signaling proteins identified are feasible drug targets but suffer from being cytotoxic. In an effort to identify additional, host targets for intervention we have been studying later trafficking steps in virus uptake. It was evident that ZEBOV particles were being trafficked deeper into the cell than the late endosomes from which most viruses escape to enter the cell cytoplasm. Here, we show that ZEBOV moves into compartments close to or including the Golgi. The evidence for this unusual trafficking step is based on observations that virus particles colocalize with Golgi markers at the time of membrane fusion. Additionally, drugs that disrupt vesicle translocation into and out of the Golgi complex, as well as expression of dominant-negative form of Rab22a, that controls this step, inhibit ZEBOV infection at the point of entry into the cytoplasm. We will present this work together with our analysis of a novel set of drugs that target the host and block these late, crucial steps in ZEBOV infection.
2000 mg/kg and the no observable effect level (NOEL) in non-human pri-
mates measured at 300 mg/kg. Drug substance and drug product processes
have been developed and commercial scale batches have been produced
using processes that conform to current Good Manufacturing Practice (cGMP)
guidelines. Human clinical trials have shown that ST-246 is safe and well tol-
erated in healthy human volunteers. To establish the effective human dose,
pharmacokinetic and pharmacodynamic modeling was used to correlate
plasma drug levels in humans to plasma drug levels in nonhuman primates
that were infected with either monkeypox or variola virus. These data were
used to determine the human dose that produces plasma drug exposures in
the range predicted to be antiviral. These data support the use of ST-246 as
therapeutic to treat pathogenic orthopoxvirus infections of humans.

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Broad Spectrum Antiviral Therapeutic Based on Iminosugar
Derivatives

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Introduction: Iminosugars inhibit processing of viral glycoproteins and im-
pair viral assembly, secretion and infectivity. They inhibit ER α-glucosidases
I/II, which trim oligosaccharide chains as part of the folding process of many
viral envelope glycoproteins. This prevents glycoproteins from entering the
calnexin/calreticulin folding cycle and results in misfolding and degradation.
Iminosugars provide a host-based antiviral approach with potential for effi-
cacy against a broad range of viruses and the ability to limit the emergence
of drug resistance. Methods: Iminosugars included compound UV-4 and
analogs. Activity was tested against representative targets from 10 virus fami-
lies: arena, bunya, corona, filo, flav, orthomyxo, pox, toga, hepadna and
troviridae. Reduction of viral titer was determined by plaque assay. Com-
ponds showing optimal potency and safety were tested in vivo against
dengue in AG-129 mice and influenza in Balb/c mice by oral gavage. Results:
Iminosugars showed activity in vitro against a broad spectrum of virus fami-
lies: 9 of 10 families tested. in vitro, the compounds showed IC50 values from
0.3uM to >250 uM. in vivo, the compounds were safe and one compound
resulted in therapeutic protection when given orally TID up to 4 days post
challenge of H1N1 influenza and in a 1 day post Dengue-2 challenge. Con-
clusion: The iminosugar platform shows promise for developing broad spec-
trum, host-based antiviral drugs. The broad-spectrum antiviral effects could
be applied to multiple known viral diseases or new, engineered viral threats.
Targeting host-cell pathways critical for production of infectious virus, imi-
osugar antivirals limit the emergence of drug-resistant viral mutants.
**Focus Session**

**001. Clinical Research on Biodefense Pathogens: Epidemiology and Diagnostics for Brucellosis and Immune Response to Anthrax**

Sunday, February 6, 2011 | 12:00 PM – 2:00 PM

**001**

**DoD Sponsored Cooperative Biological Research: Existing Program and Future Direction**

WILL CHAPMAN, MS; DTRA, Ft. Belvoir, VA.

The Defense Threat Reduction Agency’s Cooperative Biological Engagement program (CBEP) has worked for the past decade building capacity across the former Soviet Union (FSU) to detect and respond to outbreaks of dangerous disease. These investments have substantially raised the surveillance capacity of the region. Efforts now need to shift to ensuring that this capacity is being put to its full use in assist the region in the control of these dangerous diseases. Fully leveraging this capacity will require broad regional partnerships that bring together both government and nongovernmental partners.

**002**

**Molecular Epidemiology of Brucellosis in the Former Soviet Union**

MIKELJON NIKOLICH, PhD; Walter Reed Army Inst. of Res., Silver Spring, MD.

**Background:** Brucellosis, one of the most widespread and important zoonoses globally, is a bacterial disease of livestock and wildlife with high infectivity in humans that can produce a high disease burden on countries with large rural populations and limited control programs. **Methods:** The Defense Threat Reduction Agency’s Biological Threat Reduction program includes collaborative efforts in multiple countries of the Former Soviet Union where brucellosis is a significant endemic problem, including Kazakhstan and Georgia, to improve surveillance with tools such as molecular epidemiology. Initial work in Kazakhstan and Georgia used genetic typing by Multilocus Variable Number Tandem Repeat Analysis (MLVA) to characterize *Brucella* isolates from livestock and human patients that were geolocated and analyzed using Geographic Information Systems. **Results:** The combination of MLVA and GIS in a region of Kazakhstan indicated a broader genetic diversity in strains isolated from livestock, with a limited genetic diversity apparently transmitted to humans. One genetic cluster was only found in human isolates, indicating a potential need for improved animal brucellosis surveillance. MLVA using the same 8 VNTR loci indicated that distinct genetic groups of *Brucella melitensis* are circulating in Georgia and Kazakhstan. **Conclusions:** Initial MLVA results combined with GIS provide glimpses into brucellosis transmission patterns in Kazakhstan and Georgia and point to areas of potential focus for the improvement of surveillance programs.

**003**

**Clinical Evaluation of Diagnostic Tests for Brucellosis**

TAMUNA AHKVLEDIANI, MD; Techn. Management Company, Tbilisi, Georgia.

Brucellosis is an infectious disease caused by gram-negative bacilli of the *Brucella* species. It is responsible for substantial morbidity in Georgia. *Brucella* species are also considered potential biological weapon agents. Brucellosis raises many challenges for health care providers, including the difficulty of diagnosis because of the lack of ideal diagnostic techniques. In this presentation, we will review our experience in evaluating various diagnostic platforms for brucellosis infection, with a discussion on similarities and differences with previously published results. Our observations suggest that the tube agglutination test, used for laboratory confirmation of brucellosis infection in Georgia infection requires careful re-evaluation. We will highlight the applicability of different diagnostics tests during the time course of brucellosis infection. The diagnosis of Brucellosis requires complex approach, using different diagnostic tests in parallel to the clinical information.

**004**

**Immunity to Anthrax: A UK Perspective**

HUGH DYSON, MD; Defence Sci. and Techn. Lab., Salisbury, United Kingdom.

To be efficacious, a vaccine must generate immune memory of key antigens of an organism or its toxins, such that subsequent exposure stimulates a rapid inactivating response. Development of BW vaccines has traditionally relied on using animal efficacy models to test likely candidates, followed by confirmatory measurements of surrogate markers (usually antibody titers) in healthy human volunteers. We have assessed T-cell memory in recipients of the licensed UK anthrax vaccine AVP and compared this with memory responses in patients with cutaneous anthrax, in Turkey and Georgia. This approach can provide information that enhances understanding of human immune responses to an organism, and aids optimisation of the use of already licensed vaccines (such as AVA and AVP). Furthermore, novel vaccine candidates that show promise in animal models can be tested (with techniques such as ELISpot) in humans previously exposed, by natural infection or vaccination, to antigens derived from the organism concerned.

**005**

**Clinical Data and Immune Response: Cutaneous Anthrax Review**

TINATIN KUCHULORIA, MD; Tbilisi State Univ., Tbilisi, Georgia.

Approximately 30-60 cases of cutaneous anthrax occur every year in Georgia, which has allowed for the accumulation of clinical experience through observations and systematic study. We will discuss the Georgian experience with cutaneous anthrax cases, comparing our results to both historical and current publications about this infection. We will review clinical presentation, recent developments in diagnostic testing, and the currently prescribed treatment modalities during the presentation. Insights into cutaneous anthrax are important so that diagnostic testing and treatment are optimized. Additionally, there is also opportunity to explore the relationship between the pathogen, the route of infection, and the immune response.

**006**

**Immune Responses to Anthrax in Humans**

NINO TRAPALIDE, PhD; Natl. Ctr. for Diseases Control and Publ. Health, Tbilisi, Georgia.

*Bacillus anthracis* considered to be a substantial threat as a biologic weapon. Using strategies to boost the immune response to *B. anthracis* exposure, such as vaccination, can counteract the impact of this threat. Thorough understanding of the immune response to anthrax infection will contribute to development of more effective boosting strategies that remain a priority in biodefense countermeasures. We will present our findings obtained in humans with natural infection of cutaneous anthrax, as well as in recipients of various types of anthrax vaccines. In particular, we will review the long-term immune responses to anthrax infection or vaccination explored by modern immunologic techniques. Our observations, suggest that there may be previously unrecognized differences between anthrax vaccines. This discussion provides substantial insight for anthrax vaccine and immunodiagnostic development.
002. Impact of Modern Molecular Typing Methods and Whole Genome Sequencing on Bioforensics Epidemiology and Improved Diagnostics of Infectious Diseases Caused by Select Agents
Sunday, February 6, 2011  |  12:00 PM – 2:00 PM
Diplomat Ballroom

008

Focus Session

Set-Up and Current Work of the European Biodefense Laboratory Network
MATS FORSMAN, PhD; Swedish Defence Res. Agency, Umeå, Sweden.

