INSTITUTE FOR INFECTIOUS ANIMAL DISEASES
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Center of Excellence
for Emerging and Zoonotic Animal Diseases

Defining Requirements for International Field Trials for Conventional and Next-Generation Foot and Mouth Disease Virus (FMDV) Vaccines and Diagnostics

Washington, DC
June 10-12th, 2014

Workshop Report

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Executive Summary

This report describes the discussions, key findings, and recommendations that arose during a workshop held June 10th-12th, 2014 in Washington, DC, *Defining the Requirements for Performing International Field Trials for Conventional and Next-Generation Foot and Mouth Disease Virus (FMDV) Vaccines and Diagnostics*. Funding for this workshop was provided by the U.S. Department of Homeland Security Science and Technology Directorate, Homeland Security Advanced Research Projects Agency, Chemical and Biological Defense Division, Agricultural Defense Branch (DHS S&T HSARPA CBD), and participants included 49 personnel representing the National Center for Zoonotic and Animal Disease Defense (ZADD), DHS, U.S. Department of Agriculture (USDA), Pan African Veterinary Vaccine Centre (PANVAC), Commonwealth Scientific and Industrial Research Organisation (CSIRO), World Organisation for Animal Health (OIE), Food and Agriculture Organization of the United Nations (FAO), Pirbright Institute (UK), Kenya, Libya, Israel, Egypt, Turkey, Vietnam, academia (including international universities), animal health biopharmaceutical companies, and industry (full list of participants in Appendix A).

Objectives

The objectives of this workshop were to:

- Establish a common understanding of DHS goals and objectives for working with the global community on the further characterization of the FMD virus vaccine, live adenovirus vectored vaccine (hAd5-FMD) and VRMD 3B enzyme-linked immunosorbent assay (ELISA); Discuss logistical considerations and how to best succeed at the execution of an international field trial of the conditionally licensed live adenovirus vectored FMDV vaccine and VMRD 3B ELISA;
- Develop an approach to keep all interested stakeholders informed of the project progress in a transparent and timely manner;
- Identify potential field trial locations and partners;
- Establish recommendations for performing international field trials to include, but not be limited to:
    - Field trial processes, study protocols, test plans, evaluation, and regulatory requirements
    - Validation requirements for companion diagnostics
    - Concept of operations for testing;
- Develop a general understanding of the regulatory approval processes for vaccines and diagnostics in the U.S. and in countries potentially interested in collaborating on this project; and

- Discuss and understand the best approach to incentivize participation at the country and individual producer level.

Meeting Overview

The workshop was organized into presentations, large group discussions and smaller breakout groups. The first and second days of the workshop began with a series of presentations focused on: 1) the project and workshop goals; 2) regulatory processes and procedures for performing vaccine and diagnostic field trials in the U.S. and abroad; 3) challenges and opportunities for performing field trials in endemic countries; 4) the role of PANVAC in vaccine production and implementation in African countries; and 5) international regulatory requirements for FMD vaccine testing and evaluation. Each talk was designed around specific topics that were meant to form the foundation and starting point for follow on discussions. Large group discussions were held, largely at the end of each day, in an effort to summarize the recommendations/reports from individual breakout groups.

Topics covered in each of the four breakout sessions included:

- Concept of operations and infrastructure requirements for performing a vaccine field trial in an FMD endemic country;
- Regulatory considerations and requirements to consider when performing a vaccine field trial in an FMD endemic country;
- Experimental design for a vaccine field trial in an FMD endemic country; and
- Concept of operations and experimental design for evaluation of a DIVA (Differentiating Infected from Vaccinated Animals) diagnostic in an FMD endemic country.

Recommendations and discussions from breakout sessions were summarized during report out sessions during the workshop as well as during an expanded session the last day intended to capture overarching recommendations for design and implementation of an FMD vaccine and diagnostic field trial. A summary of the recommendations and next steps can be found in the next section. Additional details and a more in-depth discussion of the recommendations can be found in the sections that follow. The complete agenda can be viewed in Appendix B, with presentation abstracts in Appendix C. A set of expanded recommendations and an in-depth overview of the discussions that took place are captured throughout the remainder of this report.

