Diagnostic Technology Update-
Strategic Development and Deployment

NIAA Annual Meeting
FMD Symposium
Dr. Sarah Tomlinson
USDA, APHIS, VS, NVSL
National Animal Health Laboratory Network
Overview of Presentation

• Partners
• Process
• Scope of Projects
• Status of Key Projects
• Opportunities and Challenges
‘Partners in Preparedness’

- Multiple partners have been working together to improve our Nation’s preparedness for adverse animal health events.

- Significant progress has been made in addressing identified preparedness gaps through collaborative efforts and projects.

- New tools in our preparedness and response toolkits will require new, creative approaches to policies related to testing protocols, notification expectations, investments, and funding.
Safeguarding Animal Health

National Veterinary Services Laboratories

National reference veterinary laboratories
• Ames, IA and Plum Island, NY
The National Animal Health Laboratory Network

• Formed in 2002
• State, University and Federal veterinary diagnostic labs that deal with diseases of animals including endemic, exotic, zoonotic, and emerging diseases
• Is a partnership between:
  – USDA (APHIS and NIFA)
  – AAVLD
  – NAHLN Laboratories
• Early detection
  – Targeted surveillance based on population density & risk
• Rapid response
  – Surge capacity to test outbreak samples
• Appropriate recovery
  – Large numbers of samples tested to show freedom
USDA Agricultural Research Services

USDA’s chief scientific research agency

Safeguarding Animal Health
DHS Partnerships

• **DHS Science and Technology**
  – Chemical/Biological Defense (CBD) Division
    • Dr. Michelle Colby, Agricultural Defense Branch Chief
    • Dr. Angela Ervin, Program Manager, CB R & D
  – Office of University Programs – National Centers of Excellence for Zoonotic and Animal Disease Defense (ZADD)
    • National Center for Foreign Animal and Zoonotic Disease Defense (FAZD) – Dr. Tammy Beckham, Director
    • Center of Excellence for Emerging and Zoonotic Diseases (CEEZAD) – Dr. Juergen Richt, Director – no current projects but expected in future
  – Office of National Laboratories
    • Support for PIADC modifications/infrastructure (ex: high throughput laboratory)
  – National Bio Forensic Analytical Center (NBFAC)
    • Collaboration with DHS, FBI and USDA
  – Other collaborations include Integrated Consortium of Laboratory Networks (ICLN), foreign animal disease modeling, 3D, countermeasures development, NVS Steering Committee, and Foreign Animal Disease Threat (FADT) Working Group

• **Office Of Health Affairs** (ICLN expected to transfer here)
DHS – Lead the unified national effort to secure America... protect against and respond to threats and hazards to the Nation
√ S&T: RDT&E to Support DHS Mission

USDA - Provide leadership on food, agriculture... based on sound public policy, the best available science, and efficient management
√ Strategic Objectives for U.S. Agriculture:
  - Enhance International Competitiveness
  - Enhance Protection and Safety

PIADC
To Protect U.S. Agriculture from the threat of FADs

Programs
√ USDA APHIS FADDL – FAD diagnostics, NAHN reference lab, FAD training, NAFMDVB
√ USDA ARS FADRU – Generates scientific knowledge for FAD prevention, control and recovery - basic research, rapid diagnostics, countermeasures
√ DHS S&T – Translational product development of countermeasure discoveries for FAD

Operations
√ Provide outstanding service to science
√ Protect people, property and the environment
√ Be a trusted and valued employer and neighbor
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Ag Screening Tools Workshops

FAZD/DHS sponsored

• AST I – November 2010
  - Defined agricultural screening tools, evaluated current status, and identified and discussed the gaps and needs.

• AST II – April 2011
  - Obtained industry perspectives and input on diagnostic screening tools for transboundary, emerging, and zoonotic diseases.

• AST III – October 2011
  - Gathered input on lab-related concept of operations -- use of diagnostic assays during an outbreak, laboratory operations, and prioritization of samples and reagents.