The objective of this project is the establishment and management of a strategic European Biodefence Laboratory Network (EBLN) to increase European preparedness for protection against Biological Warfare Agents (BWA). In the case of an event, indicating the use of BWA, unmistakable identification of the agent has to be performed. The forensic proof of use of these agents must be such that it cannot be refuted. This is a very demanding task that needs increased multinational collaboration. Twelve countries, Austria, Belgium, Czech Republic, Finland, France, Germany, Italy, Netherlands, Norway, Poland, Spain and Sweden, participate in this project. The project involves the selection of reference strain collections, design and construction of a shared database with high-resolution typing data which is necessary common resource for unmistakable typing and identification of B-agents. Many different technological approaches to BWA typing are employed. Compilations of data give opportunities to compare different identification and typing methods on the same strains and judge the best method to be employed for different investigatory questions. Some examples of comparisons of methods for typing purposes will be presented.

009

Whole Genome Sequencing as a Rapid High Resolution Diagnostic Typing Tool When Tracing Bioterror Organisms in the Food and Feed Chain

Background: The rapid technological development in the field of parallel sequencing offers new opportunities when tracing and tracking microorganisms in the food and feed chain. Today, sequencing efforts are no longer restricted to one representative from each species. Thus, we are now getting information on whole genome variability within species and subspecies groups as well as between them. Especially for the highly pathogenic bacteria, many strains from the same species have been sequenced. Methods: This study is focused on how to use parallel sequencing for rapid phylogenetic analysis and screen for genetic modifications. A bioinformatic methodology has been developed to rapidly analyze sequence data with minimal post-processing. The method defines orthologous regions instead of orthologous genes and the average similarity of the core genome is calculated. Sequence regions with high diagnostic values for a certain group or subgroup can also be selected and used as high specificity biomarkers. Results: Whole genome sequence data is used to measure and give an overview of genomic variability within high pathogenic species groups and to quantify distances to closely related species. The genetic material that discriminates different groups is quantified and analyzed.

010

Typing of Yersinia pestis in a Forensic Context
DAVID M. WAGNER, PhD; Northern Arizona Univ., Flagstaff, AZ.

Various tools are available to type Yersinia pestis in a forensic context. A new global phylogeographic analysis of Y. pestis was recently published that utilized more than 900 single nucleotide polymorphisms (SNPs). As a result, these SNPs can be used to quickly place an unknown strain into a global context. Importantly, all of these SNPs can be analyzed in an unknown strain using next-generation sequencing approaches. If enough material is available a whole genome sequence almost certainly would be generated in a forensic scenario. If sequencing is not possible, trace DNA can still be analyzed using assays targeting key SNPs in the global phylogeny, which has recently been demonstrated using material obtained from humans that died during the historical plague pandemics. Variable number tandem repeat (VNTR) markers can be used to understand the relationship among very closely-related strains, especially when coupled with genetic database containing VNTR fingerprints for multiple strains. I will discuss how these tools can be used to evaluate unknown samples and identify the most likely source from a set of possible sources.

011

Typing Francisella tularensis in a Forensic Context
ANDERS JOHANSSON, MD, PhD; Umeå Univ., Umeå, Sweden.

Background: The talk will be focused on genetic characterization of the infectious agent Francisella tularensis and how to translate the results into an evidence value. Recently, a number of genotypic methods, ranging from relatively insensitive methods to full genome sequencing, have been used to investigate genetic diversity within F. tularensis. Results: F. tularensis is a pathogen of low sequence diversity with genomic pair-wise average nucleotide identities >99.2% across subspecies. Nonetheless, genetic subpopulations intermediate to the subspecies and strain level have been identified within F. tularensis by several different typing methods. These genetic subpopulations have been associated with differences in disease severity and geographic distribution. Conclusions: Information on natural epidemics of tularemia in humans and the frequency of different F. tularensis subpopulations is essential for estimating the evidence value of a genotyping result. With ample reference data available, a likelihood ratio for two competing propositions can be numerically determined, e.g., “the causative microbial strain is naturally epidemic” versus “some other strain caused the outbreak.” Quantifying the evidence value of microbial genotyping results allows for evaluation with respect to the other inputs that are prevalent in outbreak investigations, or in court.

012

Next Generation Sequencing Applications Toward Pathogen Genomics, Detection, and Characterization
PATRICK CHAIN; Los Alamos Natl. Lab., Los Alamos, NM.

Since their entrance in the sequencing market, the impact of next generation sequencers has been felt throughout most spheres of biology. For pathogen research, these next-gen platforms were first used to expand the repertoire of genome sequences in the database, in both draft and finished formats. With the throughput of next-gen sequencing, novel pathogen sequencing applications have arisen, including single cell genomics, transcriptomics (digital gene expression or RNAseq), and metagenomics. The rapid detection and characterization of pathogens from environmental and clinical samples is important for informed treatment options. We have been developing a pipeline for assembly and analysis of both reads and assembled contigs for the pur-
poses of identifying and characterizing potential pathogens within the sample. Given that quantities of DNA may be limiting for any given sample, we have also been exploring the use of sub-microgram quantities of DNA as well as small numbers of cells for amplification and sequencing. Some of these experiments and results will be discussed.

**003. Adapting the Public Health Model of Outbreak Investigation for Biodefense**

Sunday, February 6, 2011 | 2:15 PM – 4:15 PM
Diplomat Ballroom

**Surveillance, Detection and Investigation of Foodborne Disease Outbreaks in the United States**

**JOHN J. GUZEWICH, MPH; FDA, College Park, MD.**

**Background:** Local, State and Federal public health and regulatory agencies make up the complex foodborne disease outbreak surveillance, detection and response system in the United States. This presentation describes this system as well as the challenges in operating surveillance among approximately 3,000 local, approximately 75 state and 3 federal agencies that have key roles. **Methods:** Description of the components of the system and how they operate. **Results:** Governmental agencies at the local, state and federal levels constantly strive to improve the speed of the foodborne disease surveillance system to mitigate the impacts of foodborne illness. The Conference to Improve for Foodborne Outbreak Response (CIFOR) provides a model that might be useful for improving bio-defense systems. CIFOR is made up of representatives from local, state and federal agencies and professional organizations representing the key players in the foodborne disease surveillance, detection and response system: epidemiology, laboratory and environmental health/food regulatory. **Conclusions:** The challenges of operating foodborne disease surveillance in the US provide some insight into challenges that might be faced in developing and improving bio-defense surveillance in the US and approaches that might be used to address those challenges.

**015**

**The Armed Forces Health Surveillance Center Global Emerging Infections Surveillance and Response System**

**MATTHEW C. JOHNS, MPH; Armed Forces Hlth. Surveillance Ctr., Silver Spring, MD.**

**Background:** The United States Armed Forces Health Surveillance Center’s served an important role for the US military in response to the 2009 H1N1 pandemic. This was accomplished through a comprehensive response effort conducted in the days, weeks and months after the onset of the first influenza pandemic of the 21st Century. **Methods:** Disease tracking and epidemiologic support through a laboratory-based sentinel surveillance network and electronic, encounter-based medical systems for military service members and beneficiaries around the world. **Results:** Utilizing the assets of a global laboratory-based network of influenza surveillance sites, a robust electronic surveillance infrastructure and a team of subject matter experts in the field of military public health, the AFHSC mounted a comprehensive response package to the 2009 H1N1 pandemic. In the first wave of the 2009 pandemic (April-August 2009), the AFHSC tracked and monitored the global spread of the pandemic in over 4,000 DoD military health system beneficiaries in 14 different countries. Systems were developed or modified for case tracking, influenza vaccine coverage, effectiveness and safety monitoring and included reporting of findings to leadership within the DoD and US Government and the global health community at large. **Conclusions:** In the first influenza pandemic in the 21st century, the recently formed Armed Forces Health Surveillance Center of the US Department of Defense, served in a central coordinating role for US service members around the world.

**017**

**Responding to Plant Biosecurity Threats: A Role for Forensic Plant Pathology**

**JACQUELINE A. FLETCHER, PhD; Oklahoma State Univ., Stillwater, OK.**

A sound and safe agricultural system is critical to national security, but U.S. crops, a cornerstone of our nation’s economy, are vulnerable to emerging and introduced pathogens, the latter of which can be introduced naturally or intentionally. A comprehensive national biosecurity plan must include both prevention and preparedness. In cases where intentional introduction is known or suspected, microbial forensic technology is needed to determine the
source of the pathogen and provide evidence for attribution. Strategies include (a) assuring high stringency for all assays (validation, confidence, statistical significance, consistency); (b) tracing pathogen origin and movement; (c) estimating the timing and site of initial introduction; (d) identifying the perpetrators; (e) providing evidence for criminal attribution; and (f) maintaining links to law enforcement and security communities. Training and educational programs are needed to assure a pipeline of scientists familiar with both the agricultural and forensic sciences. These efforts will strengthen our agricultural enterprise regardless of whether challenges to our crops arise naturally, accidentally, or intentionally.