Background

Vaccines are perhaps the most important tool for control of livestock diseases that pose a serious risk to animal and human health, threaten food security, and halt trade in animals and animal products[1]. At present, live and attenuated vaccines produced by classical methods make up the greatest majority of the vaccine market for high consequence livestock diseases; however, for FMDV, killed vaccines have been utilized for decades. Recent estimates from Hammond, *et al* indicate an estimated 2.35 billion doses of FMD vaccine are administered annually[2]. Killed FMDV vaccines have been utilized to effectively eradicate FMDV from the majority of Europe and many other parts of the world; however, limitations of these vaccines are partially responsible for the inability to achieve global FMD eradication and have often led to ineffective vaccine control strategies. For example, inactivated vaccines lack the ability to confer cross-protection between and within serotypes/topotypes, have a limited shelf life, may require re-vaccination every 4-12 months, and may have non-structural proteins in some formulations that can interfere with the ability to serologically differentiate infected from vaccinated animals[3].

The ability to meet national goals[4] for responding to an FMD outbreak and the ability to ensure successful outcomes for the OIE/FAO global FMD control strategy[5] will most likely require next generation FMD vaccine(s) that address(es) the aforementioned limitations. The ideal FMD vaccine platform will confer protection across serotypes (or have the ability to rapidly be updated with new cassettes according to the serotype/topotype present); provide a rapid onset and long duration of immunity; not be dependent on cold chain; and demonstrate clear DIVA capabilities.

Recent advances in vaccine delivery systems and recombinant technologies offer an opportunity to develop improved vaccines for transboundary animal diseases such as FMD. In fact, a second-generation FMD vaccine (hAd5-FMD vaccine) for serotype A/subtype A24 Cruzeiro, that can be safely manufactured within the U.S. has recently been developed and conditionally licensed by the USDA Centers for Veterinary Biologics (USDA CVB). Numerous hAd5-FMD monovalent vaccine candidates for major serotypes A, O, Asia-1 and SAT2 have been tested in cattle in proof-of-concept safety and efficacy studies and have been shown to be as effective as conventional (normal potency) vaccines against experimental FMD challenge[3]. Research has demonstrated that the hAd5-FMD A24/Cruzeiro vaccine has the ability to confer complete challenge protection in cattle after one dose by 7 days post-vaccination and has the necessary antigenic markers to allow DIVA testing[6-8]. In addition, a new DIVA assay that is currently under development (the VMRD 3B ELISA), has been shown in feasibility studies to be compatible for use with next-generation FMD vaccines, including the hAd5-FMD vaccine

platform. In initial bench feasibility studies, the performance of this 3B ELISA (VMRD) was equivalent to or better than the commercially available ELISAs and can be performed in approximately 5 hours, in contrast to the 24 hours required for commercially available kits.

The development of new FMD vaccine countermeasure tools is driving a new need to design a strategy for performing vaccine field trials to assess efficacy and performance in an endemic setting. These trials should assess their safety, immunogenicity and effectiveness and allow for a performance comparison with conventional inactivated vaccines. In addition, companion diagnostics that support these vaccines must be validated in FMD-free with vaccination and FMD endemic countries where additional performance characteristics can be obtained. Because FMD has not occurred in the United States since 1929, vaccine trials of this nature must be performed in an international setting. International vaccine trials offer an opportunity to test the performance of novel FMDV vaccines in a real world production setting where FMD is endemic or controlled using FMD vaccination strategies. During these trials, the companion diagnostic assays can be utilized and information gained can be used to determine their effectiveness in supporting the DIVA characteristics of the respective vaccine. In addition, novel diagnostic kits can be evaluated for their DIVA feasibility.

Key to the success of an international field trial is the upfront planning and coordination with relevant stakeholders (to include but not be limited to): DHS S&T, USDA APHIS (Science, Technology and Analysis Services [STAS], National Import Export Services [NIES]), other federal partners, international partners and governments, FAO, OIE, and officials from countries that are candidates for participation in the field trial. Thus, the goals and objectives of this workshop were to bring together a group of national and international experts to discuss and provide recommendations for experimental design and best practices for performing a vaccine and diagnostic field trial in an FMD endemic country.

Recommendations from this workshop will be utilized for developing the necessary processes/procedures for successful selection and implementation of the vaccine/diagnostic field trial. In addition, recommendations from the experimental design working group will be the basis for designing the experimental protocol and concept of operations for performing the trial in an FMD endemic country.

Discussion Summaries

During the workshop, breakout groups focused on four primary topics (see below) to discuss challenges, opportunities, and best practices for performing vaccine and diagnostic field trials in an FMD endemic country.