• AST IV – May 2012
  - Review by NAHLN Coordinating Council and other stakeholders of laboratory-related policies including use of PCR, milk, ELISA and penside assays, prioritization of samples, biosafety requirements, and validation of assays during an outbreak.
Gaps Identified through the AST Workshops (I-IV)

**Diagnostics**

- Penside tests for FADs
- Portable technologies for FAD field diagnosis
- Multiplex assays
- DIVA assays
- High-throughput testing
- FMD RT-PCR in milk

**Assay Validation & NAHLN**

- Serology and molecular HTP diagnostic platforms deployable to the NAHLN
- Methods for sample collection, preservation, and transport
- NAHLN deployable assays for use with pooled samples
- FMD RT-PCR in various sample types
- Wildlife samples
- Policy for validation during outbreaks
Gaps Identified through the AST Workshops (I-IV)

**NAHLN laboratories**

- Laboratory capacity estimation
- Policy and laboratory concept of operations in laboratories
- Data management

**Field**

- Streamline VS Guidance 12001.1 (formerly VS Memo 580.4)
- Tools and training for Foreign Animal Disease Diagnosticians (FADDs)
Technology Transition

Needs Assessment

Gap identification
- Recommendations from stakeholder meetings
- ID of emerging disease
- Increased risk of trans-boundary disease
- Research

Gap assessment and prioritization
- Assess the need and feasibility for deployment

Determine implementation goals
- Surveillance plan
- Funding
- Training
- PT and reference materials

NAHLN Methods Technical Working Group Processes

Receive and review proposals to address specific gaps
- Validation
- Methods comparison
- Consistent with OIE guidelines
- Analytical and diagnostic sensitivity and specificity
- Repeatability

Dossier Review
- Available samples
- Fitness for purpose
- Fitness for use

Recommendations provided to VS
- Decisions made on use
- FADI
- Surveillance for early detection
- Response
- Recovery

Implementation

- Develop testing algorithm
- Finalize surveillance plan
- Finalize response and notification plans
- Distribute SOPs
- Provide training
- Provide reference materials
- Provide proficiency tests
- Determine funding mechanism
- Determine data collection
- Finalize guidance documents
- Communication across all involved stakeholder groups
- Monitor performance of assay
- Review effectiveness of surveillance program
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Scope of Projects

Assay Development
- FMD 3ABC ELISA
- FMD 3D ELISA (DIVA)
- FMD LPBE
- CSF Competitive ELISA
- Isothermal LAMP assay
  - Pan-mycoplasma
  - Capripox
  - Lumpy skin disease
- Panviral microarrays
- Multiplex platforms
- Microfluidics

Assay Validation
- FMD Penside test (SVANODIP® FMDV-Ag)
- Detection of FMDV in bulk tank milk
- Ropes for collection of oral fluid [sample preservation & transport in FTA paper and multiplex Dx]
- New assay chemistries and platforms

Enhancing Capability and Capacity
- Diagnostic support
  - Vesicular Ag ELISA reagent production
  - Monoclonal production - ASF
  - FMD antisera bank
  - High Throughput
  - Forensics
- Vaccine Bank
  - Transboundary
  - Vaccine matching
- Laboratory Capacity Estimation Model
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Assay Development and Validation
Serologic Capability

• Serologic Capability deployable to the NAHLN for FMD:
  
  ➢ Short-term:
    
    ➢ Evaluation and Validation of Current 3ABC NSP ELISA (PrioCHECK®)
      – Interlaboratory comparison, negative cohort and proficiency testing planned for Spring/Summer 2013
    
    ➢ Collaboration with FAZD on their development of new 3ABC NSP ELISA with equal/improved performance

  ➢ Longer Term:
    
    ➢ Develop new nonstructural protein ELISAs – 3ABC and 3D
    
    ➢ Advantages – faster (not overnight incubation), more amenable to high throughput conversion, additional confirmatory options (replace old assays), U.S. manufactured
    
    ➢ Expected completion: 2014
Evaluation of Penside Diagnostics for FMD

SVANODIP® FMDV-Ag

- **Project goal**: gain an understanding of performance characteristics, utility, and limitations
- A negative cohort study has been completed with two NAHLN laboratories to better assess its specificity.
- The Svanova penside lateral flow test for FMD was found to be rapid (minutes) and appears **most suitable for directly sampled lesions** (tissue or vesicular fluid).
  - **Sensitivity** (comparable to AgELISA-Asia 1 and O)
    - Bovine 95%
    - Swine 97%
  - **Windows of detection**:
    - Bovine 1-11 dpi
    - Swine 1-10 dpi
    - ↓ for SATs
- **On-going Larger negative Cohort FAZD in 2013**
  - 7 laboratories and 500 samples from cattle, swine, small ruminants