018

Reconstructing Microevolution and Global Spread of Bacterial Plant Pathogens

BORIS A. VINATZER, PhD; Virginia Tech, Blacksburg, VA.

Background: The history of our civilization has been strongly influenced by our ability to produce crops. Today, US agriculture is threatened by many diseases that are either already affecting our agriculture or may do so in the near future. We thus need to avoid introduction of new plant pathogens and, at the same time, be prepared if such an introduction were to occur anyway. Preparedness includes the acquisition of knowledge on how pathogens have spread around the world in the recent past in order to model how new pathogens may spread once they are introduced into the USA. Methods: To this end, we have sequenced the genomes of isolates of bacterial plant pathogens that have a global distribution. Correlating their phylogeny based on whole genome sequences with time of isolation and geographic location of isolation we are reconstructing their routes of global spread. Comparing gene content and allelic differences of virulence genes we also study their role in pathogenesis. Results: Data for three bacterial plant pathogens will be presented: Pseudomonas syringae pv. tomato, Ralstonia solanacearum R3B2, and P. syringae pv. actinidiae. P. syringae pv. tomato has been found to be a genetically monomorphic pathogen frequently exchanged between Europe and North America but not between Africa and South America on one hand and Europe and North America on the other. Some alleles of genes involved in plant-pathogen interactions have only been found in South America highlighting the importance of reducing strain movement between geographic areas. For R. solanacearum R3B2 and P. syringae pv. actinidiae single nucleotide polymorphisms and differences in gene content have also been identified and we have started to reconstruct how these pathogens were recently introduced into Europe. Conclusions: Genome sequencing of many isolates of bacterial plant pathogens can help unravel their routes of global spread and their recent evolution to prepare for their possible introduction into the USA.

019

Influence of Epidemiological Characteristics of Arboreal Pathosystems on Development of Mitigation Strategies

TIM GOTTWALD, PhD; USDA, Fort Pierce, FL.

Where Huanglongbing (HLB) exists, commercial citrus industries decline owing to inadequate control methods. HLB increase and regional spread are related to vector populations and inoculum dispersal and are rapid compared with other arboreal pathosystems. Disease dynamics result from multiple simultaneous spatial processes, suggesting that psyllid vector transmission is a continuum from local to very long distance. Multiple asymptomatic infections per tree, incomplete systemic distribution within trees, and prolonged incubation period make detection difficult and greatly complicate disease control. Visual detection is inadequate due to a prolonged and variable incubation period that leads to an underestimation of disease and no adequate methods currently exist to detect asymptomatic infections over the vast citrus acreage of most growing regions. However, disease spread and regional decline can be slowed with adequate vector control to reduce transmission and removal of HLB-infected trees to reduce inoculum sources as has been demonstrated in Florida and Brazil.

020

Tracking the Long Distance Movement of High Risk Plant Pathogens in the Atmosphere

DAVID G. SCHMALE III, PhD; Virginia Tech, Blacksburg, VA.

Improved technologies are needed to anticipate and respond to the introduction of high risk plant pathogens (HRPPs) into the United States. Many HRPPs are transported over long distances in the atmosphere. The ability to track the movement of HRPPs in the atmosphere is essential for establishing effective quarantine measures and forecasting disease spread. Atmospheric transport models have been used to predict the long distance transport of plant pathogens, but these models often fail to incorporate actual measurements of spore concentrations along proposed particle trajectories. We developed autonomous (self-controlling) unmanned aerial vehicles (UAVs) to track the movement of HRPPs in the atmosphere tens to hundreds of meters above crop fields. These UAVs are equipped with unique sporesampling devices that are opened and closed by remote control from the ground. Data collected with our UAVs is considered in the context of a new aerobiological framework based on atmospheric transport barriers.

Keynote Session

005. Keynote Session Sunday, February 6, 2011 | 4:30 PM – 6:00 PM Regency Ballroom

021

The Eradication of Smallpox: A Continuing Saga

DONALD A. HENDERSON, MD, MPH; Ctr. for Biosecurity of UPMC, Baltimore, MD.

This past year, the world celebrated the thirtieth anniversary of the declaration that smallpox had been eradicated. It was a triumphant celebration of a remarkable achievement of public health and disease eradication as well as a testimony to the potential of cooperation through the World Health Organization. In 1974, the program itself was instrumental for launching the successful “Expanded Program on Immunization” which has been responsible for a 99% reduction in the incidence of poliomyelitis and, in the Western Hemisphere, in the interruption of transmission of measles and rubella viruses as well as poliomyelitis. Now on the agenda for consideration as to whether the known stocks of smallpox virus should be destroyed as a further step in mitigating the likelihood of smallpox ever recurring. Final decisions are due to be decided at the World Health Assembly in May of this year.
Smallpox Eradication

**006. Progress in Orthopoxvirus Research Post Smallpox Eradication**

Monday, February 7, 2011 | 8:30 AM – 12:00 PM
Regency Ballroom

**022**

**Poxvirus Biology: Targets of Immunity and Antiviral Therapy**

**BERNARD MOSS, MD, PhD; NIH/NIAID, Bethesda, MD.**

The poxviruses comprise a large group of double-stranded DNA viruses that replicate exclusively in the cytoplasm of eukaryotic cells. The most medically significant members of the family include: variola virus, the causative agent of smallpox; vaccinia virus, used as the vaccine to prevent smallpox; monkeypox virus, responsible for a smallpox-like disease in Africa and imported into the United States several years ago; and molluscum contagiosum virus, accountable for benign persistent skin lesions in young children and more serious infections in immunodeficient individuals. Variola virus, vaccinia virus and monkeypox virus belong to the orthopoxvirus genus and share basic mechanisms of replication, although there are some differences in immune defense genes. The large number of virus-encoded proteins required for entry into cells, expression and replication of the genome, and virion assembly and spread provide numerous targets for vaccine and drug development.

**023**

**Smallpox Preparedness: Review of Antivirals and Considerations for Use**

**INGER DAMON, MD, PhD; CDC, Atlanta, GA.**

Over the past 10 years, a remarkable acceleration of research to enhance public health preparedness for a possible reintroduction of smallpox has ensued. This presentation will briefly review facets of that work, and focus on studies evaluating orthopoxvirus disease antiviral efficacy, and strategies for use of these products now in advanced stages of development.

**024**

**Determinants of the Increasing Incidence of Human Monkeypox in the Democratic Republic of the Congo**

**ANNE W. RIMOH; Univ. of California, Los Angeles, CA.**

**Background:** Studies on the burden of human monkeypox in the Democratic Republic of the Congo (DRC) were last conducted from 1981-1986. Since then, the population that is immunologically naïve to orthopoxviruses has increased significantly due to cessation of mass smallpox vaccination campaigns. To assess the current risk of infection, we analyzed human monkeypox incidence trends in a monkeypox-enzootic region. **Methods:** Active, population-based surveillance was conducted in the Sankuru District in central DRC. Epidemiological data and biological samples were obtained from suspected cases. Cumulative incidence (per 10,000 population) and major determinants of infection were compared with data from active surveillance in similar regions from 1981 to 1986. Adjusted odds ratios were calculated for selected risk factors. **Results:** Between November 2005 and November 2007, 1234 cases of suspected human monkeypox were identified in participating health zones. 806 of these cases were confirmed as acutely infected with MPX virus by polymerase chain reaction (PCR). The average annual cumulative incidence across participating zones was 5.53 per 10,000 (2.18 - 14.42). Factors associated with increased risk of infection included: living in forested areas, male gender, age <15, contact with rodents and no prior smallpox vaccination. Comparison of active surveillance data in the same health zone from the 1980s (0.72 per 10,000) and 2006-07 (14.42 per 10,000) suggests a 20-fold increase in human monkeypox incidence. **Conclusions:** Thirty years after mass smallpox vaccination campaigns ceased, human monkeypox incidence has dramatically increased in rural DRC. Our findings suggest that vaccinated individuals are at reduced risk for MPX infection, particularly in unvaccinated populations. Improved surveillance and epidemiological analysis is needed to better assess the public health burden and develop strategies for reducing the risk of wider spread of infection.