Topics of the breakout sessions included:

- **Group I:** Concept of operations and infrastructure requirements for performing a vaccine field trial in an FMD endemic country
- **Group II:** Regulatory considerations and requirements to consider when performing a vaccine field trial in an FMD endemic country
- **Group III:** Experimental design for a vaccine field trial in an FMD endemic country
- **Group IV:** Concept of operations and experimental design for evaluation of a DIVA diagnostic in an FMD endemic country

Group I: Concept of operations and infrastructure requirements for performing a vaccine field trial in an FMD endemic country

Moderated by Dr. Elizabeth Parker

Summary

Although there are a vast number of critical steps for successful implementation of an international vaccine trial, probably the single most important, and the one that all other challenges/opportunities/processes will depend on is the selection of an appropriate international partner (i.e. country willing to participate in the study). The country chosen as a collaborating partner will need to clearly understand the objectives of the study, define their own objectives and incentives for participation, understand the resource requirements, be fully committed to successfully completing the study and have the infrastructure and processes in place to support such a study. The U.S. partners will need to clearly define the objectives, identify required resources, understand partner country expectations for participation, understand the regulatory processes and infrastructure within the partner country and obtain "buy-in" from the partner for participating and successfully completing the study. For successful implementation, this relationship will need to be one of transparency and benefits for both partners. Relevant stakeholders/participants within the country should be aware of this transparent process (e.g. producers, veterinarians). Incentives for the collaborating country should be identified early in project planning, prior to country selection. The DHS and U.S. partners will need to determine which incentives they are capable of delivering to the international partner and, if accepted by the country, this should be documented early during the project planning stages.

The process for down-selecting an international partner should be transparent and involve a call for an expression of interest (EOI) as well as subsequent site visits. Criteria for initial down-selection of countries should be based in part on criteria outlined in Appendix D. Site visits will be necessary to validate the information provided in the expression of interest, to develop relationships for future implementation of the study, and to gain a greater understanding of the test environment and challenges. Depending on the outcome of the visit, the study plan may need to be modified in order to meet the stated objectives.

Once a nearly final experimental protocol and solidified set of requirements (ideal traits) for partner countries is established, a "one-pager" that describes the project and products should be developed. This document, combined with support from the U.S. Chief Veterinary Officer (CVO), would aid in generating responses to an EOI. Following release of the EOI and review of responses, DHS, the selected performer, and the Integrated Project Team (IPT) for this effort should work to down-select 2-3 countries for site visits. This would allow for in-depth engagement with potential partner country governments, laboratories, officials, and other project participants to gain and "on the ground" understanding of country capacity. A partner country would then be chosen based on information obtained in the EOI response and on-site visit.

During each site visit, the U.S. team should visit with the CVO, the Ministry of Agriculture (or equivalent), the reference laboratory (if one exists in country), potential sites for the study, industry representatives, regional and country regulatory authorities, and any available animal holding/challenge facilities. The U.S. team should coordinate the expression of interest and site visits with the USDA CVO and U.S. Department of State (DoS); and the visiting team should at a minimum consist of representatives from DHS, USDA, the DHS S&T Office of University Programs (OUP) Centers of Excellence, OIE, and FAO. Selected members of the Integrated Product Team will be invited on each site-visit. After the country is selected, a formal agreement should be established that outlines expectations, roles and responsibilities, resources, and length of study. Throughout the project it will be critical to maintain active and transparent communications with the ministries, the CVO and other field staff. Staff participation from the OIE and FAO will also be critical to the success of this project.

Because the success of this study will be heavily dependent on accurate, up-to-date knowledge of circulating serotypes/topotypes and the epidemiology of FMD in the country, effective communication with the partner country will be critical. Accurate knowledge of epidemiology and serotypes/topotypes can only be gained if adequate surveillance and monitoring are being performed in country. This type of surveillance and epidemiological analysis will require a

strong relationship with a reference laboratory and relatively robust veterinary services infrastructure. While both of these items are considered as part of the criteria for down-selection, it will only be after a country is selected that this type of data can begin to be shared and discussed to help formulate the most robust experimental design possible.