Safeguarding Animal Health
Optimization and Validation of a real-time RT-PCR Assay for Rapid Detection of FMD Virus in Bulk Tank Milk

Collaboration: FADDL, FAZD, DHS, Pirebright, NAHLN laboratories

Objectives:
• Develop and validate an extraction and rRT-PCR for FMDV suitable for milk testing
• Validate on virus-spiked milk, milk from experimentally infected dairy cows (Pirbright, UK) and on international samples from countries endemic for FMD
• Deploy to the NAHLN and use in business continuity plans

Status: Interlaboratory comparison and negative cohort completed in 5 NAHLN laboratories

Estimated analysis completion: August 2013

Safeguarding Animal Health
Evaluations of oral fluid samples from swine as a potential new sample type for FAD diagnostics

- Evaluate rope collection of oral fluid from pigs as a new sample type for foreign animal disease diagnosis (FMD, CSF, and ASF)
- Develop a multiplex RT-qPCR for rapid surveillance of foreign (FMD, CSF, ASF) and endemic (SIV, PRRSV, PCV2, Mhyo) diseases in pigs
- Validate the use of Whatman FTA cards for oral fluid sample preservation and nucleic acid extraction.
- Collaboration with FAZD and TVMDL
- Status: Feasibility of rope collection for ASF, CSF and FMD completed. Multiplex assay is being optimized.
- Estimated completion: 2014

**Multiplex RT-qPCR**

**Pathogen targets**

- PCV2
- Mhyo
- ASFV
- CSFV
- FMDV
- PRRSV
- SIV
- Control
## Summary of Assay Development

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<th>ASF</th>
<th>Rinderpest</th>
<th>FMD Milk</th>
<th>FMD Serum</th>
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<td>FADDL</td>
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Enhancing Capability and Capacity
### Reagents and Vaccine Projects

#### FMD Antisera Bank
- Produce, collect, characterize, and standardize FMD polyclonal antisera to fulfill the needs for FMD diagnosis, assay validation, proficiency testing, and research

#### Transboundary Vaccine Projects
- Testing master seed and commercial available vaccines to determine cross protection

#### Pilot Study of In Vivo Cross-Protection for FMD Vaccines
- Evaluated cross-protection with three different A vaccines with challenge at 7 days; data analysis is underway
Laboratory and Field Preparedness

- FMD controls distributed to approved NAHLN laboratories for FAD investigation testing and preparedness
- Quality Management System Trainings to support
- NAHLN Portal
  - Secure mechanism for labs to share information
- Web-based exercises
  - Program to leverage and integrate existing tools for enhancing preparedness by regularly practicing outbreak response
- VS Guidance 12001.1– FAD investigations
- FADD Refresher Courses
Laboratory Capacity Estimation Model

- Collaboration between NAHLN/FAZD/AAVLD
- Software tool for evaluating/monitoring NAHLN capacity
  - Allows for labs to define their specific processes and identify their rate limiting steps
  - Allows for estimation of network capacity and sample allocation during an outbreak
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Opportunities with Progress in Addressing Gaps

• New Tools!
  - Serologic capabilities for FMD in NAHLN laboratories
  - Streamlined, simultaneous diagnostics for endemic and FADs (multiplex)
  - New sample types (milk and oral fluids) for screening and potential use in business continuity plans
  - Information about use of rapid field deployable diagnostics (penside)
Challenges with Progress in Addressing Gaps

- Availability of new assays in NAHLN laboratories
  - Need for policies, notification and communication with field and laboratory staff when to use/not use assays – deployment strategies
    - Surveillance vs. FAD investigations vs. routine diagnostic rule outs
- Technology transfer and commercialization, process for use of kits
- International collaborations for development and validation
Conclusions

• Partnership is key in identifying and filling many gaps in gathering stakeholders’ input and the diagnostic development and deployment processes.
Questions?

Safeguarding Animal Health