**025**

**The HPA’s Role in Smallpox Vaccine Research**

**SIMON G. P. FUNNELL, PhD; Hlth. Protection Agency, Salisbury, United Kingdom.**

The UK’s Health Protection Agency has been funded by NIAID to develop, characterise and utilise a respiratory model of Monkeypox in cynomolgus macaques to support research aimed at finding a safer, yet similarly effective, vaccine against Smallpox. HPA-Porton Down has previously shown and reported that UK-bred cynomolgus macaques are highly susceptible to aerosolised Monkeypox. At a presented aerosolised dose of 10^5 pfu, Monkeypox Zaire 79 induces severe disease in all animals and results in pathology which consistently require euthanasia. In more recent studies, HPA-Porton Down has shown that a single dose of the replication competent Vaccinia vaccine (ACAM2000TM) or two doses of MVA vaccine are able to fully protect cynomolgus macaques from aerosolised Monkeypox challenge. A single dose of MVA was, however, not sufficient to provide complete protection. Both sets of protective immunisations provided complete protection from lethal disease, but differences were detected, in our virological assay outcomes, between the two vaccines.

**026**

**Susceptibility of Marmosets (Callithrix jacchus) to Monkeypox Virus**

**ERIC MUCKER; USAMRIID, Ft. Detrick, MD.**

**Background:** Although current nonhuman primate models of monkeypox and smallpox diseases provide some insight into disease pathogenesis, they require a high titer inoculum, use an unnatural route of infection, and/or do not accurately represent the entire disease course. In our studies, we altered half of the test system by using a New World primate species, the common marmoset. **Methods:** Adult male marmosets were intravenously infected with 2.4x10^7, 9.5x10^6, and 7.8x10^6, 5.0x10^6, 510, and 48 PFU. Clinical, hematological, and viral load data were assessed. **Results:** Animals succumbed to disease between 6 and 15 days post-infection, in a dose dependent manner. The animals exhibited signs of hemorrhage, had high genome viremia, and altered blood cell hematology. At the three lowest doses, rash was more demarcated and some short-lived macules were observed. **Conclusions:** As is, our model is roughly 6 logs lower than the current intravenous cynomolgus model and roughly 4 logs lower than the respiratory model. The aggressive nature of the disease manifested in these animals implicates an even lower lethal dose and warrants exploration of other infection routes that could alter the disease course to reflect clinical and subclinical phases indicative of smallpox. Also, these data should invoke consideration for variola experimentation in marmosets.
026a  
**Perspectives on the Development of Primate Models for Evaluating Countermeasures against Human Smallpox and Monkeypox**  
**PETER B. JAHRLING, PhD; NIH/NIAID, Ft. Detrick, MD.**

**Background:** The potential for variola virus to be exploited as a bioterrorist weapon is widely understood. In addition, the re-emergence of monkeypox as a public health concern has increased the urgency of developing improved countermeasures, including vaccines and antiviral drugs, for these orthopoxviruses. It is generally recognized that animal models will be needed to demonstrate efficacy of these countermeasures and that for pivotal studies in compliance with the Food and Drug Administration “animal rule” (US 21CFR310.610), challenge with authentic variola may be required. **Methods:** Cynomolgus macaques were exposed to graded doses of variola via the intravenous route. The resulting disease patterns were evaluated by conventional virology, PCR, microarrays, immunohistochemistry, and a variety of clinico-pathologic parameters. **Results:** Various combinations of variola virus doses and routes of exposure in primates (cynomolgus monkeys) lead to predictable disease patterns that replicate some, but not all, features of human smallpox. Although the models require further refinement, they have been adequate to demonstrate the efficacy of several candidate antiviral drugs, including cidofovir and ST-246. **Conclusions:** It is likely that no single combination of conditions will result in a model that will simultaneously satisfy all of the criteria required under the Animal Rule; different models may be required to assess different indications. Special attention should also be paid to finding biomarker patterns that could be used in a clinical setting as triggers for early intervention, thus increasing the likelihood of successful intervention and facilitating the licensing of countermeasures. Although much of this developmental work can be accomplished using surrogate orthopoxviruses in rodents and primates, increased confidence in countermeasures against variola virus can be obtained only by efficacy testing in primate models using variola virus.

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**Symposium Session**

**011. Coxiella burnetii: An Emerging Biothreat**  
**Monday, February 7, 2011 | 4:15 PM – 6:15 PM**  
**Diplomat Ballroom**

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**125 Q Fever in the Netherlands: Queries Raised and Queries Answered**  
**YVONNE VAN DUYNHOVEN, PhD; Nat. Inst. of Publ. Hlth. and the Environment, Bilthoven, Netherlands.**

In the Netherlands, from 2007 onwards an increasing number of human Q-fever cases have been reported, with about 170 cases in 2007, 1000 cases in 2008 and over 2300 cases in 2009. Before 2007, 10-20 cases were recorded annually. In 2010, following stringent measures, including the culling of bearing goats at bulk-milk positive farms and mandatory vaccination of small ruminants, the number of human cases decreased, although the about 500 cases were still exceeding the normal frequency. Preceding (since 2005) and parallel to the increase in human Q-fever cases, Q-fever abortion waves were observed in about 30 dairy goat farms and, to a lesser extent, dairy sheep farms, with farms matching the geographical area of the human cases. This unprecedented large-scale outbreak triggered an extensive, multidisciplinary research portfolio. The research focussed on 1) assessment of the magnitude of the human and animal public health problem due to Q-fever and identification of non-human reservoirs; 2) identification of risk factors and transmission routes for Coxiella burnetii in human and animals; 3) evaluation of intervention measures and 4) improvement of preparedness and response of the Q-fever and future zoonotic outbreaks. More recently, several clinical studies have been implemented to study more in-depth the incidence of long-term sequelae, the prognostic characteristics of the bacterium and the host (response) and optimization of diagnostic tools. During the ASM meeting a selection of some of the main findings in research areas 1 to 3 will be presented.

126  
**The Clinical Spectrum of Q Fever**  
**THOMAS J. MARRIE, MD; Dalhousie Univ., Halifax, Canada.**

Q fever is the name given to a number of illnesses caused by Coxiella burnetii. Most individuals acquire this organism through inhalation. It is so infectious that a single inhaled microorganism can cause disease. The most common manifestation is self-limited fever — thus most individuals in a community do not know that they have had Q fever. Seroprevalence in the general population in North America is the range of 5 – 10%. Pneumonia, endocarditis, hepatitis and Q fever during pregnancy are the most common manifestations. Q fever can occur sporadically or as outbreaks. It is a zoonosis with the most common animal reservoirs being cattle, sheep and goats. In some areas cats and rarely dogs serve to spread this infection to man. There are extensive wildlife reservoirs of Q fever in North America. With the exception of endocarditis, mortality is uncommon. Q fever is classified as acute (fever, pneumonia and hepatitis) or chronic (endocarditis and Q fever in pregnancy). Treatment of acute Q fever with doxycycline or a fluoroquinolone is usually successful. The treatment of chronic Q fever is complex and should be under the direction of some experienced in the treatment of chronic Q fever.

127  
**Q Fever in the USA: A Rare Infection or a Bioweapon in Your Backyard?**  
**ROBERT MASSUNG, PhD; CDC, Atlanta, GA.**

**Background:** Coxiella burnetii, the causative agent of the zoonotic disease Q fever, is an obligate intracellular bacterium with worldwide distribution. Bacteria are shed primarily in the urine, feces, milk and birth products of livestock such as goats, sheep and cattle, with human infection by inhalation of aerosolized organisms. Despite Q fever being a notifiable disease in the USA, fewer than 200 cases are reported to the CDC per year. **Methods:** The prevalence of Q fever in the USA was evaluated by seroprevalence studies on the general population, among practicing veterinarians, and in residents of a region with a high reservoir (cattle) population. The presence of C. burnetii in the environment was evaluated by the collection and molecular analysis of environmental samples from 6 states across the USA. **Results:** Recent studies have shown 3.1% seroprevalence in the general US population among adults > 20 years of age, 22.2% among practicing veterinarians, and 10.8% in residents of west Texas. Molecular testing of > 1,600 environmental samples collected across the USA showed 23.8% to be positive for C. burnetii. **Conclusions:** These results suggest that C. burnetii is quite common in the environment and that Q fever is underreported in the USA primarily due to a lack of physician awareness and diagnostic capacity.

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**Phylogeography and Subtyping of Coxiella burnetii**  
**TALIMA PEARSON, PhD; Northern Arizona Univ., Flagstaff, AZ.**

Coxiella burnetii is found throughout the world, is ubiquitous in domestic food producing animals and is classified as a select agent. Despite this, large scale sample collection has been hindered by culturing, handling, and regu-
Programmable Bio-Nano-Chips and Advanced Diagnostic Devices for the Point of Need

NICOLAS CHRISTODOULIDES, PhD; Rice Univ., Houston, TX.

Our group has sustained efforts for the implementation of bio-nano-chip (BNC) sensors for a number of important clinical applications. The first class of miniaturized BNCs is a “chemical processing unit” that involves an array of chemically-sensitized bead “microreactors” within a micro-fluidic cell. Instead of handling electrical signals passing through conductors this technology processes fluids so as to provide a digital proteomic fingerprint of complex biological fluids. The second class of BNC sensor is a “cellular processing unit” based on a membrane capture element integrated into a fluids structure servicing cell identification, characterization and enumeration. For non-invasive testing BNCs are now being applied in six clinical trials targeting ovarian, prostate and oral cancers, trauma, drugs of abuse and cardiovascular disease. This work is culminating in the creation of a testing infrastructure, a universal analyzer with functional integrated mechanical/optical interfaces, and a flexible microchip architecture that may be adapted for biodefense-relevant applications at the point of need.