PARTNER COUNTRY SELECTION: RECOMMENDATIONS/NEXT STEPS

1. Develop a "one pager" that clearly outlines the project purpose/objectives and potential incentives for collaborating countries;
    a. Utilize this one-pager when speaking to countries to gauge interest.
2. Determine objectives and incentives that are available for partner countries
3. Finalize the set of requirements for partner country participation that will result in field trial goals and objectives being met;
4. Develop and execute an EOI to allow prospective partner countries to define capabilities/capacity and interest in performing study
    a. Before executing the EOI, notify U.S. CVO of intent to execute.
    b. Develop EOI scoring system.
    c. Information to be obtained from EOI and verified during follow on site visits should include, but not be limited to:
        i. Information on status/maturity of regulatory infrastructure;
            1. Determine capability to issue decisions and permits needed for conducting a field trial in a timely manner.
            2. Define roles of governing bodies/vaccine regulatory bodies within each nation/region (i.e. PANVAC and others).
        ii. Determine regulatory processes and procedures that will be required to successfully execute the project in partner country;
        iii. Ensure down-selected country will permit use of a recombinant vaccine in a field trial;
            1. Discuss possible implications of such a study on a country's FMD control program and trade.
            2. Determine whether participation in this trial will affect an ongoing eradication campaign (if one exists) and how on-going eradication program(s) might affect the study outcomes.
        iv. Determine the safety and efficacy data required to obtain an import permit for the hAd5-FMD and conventional vaccines;
            1. For example, will the country require U.S. approval (conditional licensure) prior to accepting its use in the field trial? What if the product is still experimental?

v. Determine numbers and types of circulating serotypes/topotypes of FMD in the selected country;
vi. Determine status/availability of surveillance data;
vii. Determine epidemiological status of FMD in potential partner country and associated contact rate/viral load (contamination?);
viii. Establish the availability of naïve animals for use in the study and determine whether or not the animals will need to be purchased in an FMD free country or zone and shipped to the test site (and can be);
    1. If naïve animals are not available, ascertain availability of previously vaccinated and/or previously exposed animals.
ix. Determine ability to track/tag animals in the country;
x. Determine objectives and incentives for the potential partner country and assess the relative probability to meet these objectives and incentives; and
xi. Ascertain capacity of the laboratory in country and infrastructure to support sample collection and shipment and provide training and resources where necessary;

d. Down-select 2-3 potential partner countries based on a pre-defined scoring system
e. Perform site visits to each down-selected country to include but not be limited to:
    i. Chief Veterinary Officer
    ii. Ministry of Agriculture
    iii. Potential sites for study implementation
    iv. Veterinary diagnostic laboratory
    v. Regulatory agency(ies)
    vi. Industry representatives
    vii. Any available animal holding/challenge facilities
    viii. Vaccine manufacturing plant (if one exists within country)
f. Execute a written agreement with the partner country that defines and includes at a minimum:
    i. Incentives, resources, expectations, objectives, regulatory requirements;
        1. Levels of safety and efficacy data that will be necessary for implementing a field trial in the partner country.
        2. Disposition of animals at the termination of the study.
        3. Required risk assessments.
    ii. Established roles, responsibilities, and resources needed to accomplish the objectives of the study;

iii. Expected outcomes of the study (for both the U.S. and the partner country);
iv. Communication pathway for successful implementation; and
v. Length of study.

Group II: Regulatory considerations and requirements to consider when performing a vaccine field trial in an FMD endemic country

Moderated by Dr. Larry Elsken

Summary

Regulatory requirements will play a critical role in determining both the pace and success of this project. Once countries that will allow the use of a recombinant product have been identified, it will be critical to understand the levels of safety and efficacy data required for obtaining an import permit for use within the country. Countries that are down-selected for a site visit must demonstrate the willingness to utilize a recombinant product. They must also demonstrate the presence of a robust regulatory infrastructure with processes and procedures in place to make decisions in a timely manner. One of the key elements that must be determined immediately, perhaps prior to selection of the partner country is the levels of data required by a potential partner country to demonstrate safety and efficacy prior to issuance of an import permit for the hAd5-FMD A24/Cruzeiro vaccine. The country must demonstrate the ability to issue import permits for conventional vaccines as well as for diagnostic test kits.

The regulatory working group noted that the serotype/topotype selection (and how many) will determine how long the regulatory process will take and how many times it would need to be repeated. This would be dependent on the country, the number of serotypes present, and the species that are currently being vaccinated. It was generally felt that the international partner's regulatory authority would want to see U.S. approval for the hAd5FMD product. However, this would be determined on a country-by-country basis and in fact may not be the case for every country. If the partner country expects U.S. approval of the new hAd5FMD constructs, then a masterseed virus, summary information format (SIF), federal register notice, and a finding of no significant impact (FONSI) would need to be completed for each construct. The estimated time to complete all of these activities is approximately 18-24 months. Vaccine efficacy studies would need to be performed at the Plum Island Animal Disease Center (PIADC) to demonstrate performance in a multivalent format. Obvious issues with this timeline include whether, after 18-24 months, the strains included in the vaccines are the same strains now circulating in the region of interest. The group noted that one way to circumvent the long regulatory process for a multi-valent vaccine might be to produce a monovalent vaccine to fill an "immunogenicity

gap" for a country that uses one vaccine and has a newly emerging strain. This in effect would allow for a multivalent study.

The working group outlined the steps in the USDA CVB process that should be considered for regulatory approval. The requirements that are outlined below are the strictest and most lengthy possible, to include CVB approval for international shipping and documented foreign government acceptance of the product. It was recommended to follow a study protocol that adheres as closely as possible to U.S. requirements for licensure in the international setting (the same ones that were followed for hAd5FMD A24/Cruzeiro serotype conditional licensure).

- CVB regulatory requirements to export/transport vaccine to international partner country in which FMD is endemic:
    - Completion of National Environmental Policy Act (NEPA) process, if needed, for each new serotype or strain* (*see italicized sub-bullet)*
        - Preparation of master seed virus
        - Firm's testing of master seed
            - At minimum, experimental safety in target animals with 20+ vaccinates
            - Back-passage/reversion to virulence studies
        - Preparation of SIF(s) (one for each construct)
        - FONSI or environmental impact statement as needed
        - Confirmatory testing of master seed by CVB
        - Federal register notice – number depends on construct, potentially with description of field safety testing to be determined by CVB
        - Comment period of 30 days
        - Field safety testing
            - Strictest interpretation to be in U.S. in naïve cattle—in three or more locations in 500 animals—for each construct including a multivalent option
            - As of now, CVB recognizes the platform concept only for killed virus vaccines. Recombinants would require a separate field safety study for each construct.
        - **This will be performed based on circulation in country at time of NEPA process initiation. Will this still be the target strain in 18-24 months when in-country field test can begin?*

The working group also identified critical questions/topics that should be addressed and answered when selecting a partner country and before approval of the country for participation in the study. These topics include, but are not limited to:

- Identification of a partner who is willing to allow use of recombinant vaccine within a field trial study
    - In some countries, if the recombinant product passes environmental and other species risk assessments acceptable to the USDA, then it would likely be accepted for use in a vaccine trial (Egypt was one example).
        - It is not immediately known if this will also be the case for other countries
        - Whether additional documentation is needed will likely vary from country to country
        - Some countries/regions may be resistant to the utilization of recombinant vaccines, though the EU is currently using a gE-gene deleted infectious bovine rhinotracheitis (IBR) marker vaccine for its IBR eradication program
    - Engage partner country ministries and regulatory authorities early and throughout the process.
        - Should be engaged by questions in the EOI and certainly during site visits
        - Should work transparently with partner country regulatory officials and expect reciprocation of this transparency
    - Government/Regulatory officials of international partners will need to express their expectations of the products to be tested and concessions (if any) for product acceptance
        - Levels of safety and efficacy data
- Determine what regulatory approvals (in U.S. partner country) will be required for approval of study
    - Define number of serotypes and strains to be utilized in the vaccine (this will determine the number of SIFs required)
    - Determine early in the process the amount of safety data required for the participating country to issue an import permit
        - Document this requirement from participating partner
    - The Code of Federal Regulations (9CFR) states that experimental product cannot be shipped until written approval to import is received from the international regulatory authorities
- Determine if there is a defined/well-documented process for obtaining “in country” approvals for use of a recombinant vaccine and performance of a field trial.

Other regulatory issues that were listed for consideration prior to designing the study included:

- Adjuvants

- Choice of adjuvant will be dependent on type of study and the disposition of animals
- Consider food safety regulations with regards to disposition of animals after the study
  - Will be country specific
  - U.S. has stricter regulations on withdrawal/slaughter withholding times for meat animals than most countries
- Label dependent, manufacturer's label instructions
- Testing may be performed to look for recombinant product in milk