Q Fever and War: Past, Present, and Future

JOSHUA D. HARTZELL, MD; Walter Reed Army Med. Ctr., Washington, DC.

Q fever caused by Coxiella burnetii is a highly infectious worldwide zoonosis. It has a myriad of presentations including a flu-like illness, pneumonia, and hepatitis which can present diagnostic challenges to providers especially in settings of limited resources. Q fever has impacted military campaigns since World War II and has long been considered a potential agent of bioterrorism/biowarfare. Although rare in the US, there have been over 200 cases reported among military personnel deployed to the Middle East. The management and follow-up of these cases have increased the understanding of Q fever in this relatively young and healthy population. We will review these cases and highlight areas where research is needed to improve the diagnosis, treatment, and prevention of disease. The role of Q fever as a potential agent of biowarfare will be discussed.
In the diagnosis of acute respiratory and diarrheal illnesses as well asdescribe ongoing applications of the Virochip for outbreak investigation andviral discovery.

134
Rapid Detection and Functional Assay for Butulimum Toxins
SHASHI K. SHARMA, PhD; FDA, College Park, MD.

Over the past decade, a multitude of new detection methods for *C. botulinum* toxins have been introduced into bioresearch and the bio-defense community, all based on the premise that the detection of *C. botulinum* would be comparable to mouse bioassay. Furthermore, a few of these methods would now be leveraged as high throughput detection for counter terrorism. Devising detection measures for botulinum toxins has always puzzled researchers. This is perhaps due to its versatile nature, different targets, and numbers of serotypes, subtypes, and variations in the immunological response amongst serotypes. Few truly novel detection methods have made it far along the development and commercialization path because of these advances. We report the development of a functional cell based assay, utilizing a fluorescence reporter construct using full length SNAP-25 as the linker, stably expressed in neuronal cell model that more closely approximates the in vivo model. This cell-based assay is designed to replicate BoNT’s cell-binding, cellular internalization, endosomal translocation, and protease activities. Our data indicates that the pc-12 cells have significant sensitivity to BoNT/A and E action and the assay can detect as little as 100 pM BoNT/A within living cells. Development of this assay would further limit the animal use in biodefense applications.

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Recombinant Antigen-Based Immunodiagnostics for Lassa Fever
ROBERT F. GARRY, PhD; Tulane Univ., New Orleans, LA.

Background: Lassa fever (LF) is a severe, often fatal viral hemorrhagic fever (VHF) that is of major public health importance in Sierra Leone, Nigeria, Guinea, Liberia and other countries in West Africa. There is a need for sensitive, reliable, simple, and cost-effective LF diagnostic assays that can be performed in resource-poor settings, and can also be readily deployed and implemented in the event of a deliberate release. Methods: A comparison of traditional BSL-4 antigen-capture and immunoglobulin M-capture enzyme-linked immunosorbant assays with recombinant antigen-based ELISA was initiated to determine whether or not recombinant LAVS proteins produced in Escherichia coli and mammalian cells, and antibodies raised to the recombinant proteins can circumvent the need for a BSL-4 laboratory in diagnosis of LF. BSL-4 LF ELISA similar to assays previously employed by the CDC and USAMRIID were re-established both to diagnose LF patients at the Lassa Ward at Kenema Government Hospital in Sierra Leone and to serve as a standard for comparison to the recombinant ELISA under development. The BSL-4 and recombinant LF ELISA were evaluated on patient serum collected from febrile patients admitted to the KGH Lassa Ward. Results: The LF Ag- and IgM-capture ELISA using recombinant LAVS proteins produced in E. coli or mammalian cells have sensitivity and specificity comparable to or exceeding traditional BSL-4 antigen-capture and immunoglobulin M-capture enzyme-linked immunosorbant assays based on LAVS grown under BSL-4 conditions. LF IgG-capture assays based on recombinant LAVS proteins have also been developed that can assist in determining whether a patient is experiencing acute LAVS infection or has had a past infection and can also be used in surveillance, ecology and natural history studies of LF. The recombinant antigen-based ELISA have been adapted to a lateral flow immunoassay format that will permit point-of-care diagnosis of LF. Conclusions: LF immunodiagnostic assays using recombinant LAVS proteins can be developed with sensitivity and specificity comparable to currently available assays based on LAVS grown under BSL-4 conditions.

135
Organ Specific Regulation of Innate Immunity: Implications and Applications
EYAL RAZ, MD; Univ. of California, La Jolla, CA.

Innate immunity is paramount for host survival. It employs germline-encoded pattern recognition receptors (e.g., TLR) to recognize signature microbial compounds. Numerous in vitro and in vivo studies indicate that TLR triggering activates an inflammatory cascade, launches a quick anti-microbial reaction and directs adaptive immunity to mount a protective response. However, these studies fall short in explaining how innate immunity operates in organs such as the gut or skin, which are laden with symbionts rather than pathogens. Organ compartmentalization of host-bacterial interaction leads to different defense strategies of innate immunity, that is, the innate immune game played by the spleen is very different from that played by the lung and both are very different from that played by the colon. This topic of organ-specific innate immunity is the focus of this presentation.

136
Differential Host Response for Control of Francisella tularensis in the Lung and Spleen
REBECCA V. ANDERSON, PhD; Rocky Mountain Lab/NIAID/NIH, Hamilton, MT.

*Francisella tularensis* is a Gram negative, facultative intracellular pathogen. Lethal *F. tularensis* infections are a result of replication at the site of infection followed by wide spread dissemination of the bacteria to internal organs, and finally into the blood. Since the bacterium is required to replicate at the site of infection for several days prior to dissemination there are two sites of host-pathogen interaction that must be considered when developing novel vaccines and therapeutics, the site of inoculation and peripheral organs. To address these issues, we have dissected the cellular components responsible for controlling virulent *F. tularensis* in different organs following either vaccination or antibiotic therapy. Our evidence suggests that at early time points after infection, limitation of bacterial replication in the lung and spleen in vaccinated animals is mediated by different host effector cells. In the lung, control is associated with NK cells, whereas T cells are consistently associated with limiting bacterial replication in the spleen. Understanding the underlying mechanisms for compartmentalization of these responses and the specific mechanisms by which these cells participate in protection may impact design of novel vaccines and therapeutics for pneumonic tularemia and will be discussed herein.

137
Salmonella and the Gut Mucosal Barrier: Dichotomies of Help and Harm
MANUELA RAFFATELLU, MD; Univ. of California, Irvine, CA.

*Salmonella typhimurium* is a leading cause of inflammatory diarrhea. The gut inflammatory response has the important function of containing *S. typhimurium* infection and preventing dissemination of this pathogen to systemic sites (barrier function). On the flip side of the coin, pathogens like *S. typhimurium* have evolved to survive in an inflamed gut in spite of the host
defense mechanisms. Thus, while some aspects of inflammation are beneficial to the host, others are exploited by *S. typhimurium* to achieve greater colonization when competing for a niche with other microbes. In my talk, I will discuss which responses control *S. typhimurium* dissemination from the gut, thus providing a benefit to the host, and which ones may favor *S. typhimurium* colonization, thereby benefiting this pathogen.

### 138

**Immune Mechanisms in Dengue Virus Infection—Protection Versus Pathogenesis**

ALAN L. ROTHMAN, MD; Univ. of Massachusetts Med. Sch., Worcester, MA.

Infection with any of the four serotypes of dengue virus (DENV) can produce a mild illness or a more severe vascular leakage syndrome termed dengue hemorrhagic fever (DHF) that is associated with high circulating levels of various cytokines. Secondary DENV infection—infected in a previously exposed individual—is one of the strongest risk factors for DHF. Secondary DENV infections involve viral serotypes heterologous to the previous DENV infection. DENV-specific memory T cells induced by the prior DENV infection recognize heterologous DENV antigens during secondary infection. However, sequence variation between viruses induces quantitatively and qualitatively different effector responses. Immunologic studies of blood samples collected prior to and during acute dengue illness reveal profiles associated with either elevated or reduced severity of infection—subclinical vs. mild vs. severe illness. These studies support a model in which pre-existing memory DENV-specific T cells, along with other viral and host factors, determine the clinical outcome of DENV infection. Identification of the critical immunopathogenetic determinants of dengue disease should lead to targeted interventions and improved vaccines.

### 143

**Blocking Botulism: A Journey into Modules and Modulators**

MAURICIO MONTAL, MD, PhD; Univ. of California, La Jolla, CA.