- Additional international approvals or acceptance may be required
  - Dependent on country or region, such as regional economic bodies
    - PANVAC in Africa
    - Association of Southeast Asian Nations (ASEAN) or South Asian Association for Regional Cooperation (SAARC) in Asia
  - Should also request OIE and FAO assistance as needed
- Other
  - When comparing to the conventional vaccine, it will be critical to define which vaccine is appropriate for comparison
    - The "fairest" comparative study with traditional FMD vaccines would involve utilization of the "in-country" product
    - Only "purified" vaccines should be utilized for comparative study
    - How should study be designed to coordinate a multivalent trial with comparable conventional vaccine strains?
      - It is imperative to engage vaccine manufacturer to determine process with regards to strain disclosure
        - Critical to obtaining vaccines that are closely matched to recombinants utilized in the field.
      - Will be difficult to get an exact match between the commercial vaccine and the hAd5FMD vaccine, however it should be within the same topotype and same lineage if possible
      - Will need *in silico* analysis up front to ensure that structurally the hAd5FMD and conventional vaccines match as closely as possible so that the study isn't biased from the start
  - Determine how the inoculation requirements for field study will align with local vaccine campaign strategies
  - Define data required to support the objectives of the study
    - NOTE: This recommendation was addressed in Group III during the workshop

- Determine treaty compliance, regulatory compliance and export controls for DHS-funded efforts

Group III: Experimental design for a vaccine field trial in an FMD endemic country

Moderated by Dr. David Brake

Summary

Discussions in Group III largely centered on identifying best practices and the most appropriate experimental design for performing a vaccine field trial in an FMDV endemic country. The group set out to provide recommendations on experimental design and they based their recommendations for this design on the stated goals of the project. The recommendations from this group will be utilized to guide future development of the experimental protocol.

The goals for the vaccine field trial were discussed at length both within this breakout group and as part of the larger workshop. The general consensus among participants of the workshop was that it was appropriate to evaluate the new vaccine technology to determine if the product was as safe and effective as the conventional FMD vaccines but that the goals of determining if the new product was "as safe and more efficacious" compared to conventional FMD vaccines would be difficult to achieve without an extremely large number of animals included in the study. Additional considerations such as vaccine manufacturer, defining the appropriate conventional vaccine for comparison, and number of administered doses will also contribute to study complexity. Therefore, the goals as defined by DHS leadership and as agreed on by the participants of the workshop are as follows:

- To demonstrate that an hAd5-FMD cattle experimental product is as safe and effective (immunogenic) as current commercial high potency FMD vaccines in an endemic setting, and
- To demonstrate that an hAd5-FMD cattle experimental product can be used with non-structural protein (NSP)-based serological assays to differentiate infected from vaccinated animals.

To frame the discussion for experimental design, the moderator utilized a template containing the broad categories required in a USDA CVB field safety and efficacy study protocols. The group focused most heavily on the "Study Objectives" and the "Materials and Methods" sections of this template. For reference, the broad categories in these two sections are listed below:

Study Objectives

- Primary Outcome
- Measurement of Observation
- Acceptance Criteria

Materials and Methods

- Animals
    - Inclusion/Exclusion Criteria
    - Post-inclusion Removal Criteria
    - Identification and Management
    - Allotment/Randomization
    - Veterinary Care and Intervention
- Study Design
    - Experimental Unit(s) and Models
    - Masking
- Investigational Veterinary Product
    - Pre-Licensing Serial/Adjuvant
    - Vaccination Route
    - Labeling
    - Testing Requirements
    - Safety Precautions
- Procedures
    - Pre-treatment
    - Treatment
    - Reporting Adverse Events
- Safety Assessment
    - Criteria for a Valid Test
    - Outcome Criteria
- Efficacy Assessment
    - Criteria for a Valid Test
    - Outcome Criteria
- Data Summary and Analysis
- Disposition of Animals/Samples/*In vitro* products (IVPs)
- Regulation and Compliance Requirements
- Records to be Maintained

To begin the discussion, it was assumed that a partner country had been chosen and that they were in Stage 2 of the FAO progressive control pathway (PCP) for FMD. It was noted that a country in PCP Stage 2 may or may not be vaccinating yet. If a FMD vaccination program is in

place, it may not be countrywide or cover the entire national herd. The working group also recommended that due to the complexity and availability of hAd5-FMD master seed viruses, the hAd5-FMD vaccine valency used in this study should not exceed three (trivalent). Although many countries utilize pentavalent vaccines (example: Israel), and some even use septivalent vaccines (example: Saudi Arabia), the most likely hAd5-FMD product achievable for this study was a trivalent vaccine.

Discussions about frequency of vaccination led to the recommendation that it would be preferable to follow the labels for both the conventional and hAd5-FMD vaccine. DHS would prefer to implement a one-dose efficacy and duration of immunity study, but agreed to consider the prime/boost strategy for the hAd5-FMD vaccine if this was necessary (this may or may not be influenced by the vaccination strategy that is currently utilized in the partner country). In addition, field studies should be designed based on previous knowledge of circulating virus and potential contact rates. It will be critical to have a robust understanding of the epidemiology of FMD in the partner country. This knowledge will help inform the attack/hazard rate, which is dependent on circulating virus load.