Botulinum neurotoxin (BoNT), the causative agent of botulism, is acknowledged to be one of the most feared biological weapons of the 21st century (CDC Category A). The imminent threat of a terrorist attack empowered by BoNT remains an issue of major concern nationally and globally. For BoNT, a modular nanomachine, functional complexity emerges from its modular design and the tight interplay between its component modules—a partnership which effector proteins pass to reach the cytosol. In principle, blocking such channels should prevent transport of the effectors and thereby inhibit toxin action. In this presentation I will describe current understanding of the structure and function of the anthrax toxin channel, formed by the protective antigen moiety, and indicate some ways that have been found to block the channel.

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**Plugging Toxin Channels**

JOHN COLLIER, PhD; Harvard Univ. Med. Sch., Boston, MA.

Many intracellularly acting toxins form transmembrane channels (pores) through which effector proteins pass to reach the cytosol. In principle, blocking such channels should prevent transport of the effectors and thereby inhibit toxin action. In this presentation I will describe current understanding of the structure and function of the anthrax toxin channel, formed by the protective antigen moiety, and indicate some ways that have been found to block the channel.

### 145

**Models of Viral Assembly: Ebola and Lassa**

ERICA OLLMANN SAPHIRE, PhD; The Scripps Res. Inst., La Jolla, CA.

We will present crystal structures that illustrate three different viral assembly proteins. I. Ebola virus has a filamentous morphology, which is governed by the viral matrix protein VP40. VP40 is capable of existing in a number of different oligomeric states. However, a structure of full-length VP40 in an oligomeric state or the arrangement of VP40 within viral matrix has yet to be elucidated. Here, VP40 crystal structures from two ebolavirus species, accompanied by biochemical evidence demonstrate that VP40 is a dimeric protein that assembles into linear filaments. Analysis of the VP40 crystal packing, corroborated by several microscopy techniques, validate the VP40 dimer as the basic building block of the ebolavirus matrix. II. The arenaviral nucleoprotein, NP, has been implicated in suppression of the host innate immune system, but the mechanism by which this occurs has remained elusive. Our structure of the immunosuppressive domain illustrates that NP is a member of the DEDDh family of exonucleases. Accompanying biochemical experiments demonstrate that NP indeed has a previously unknown, bona fide exonuclease activity, with strict specificity for dsRNA substrates. We further demonstrate that this exonuclease activity is essential for the ability of NP to suppress translocation of interferon regulatory factor-3 and block activation of the innate immune system. III. The matrix protein Z of the arenaviruses is necessary and sufficient for viral egress from the cell and plays an important role in the suppression of both viral and host cell translation. Z has a RING-domain, which is important for the self-assembly properties of the protein. In order to gain insights into how LASV Z self-assembles, we solved the structure of the oligomeric form of LASV Z, which provides insights into how the Z might be organized within the viral particle.
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Atomic Model of the Human Adenovirus by cryoEM

ZHONG ZHOU, PhD; UCLA, Los Angeles, CA.

Construction of a complex virus may involve a hierarchy of assembly elements. In this talk, I will present the structure of the whole human adenovirus virion at 3.6Å by cryo-electron microscopy (cryoEM) and the in situ atomic models of three minor capsid proteins (IIIA, VIII and IX), extensions of the major (penton base and hexon) proteins. Our model reveals the interactions within three protein-protein networks. One network is mediated by protein IIIA within Group-of-Six (GOS) tiles — a penton base and its five surrounding hexon - at vertices. Another is mediated by ropes (protein IX) that clench hexons to form Group-of-Nine (GON) tiles and bind GONs to GONs. The third, mediated by IIIA and VIII, binds each GOS to five surrounding GON tiles. Optimization of adenovirus for cancer and gene therapy could target these networks.

Symposium Session

020. Optimizing Clinical Care of Viral Hemorrhagic Fevers

Tuesday, February 8, 2011 | 4:15 PM – 6:15 PM Diplomat Ballroom

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Current Medical Care of Patients with Viral Hemorrhagic Fevers

GIUSEPPE IPPOLITO, MD; Natl. Inst. for Infectious Diseases Lazzaro Spallanzani, Rome, Italy.

The management of viral hemorrhagic fevers (VHF) have focused on the application of infection control measures. Standard clinical interventions, offered to critically ill patients with other life-threatening infections, have largely not been applied. The course of VHF can be divided in three phases: incubation, pre-coagulation impaired, and coagulation impaired phases. The first phase begins with exposure to the virus; immunization and antiviral molecules interfering with viral entry could be effective. In phase 2, viral replication occurs in monocytes with spreading in the bloodstream; antiviral drugs that block viral replication or small RNA molecules that interfere with the transcription of viral genes are most likely to be effective. The increasing release of pro-inflammatory cytokines determines the third phase. Immune modulating agents could be considered at this stage: steroids, inhibitors of activated protein C or of different coagulation factors could be assessed. With the support of WHO, a standardized clinical protocol that integrates comprehensive care for ill patients while allowing for the compassionate use of experimental therapeutics is a feasible goal.

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Improving Clinical Care for Hemorrhagic Fever Viruses

JAMES V. LAWLER, MD; NIAID, Ft. Detrick, MD.

The full syndrome of viral hemorrhagic fever (VHF) is associated with a high mortality rate. While caused by an array of RNA virus infections that have diverse clinical presentations and mechanisms of pathogenesis, severe diseases that manifest as hemorrhagic fever has a more conserved set of symptoms and signs. Whether these similarities predict common interventions or approaches to management that may reduce morbidity and mortality across the broad VHF syndrome is unclear. We know little about the optimal management of severe infection with any of the hemorrhagic fever viruses. Research regarding prophylaxis and treatment generally occurs in animal models, but a lack of correlation between animal model and human disease often makes translation of results to clinical practice difficult. Unfortunately, the sporadic nature and endemic areas for most of these diseases creates challenges in generating sufficient clinical data for these specific diseases. In this talk, we will discuss strategies for clinical research relevant to improving the management of VHF, and we will outline the approach that we are attempting at the NIAID Integrated Research Facility at Ft. Detrick.

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Ebola Virus Survivors from Bundibugyo, Uganda

DANIELLE V. CLARK, MPH; Integrated Res. Facility, NIAID, Ft. Detrick, MD.

Infection with Ebola virus typically results in a nonspecific initial presentation, progressing to hypotension, coagulation disorders, vascular permeability, and hemorrhage. The clinical course varies depending on the strain of the infecting virus. The long-term health effects of EBOV infection have not been well described. We conducted a case-control study to evaluate the occurrence of long-term health effects in survivors of Bundibugyo ebolavirus. Survivors were significantly more likely to report ocular deficits, hearing loss, depressed mood, difficulty sleeping, severe fatigue, neurologic abnormalities, and joint stiffness controlling for age and gender. Ebola survivors also reported significant limitations in their physical and mental function, compared to controls of similar age and gender. Survivors reporting melena during their acute Ebola virus infection were 5.8 times more likely to report hearing loss than survivors without melena. This study provides information on the long-term sequelae following Ebola virus infection, which may improve patient care for survivors as well as potentially contribute to our understanding of the mechanisms of pathogenesis of this infection.

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Profiling the Human Humoral Immune Response to Ebolavirus Sudan Gulu

LESLIE LOBEL, MD, PhD; Ben Gurion Univ., Beersheva, Israel.

Ebola virus is a member of the family Filoviridae, and the cause of Ebolavirus hemorrhagic fever (EHF). During the last decade, intensive research on the human immune response to ebolavirus has been performed, however the profile of specific humoral immune reactivity to viral proteins and the role of specific antibodies and epitope targets in recovery from disease is poorly understood. We have conducted a pilot study to characterize the human humoral immune response against the individual viral proteins of ebolavirus Sudan (strain Gulu). The aim was to examine the profile of humoral immune reactivity in both survivors of ebolavirus infection and those that perished from the disease. To this end, genes for all seven ebolavirus Sudan (Gulu) proteins were cloned and sequenced. Construction and expression of full-length recombinant proteins consisting of the gene products of ebolavirus Sudan Gulu strain was performed, as well as a thorough analysis of sera immunoreactivity, to the recombinant proteins, from survivors and non-survivors of the ebolavirus Sudan outbreak in 2000-2001 in Uganda. This work contributes to our understanding of humoral immunity during ebolavirus infection. Studies in progress to identify specific epitope targets of the native human immune response will support development of protective and/or therapeutic antibodies for ebolavirus infection.
**TUESDAY INVITED SPEAKER ABSTRACTS**

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**Development of an Immunoprotectant for Ebola Virus Infections**

**GENE OLINGER;** USAMRIID, Ft. Detrick, MD.

Ebola, a hemorrhagic fever virus (HFV), causes severe morbidity and mortality during episodic outbreaks in regions where it is endemic. In addition, there are concerns that HFVs could be used in a bioterror attack. No prophylaxis or therapy for Ebola currently exists. We have produced both CHO cell-derived and N. benthamiana-derived immunoprotectant product consisting of three previously described Ebola virus monoclonal antibodies (Wilson et al. Science 2000). Continued efforts have demonstrated potential mechanisms of protection in vitro and in the mouse model of Ebola Zaire. In addition to these data, the results of challenge studies in non-human primates and progress in cGMP manufacturing of the mAb product will be presented.