The group agreed by consensus that the overarching objective of the field study would be to "investigate the safety and immunogenicity of the experimental vaccine, Foot and Mouth Disease Virus Vaccine, Serotypes XXX, XXX, and XXX Live Adenovirus Vector, Product Code XFMX.RO (unlicensed)". The study would be conducted in cattle under field conditions in regions where FMD virus(es) have been circulating in the 12 months prior to study initiation.

The group first discussed the sub-objectives of the study, appropriate measurements and acceptance criteria. Study objectives were defined and primary outcomes, measurements or observations, and acceptance criteria for each were established. Summary recommendations from this discussion are shown in Table 1.

Table 1: Objectives of Study and Measurements			
Objective	**Primary Outcome**	**Measurement or Observation**	**Acceptance Criterion**
Demonstrate the hAd5-FMD vaccine is as effective as conventional, killed vaccine in a FMDV endemic field situation	Absence of clinical disease (assumes natural exposure to conspecific/same species)	Percentage or number of positive animals based on clinical observations (using formalized standardized observation and scoring sheet)- Day 0, 1-3, then 'vet on call' and when daily observation suggests outbreak, enter into intensive period of observation with at least weekly observations	Cattle immunized with hAd5-FMD vaccine will have similar total number of elapsed days compared to cattle vaccinated with conventional killed vaccine
		Percentage or number of positive animals based on clinical observations (using daily observation [less formal]; observations made by animal handler/workers – provide opportunity to be trained in formal scoring methods	Cattle immunized with hAd5-FMD vaccine will have similar total number of elapsed days compared to cattle vaccinated with conventional killed vaccine
		Severity of disease based on quantitative method i.e. scoring sheet for severity (combine with above?) – coincides when trigger is made that outbreak may be starting, then daily until outbreak declines to 'baseline' level	Cattle immunized with hAd5-FMD vaccine will have similar total number of elapsed days compared to cattle vaccinated with conventional killed vaccine
		Estimation of exposure time based on age of lesion (based on pictures in reference manual from Pirbright)	Cattle immunized with hAd5-FMD vaccine will have similar total number of elapsed days compared to cattle vaccinated with conventional killed vaccine

Table 1: Objectives of Study and Measurements			
Objective	**Primary Outcome**	**Measurement or Observation**	**Acceptance Criterion**
		If naïve, unvaccinated treatment group, total number of elapsed days between FMD vaccination and 1st presumptive FMD diagnosis based on clinical observations (using standardized observation and scoring sheet)	Cattle immunized with hAd5-FMD vaccine will have similar total number of elapsed days compared to cattle vaccinated with conventional killed vaccine
	Duration of immunity	Serological measurements	
	Onset of immunity	Serological and potentially other testing measurements/observations	
	Absence of infection (assumes natural exposure)	Antigen (Ag) detection pen-side test	Cattle immunized with hAd5-FMD vaccine will have similar of lower incidence of FMD compared to cattle vaccinated with conventional killed FMD vaccine Cattle immunized with hAd5-FMD vaccine will have similar or higher geometric mean titer (GMT) to each fraction compared to cattle vaccinated with conventional killed FMD vaccine
		Proportion or number of positive animals by lab diagnosis using one or more of the following:	Cattle immunized with hAd5-FMD vaccine will have similar or lower incidence of FMD

Table 1: Objectives of Study and Measurements			
Objective	**Primary Outcome**	**Measurement or Observation**	**Acceptance Criterion**
		-Virus Isolation (VI) -RT-PCR -SP ELISA -NSP ELISA	compared to cattle vaccinated with conventional killed FMD vaccine Cattle immunized with hAd5-FMD vaccine will have similar or higher GMT to each fraction compared to cattle vaccinated with conventional killed FMD vaccine
	Absence of carrier state	Proportion or number of positive animals by lab diagnosis: -3 probang samples (and Day 0 baseline) tested by RT-PCR	Cattle immunized with hAd5-FMD vaccine will have similar or lower incidence of FMD compared to cattle vaccinated with conventional killed FMD vaccine Cattle immunized with hAd5-FMD vaccine will have similar or higher GMT to each fraction compared to cattle vaccinated with conventional killed FMD vaccine
hAd5-FMD vaccine is as safe as traditional killed vaccines in a field situation	Absence of untoward local events secondary to vaccination	Number of injection site reactions post-vaccination	Cattle immunized with hAd5-FMD vaccine will have similar or fewer total number of injection site reactions compared to cattle vaccinated with conventional killed FMD vaccine