**Symposium Session**

**021. Molecular Discovery of Virus-Host Interactions**

Tuesday, February 8, 2011 | 4:15 PM – 6:15 PM
Palladian Ballroom

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**Strategies to Discover Small Molecules as Probes of Virus-Host Interactions**

**COLLEEN B. JONSSON, PhD;** Univ. of Louisville, Louisville, KY.

High-throughput screening (HTS) approaches for the identification of small molecule therapeutics have been appreciated and utilized by pharmaceutical and biotechnology companies, and more recently, the academic sector. With the availability of high quality commercial libraries, sequencing of the human genome, technology developments, and cheminformatic tools, HTS can now be realized as a drug discovery tool for a wide array of diseases. Clearly, one advantage of HTS over earlier approaches in drug discovery lies in its ability to enable surveys of a wider chemical space for the discovery of novel scaffolds and accelerate the identification of potential lead compounds. Industry trends reveal HTS as a valuable resource for increasing the number of new leads which move into preclinical phases. In this short seminar, I will review some general concepts in HTS, current approaches and resources available for HTS of viruses, and the progress of in HTS in discovery of promising leads for viral infectious diseases.

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**Genetic Strategies to Investigate Host-Virus Interactions**

**ABRAHAM L. BRASS, MD, PhD;** Ragon Inst. at Massachusetts Gen. Hosp., Charlestown, MA.

The outcome of the struggle between the host cell and an invading virus pivots upon several key factors: viral exploitation of cellular resources, the opposing actions of host cell restriction factors, and viral countermeasures that aim to neutralize the host’s defenses. We have performed genetic screens for host factors that modulate the replication of three human pathogens, HIV-1, influenza A virus, and hepatitis C virus (HCV). These studies significantly enriched for macromolecular complexes and biologic pathways with both known and previously unappreciated connections with viral pathogenesis. In addition, the genetic screen to find modulators of influenza A virus replication resulted in the discovery of a new intrinsic immune factor, the IFITM protein family, which blocks viral infection. We have focused on investigating the actions of these antiviral restriction factors which are critical in defending host cells against influenza A virus, as well as the pathogenic flaviviruses, dengue virus and West Nile virus. In this presentation I will discuss our experiences using genetic strategies to study the pathogenesis of HIV and influenza virus, including screen design, caveats, and interpretation, as well as the role of IFITM3 in restricting influenza A virus infection in vitro.

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**Knock Down Approaches Identified Kinases and Phosphotases as Host Targets for Multiple Highly Pathogenic Viruses**

**SINA Bavari, PhD;** USAMRIID, Ft. Detrick, MD.

RNA viruses from the families Arenaviridae, Bunyaviridae, Paramyxoviridae and Filoviridae encode protein repertoires consisting of only several mature proteins yet are among the least of known pathogens. How do these viruses outmaneuver human host cells which encode thousands of proteins? It is clear that these viruses are able to hijack their hosts, suppressing innate immunity and providing molecular machinery for virtually all aspects of their lifecycles. This dependency could be exploited and used to develop anti-viral therapeutics by targeting essential host factors. Further, if host factors are conserved between viral families, broad-spectrum therapeutics could be developed. Our long-term research objective is to establish a pipeline for the identification and validation of essential/restrictive host factors that will serve as targets for broad-spectrum anti-viral development. Using a multi-pronged approach consisting of phospho-proteomics, siRNA gene silencing and chemical library screening, we have identified multiple common host kinases and phosphatases essential for infection of several viral families which can be therapeutically targeted. During my talk I will discuss 1) our approaches to identification of pathways which are modified by host “traitor” kinases and phosphatases, and 2) how alteration of these signaling proteins can be used to correct the balance in favor of the host during viral infections.

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**Identification of Small Molecules that Suppress MicroRNA**

**KUAN-TEH JAEONG, MD, PhD;** NIH, Bethesda, MD.

**Background:** There are approximately 1,000 microRNAs identified in the human genome. MicroRNAs have been shown to serve roles in development, gene regulation, metabolism and many physiological processes. Currently, it remains incompletely understood whether microRNAs function to defend cells against viral infection. **Methods:** We have investigated microRNA profile changes in PBMCs from individuals infected with HIV-1 compared to those from uninfected controls. **Results:** We find signature changes in the microRNA pattern in cells from infected individuals, and we demonstrate evidence that microRNA expression can modulate viral replication in cells. **Conclusions:** Our findings agree with the hypothesis that host cell microRNAs could modulate viral replication in vivo.

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**Development of ER Targeting Liposomes for Potential Use in Broad Spectrum Antiviral Therapy**

**NICOLE ZITZMANN, PhD;** Univ. of Oxford, Oxford, United Kingdom.

Antiviral iminosugars target both host cell factors like endoplasmic reticulum (ER) alpha glucosidases, as well as virally encoded proteins such as the Hepatitis C Virus (HCV) ion channel p7. In cases where delivery of free drug is insufficient to achieve physiologically relevant therapeutic concentrations, liposomes can serve as cargo delivery vehicles. In addition, certain liposo-
mal formulations can act as cholesterol lowering agents which have antiviral properties in their own right. Viruses that depend either on calnexin-mediated folding of their envelope glycoproteins and/or on cholesterol to complete successful infection cycles, present targets. Amongst others these include human immunodeficiency virus (HIV), hepatitis B virus (HBV), HCV, dengue and influenza A viruses.

**Symposium Session**

**022. Innate Immunity and the Host Response to Infection**

Tuesday, February 8, 2011 | 4:15 PM – 6:15 PM
Ambassador Ballroom

**249 The Nucleotide-Binding Domain, Leucine-Rich, Repeat-Containing Protein (NLR) Family and Select Agents**

**JENNY P. Y. TING, PhD; Univ. of North Carolina, Chapel Hill, NC.**

The nucleotide-binding domain and leucine-rich-repeat-containing (NLR) family of molecules mediate host immunity to various pathogenic stimuli. In humans, this is comprised of a 20+ member gene family. Proteins within the NLR family show remarkable conservation in evolution and are preserved from plants to animals. They also bear structural similarities to the death-inducing molecule, APAF-1. These proteins regulate various key signaling pathways, such as NF-kB, MAPK, caspase activation, interferon production, apoptosis and necrosis. We will discuss the roles of NLRs in host response to microbial pathogens that are of interest to biodefense and emerging infections. Both host defense and viral anti-host mechanisms will be discussed. The link of NLRs with bacterial as well as viral diseases will be reported. Together, these data place NLRs as key regulators of immune signaling and infection that would impact biodefense and emerging infections.

**251 IL-1 Cytokines During Mycobacterium tuberculosis Infection**

**KATRIN MAYER, PhD; NIH, Bethesda, MD.**

IL-1beta and IL-1R1 are critical for host control of *Mycobacterium tuberculosis* (Mt) infection. We recently observed that mice deficient in IL-1alpha succumb rapidly to low dose aerosol infection with Mt, indicating that both IL-1alpha and IL-1beta are essential for host resistance to this pathogen. Interestingly, we found that IL-1alpha and IL-1beta were co-expressed in the same cellular subsets. Some of the IL-1-producing cells also expressed TNF-alpha, iNOS and/or IL-10, but these multifunctional cells were distinct from the IL-12/23 p40-producing population in the lung. We found that the IL-1-producing cells expressed surface markers downstream of IFN signaling and that expression of both IL-1 species was negatively regulated in vivo by Type I and Type II IFNs. These findings indicate that IL-1 in the lungs of Mt-infected mice primarily originates from unique multifunctional myeloid subsets which co-express other effector molecules critically involved in host resistance to Mt. Inhibition of IL-1 by IFNs might reflect an important mechanism for preventing detrimental side-effects of these potent pro-inflammatory cytokines.

**252 Subversion of Signal Transduction Pathways in Macrophages by Yersinia pestis**

**JAMES B. BLISKA, PhD; Stony Brook Univ., Stony Brook, NY.**

Pathogenic bacteria in the genus *Yersinia* use multiple virulence determinants to counteract innate immunity and facilitate infection. A type III system in *Yersinia* translocates an effector called Yop that elicits cell death in macrophages. Yop inhibits the production of survival factors in macrophages, causing them to die by apoptosis, which is generally considered to be immunologically silent. However, recent studies show that caspase-1, a key regulator of pro-inflammatory responses, is activated in *Yersinia*-infected macrophages undergoing apoptosis. How caspase-1 is activated during Yop-induced macrophage apoptosis is not known. We have identified a distinct isoform of Yop in *Y. pestis* (YopKIM) that induces high levels of apoptosis and caspase-1 activation in infected macrophages. The molecular basis for the increased activity of YopKIM was studied with the goal of better understanding the underlying mechanism of caspase-1 activation. The data show that YopKIM has two amino acid changes that give it an enhanced ability to inhibit survival signals in macrophages. The increased apoptosis that results appears to cause membrane permeability, efflux of ions and activation of caspase-1. Therefore, apoptosis of macrophages infected by highly virulent pathogens may not be immunologically silent.

**253 Bacillus anthracis Relies on Secretion of Anthrax Toxin to Impair the Scavenger Functions of Myeloid Cells to Establish Successful Infection**

**SHIHUI LIU, PhD; NIH, Bethesda, MD.**

Bacterial Toxins and Therapeutics Section, Laboratory of Bacterial Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

Anthrax toxin is the major virulence factor of *Bacillus anthracis*. Mice deleted for the anthrax toxin receptor CMG2 in only myeloid-lineage cells were generated to examine the roles of anthrax toxin targeting of macrophages, neutrophils, and other myeloid cells in anthrax pathogenesis. Macrophages and neutrophils isolated from the myeloid-specific CMG2-null mice were, as expected, resistant to anthrax toxin. Interestingly, the myeloid-specific CMG2-null mice remained fully sensitive to the lethality induced by both anthrax lethal toxin and edema toxin, demonstrating that targeting of myeloid cell types and tissues is not responsible for the lethality induced by anthrax toxin. Surprisingly, however, the myeloid-specific CMG2-null mice were completely resistant to *Bacillus anthracis* infection. Neutrophil depletion experiments suggested that *B. anthracis* relies on secretion of anthrax toxin to evade the scavenging functions of neutrophils and thereby successfully establish an anthrax infection. This work demonstrates that anthrax lethal toxin uptake through CMG2 and the resulting impairment of myeloid cells, probably specifically neutrophils, is essential to anthrax infection.
024. New Interventions and the Challenges of Emerging Infections

Wednesday, February 9, 2011 | 8:30 AM – 12:00 PM
Regency Ballroom

258 Emerging Arbovirus Disease in Central Asia
ROGER HEWSON, PhD; Hlth. Protection Agency, Salisbury, United Kingdom.

Arthropod borne viruses are global security challenges. At the least, they account for enormous burdens of disease, severely affecting economies and generally lowering the quality of life. In the extreme, they can threaten international relationships and regional stability. Research on arboviruses in relation to their geographic distribution and nature of human disease has underpinned our knowledge and scientific understanding. Nevertheless, vast geographical regions are still poorly understood. As a region, Central Asia is an intersection of international security issues and scientifically important arboviral disease. The collapse of the former Soviet infrastructure left a legacy of capable scientists, inadequately maintained collections of pathogenic microbes, poor disease surveillance and unchecked disease emergence. Collectively these issues give the impression of Central Asia as either a source of disease or a cross-roads for infectious agents. Our research in this area has provided a snap shot of virus activity in this part of the world.

259 The Q Fever Epidemic in the Netherlands, Risk Factors and Lessons Learned
ROEL COUTINHO, MD PhD; Netherlands Ctr. for Infectious Disease Control, Bilthoven, Netherlands.

In 2007 an outbreak of Q fever occurred in a village in the Netherlands. This was first considered as an isolated incident. But in 2008 1000 human Q fever cases were reported in a much larger rural area surrounding the village and in 2009 a further increase in a wider area was observed resulting in a total number of 2350 cases reported in that year. Pneumonia has been the predominant clinical presentation with about 20% of reported cases being hospitalised. Epidemiological studies identified abortion storms in large goat farms as the most likely source of infection. Dairy goat farming in the country started about 10 years ago with the number of goats increasing from 7500 in 1983 to 375,000 in 2009. Strict hygienic measures in affected goat farms appeared to be insufficient to stop transmission. End 2008 vaccination of goats with an unregistered phase-I vaccine was started and further expanded in 2009. Additional measures were taken at the end of 2009 including culling of all pregnant goats on Q fever positive farms as identified by positive PCR in bulk tank milk. In 2010 the number of reported Q fever cases declined. Different aspects of this very large — possibly the biggest in the world — epidemic will be discussed including host and environmental risk factors for infection, longitudinal detection of C burnetii in aerosols and the high risk for culling workers despite protective measures taken.

260 Emerging Challenges in Antimicrobial Resistance: Resistance in Foodborne Pathogens
PATRICK MCDERMOTT, PhD; Food & Drug Admin., Laurel, MD.

After decades of widespread antibiotic use in agriculture and medicine, many bacterial pathogens of human and animal origin have become resistant to numerous antimicrobial agents. Resistance patterns frequently result from the acquisition of external genes that may confer resistance to an entire class of antibacterial compounds. These genes are frequently associated with large transferrable DNA elements, on which may reside other mobile components. A broad array of resistance genes may accumulate on a single mobile element, presenting a situation in which extensive multiple antibiotic resistance can be acquired in a single genetic event. In the foodborne pathogen Salmonella, plasmid-mediated resistance can be extensive, and shared by common mechanisms with other enteric bacteria recovered from animals and foods. Historical susceptibility and genetic data show how this resistance has accumulated over time leading to the current genetic arrangements underlying different resistance patterns. Successful management of current antimicrobials, and the continued development of new ones, is vital to protecting human and animal health.

261 Emergence of Tick-Borne Encephalitis Viruses
ALAN BARRETT, PhD; Univ. of Texas Med. Branch, Galveston, TX.

Background: The disease tick-borne encephalitis (TBE) is caused by three genetically and serologically related subtypes of TBE virus, which are classified in the mammalian tick-borne virus group of the genus Flavivirus. The subtypes are termed central European, far eastern and Siberian subtypes, which are found in Europe, Asia, and the former Soviet Union. Methods: With improving surveillance and better diagnostic approaches the increasing magnitude of TBE as a public health problem is becoming apparent Results: The incidence of TBE disease, in particular morbidity, has been increasing in many areas with the viruses and disease expanding its geographic area and becoming endemic in new regions. This includes movement of the virus in to Northern Europe and westwards in to Japan and Korea. Many factors contribute to emergence of TBE. These include expanding tick populations because of climate change, social and behavioural changes, and changes in land use and leisure activities. Conclusions: There are effective inactivated vaccines to control different subtypes of TBE virus, which will be important for the future control of this disease.

262 Anthrax: Investigation of a New Presentation
TIM BROOKS, PhD; Hlth. Protection Agency, Wiltshire, United Kingdom.

Since December 2009 the UK has seen 52 cases (17 deaths) of a new form of anthrax affecting injecting drug users. All cases had injected heroin, mainly into muscle. Presentations varied from self limiting local lesions diagnosed by seroconversion, through serious soft tissue infections with massive local oedema to overwhelming sepsis with systemic toxicity. Two cases presented as sudden cerebral haemorrhage. Although all the features can be readily related to the pathology caused by B. anthracis and its toxin, none of the presentations resembled the classic forms of cutaneous, inhalational or gastro-intestinal anthrax. Diagnosis was performed using culture, PCR and toxin and anti-toxin assays. Treatment centred on surgery and antibiotics including ciprofloxacin and clindamycin, with a few patients receiving anthrax immune globulin. Infection control procedures for hospitals, mortuaries and forensic units were developed.
Anthrax: Experimental Therapeutics

STEPHEN MORRIS, PhD; Biomedical Advanced Res. and Dev. Authority, Washington, DC.

The Office of the Biomedical Advanced Research and Development Authority (BARDA) within the Office of the Assistant Secretary for Preparedness and Response in the Department of Health and Human Services is committed to creating a robust and dynamic pipeline of candidate medical countermeasures (MCMs) consisting of vaccines, therapeutics, antimicrobials, and diagnostics for chemical, biological, radiological, and nuclear (CBRN) threats as well as pandemic influenza, and new and emerging infectious diseases. In response to the threat of bioterrorism using anthrax, the United States Government (USG) has developed a preparedness strategy that includes a three-pronged approach: antibacterials, vaccines, and antitoxins. In 2005, BARDA awarded contracts to Cangene Corporation and Human Genome Sciences for the development of anthrax antitoxins. The scope of these contracts includes clinical and nonclinical studies necessary for licensure, and the manufacture and delivery of specified numbers of treatment courses to the Strategic National Stockpile. Through NIH contracts awarded in 2007 and 2008 to Emergent BioSolutions, Elusys, and PharmAthene, BARDA is funding the advanced development of four additional anthrax antitoxins to mitigate the risks inherent in product development. One of these contracts (Elusys) has transitioned to BARDA management and is currently funded to support studies necessary for licensure. All of these products are pursuing licensure under the FDA’s Animal Rule.
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1) Login with your ASM eStore user name and password. If you do not have a user name and password, select the “Need Help Logging In?” link.

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**Important Dates in 2011**

**Registration/Housing**
- **April 3**: All housing reservations must be made
- **April 11**: Advanced registration closes
- **April 12**: Onsite registration rates activate

**Abstracts**
- **February 25**: Abstract withdrawal deadline
- **February 26**: Abstract presenters of record will be notified of abstract status
- **February 28**: Dispositions emailed to presenting authors
- **March 23**: Late-Breaker abstract deadline

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**Important Dates in 2011**

**Registration/Housing**
- **March 14**: Registration and housing open

**Abstracts**
- **May 6**: Abstract submission deadline
- **June 1**: Late-Breaker abstract submission site opens
- **July 1**: Travel grant nominations deadline
- **July 29**: Late-Breaker abstract submission deadline

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