Table 1: Objectives of Study and Measurements			
Objective	**Primary Outcome**	**Measurement or Observation**	**Acceptance Criterion**
		Severity of injection site reactions (size/mm^3, color)	Cattle immunized with hAd5-FMD vaccine will have similar or less severe injection site reactions compared to cattle vaccinated with conventional killed vaccine
		Number of days of milk drop or number of days off feed (would have to include sham vaccinates in study)	Cattle immunized with hAd5-FMD vaccine will have similar or fewer total number of days off feed compared to cattle vaccinated with conventional killed FMD vaccine
	Absence of untoward systemic events secondary to vaccination	Number of fever days in first 3-4 days post-vaccination	Cattle immunized with hAd5-FMD vaccine will have similar or fewer total number of fever days in the first 3-4 days post-vaccination compared to cattle vaccinated with conventional killed FMD vaccine
		Number of adverse animal health events such as lameness, abortion requiring medical treatment up to 1 week post-vaccination	Cattle immunized with hAd5-FMD vaccine will have similar or fewer total number of adverse health events compared to cattle vaccinated with conventional killed FMD vaccine

The group agreed that the most critical primary outcome for the study was demonstration of the absence of clinical disease. It was noted by the group that demonstrating absence of infection was also an important primary outcome, but not as critical as demonstrating the absence of clinical signs. Observation and standardized scoring will be used to measure efficacy of the vaccine. In order to measure absence of clinical disease it was recommended to have a

clearly defined protocol in place for scoring clinical observations. Observations should be made at the individual animal level (not the herd level) and should occur daily by the animal handlers/workers trained in observational methods. The group recommended individual animals be scored (not at the herd level) using a protocol stringent enough to make a presumptive clinical diagnosis. The protocol for clinical scoring should be quantitative so as not to overwhelm the field staff. The standardized observational scoring component of this study will also require a training module for field staff responsible for performing this work. It was also recommended to consider using a penside Ag test (panserotypic lateral flow or loop-mediated isothermal amplification [LAMP]) to *rule in* animals with clinical signs. Samples from animals showing clinical signs would also be sent for laboratory confirmation, but the use of a penside assay would provide immediate verification (rule in: if positive) that the animal had been exposed and was infected. Formal observations should be performed by the research team (a veterinarian and other research team members) on a prescribed schedule (Days 0, 1-3 and then 'vet on call' and when daily observation suggests outbreak, enter into intensive period of observation with at least weekly observations) as well as on demand (when observation suggests an outbreak and/or other disease occurrence).

In order to demonstrate absence of infection, samples should be taken at defined time points, irrespective of clinical presentation. Samples should be sent to a laboratory capable of performing virus isolation (VI), RT-PCR, antigen ELISA and NSP ELISA. In a herd demonstrating clinical signs and known to be positive for FMDV, the NSP ELISA can be utilized to determine which animals are infected but not demonstrating clinical signs. Viral load can be easily determined by quantitative RT-PCR. Prior to initiation of the study, sampling schemes and concept of operations should be well-defined.

In addition to absence of clinical disease and infection, another important measurement will be the number of carriers in an exposed/infected herd. Determining the numbers of carriers in a herd post-vaccination may reveal significant differences between the hAd5-FMD and conventional vaccines. Probang samples will be required for evaluation of this outcome. Probangs should be taken at Day 0 (for a baseline) and again at least at 3 subsequent time points. Each probang sample should be tested by RT-PCR.

Safety of the vaccine will be measured through observation. Measurements will involve observation for the presence/absence and severity of injection site reactions; number of days off feed; drop in milk production; presence/absence of fever; anaphylactic reactions; and the number of adverse animal health events such as lameness and abortion up to one week post-vaccination.

Next, the group discussed each category in the Materials and Methods sections of the protocol. The study design was discussed in depth and treatment groups were defined for the protocol.

One of the most discussed questions for study design was the appropriately matched killed (inactivated) vaccine to be utilized for comparison. It was generally agreed that the killed vaccine should be of high potency, NSP-free, and contain a known PD50 of at least 6. The reasoning behind this recommendation was that concentrates stored in the North American Foot and Mouth Disease Vaccine Bank (NAFMDVB) have a PD50 of at least 6, so for emergency vaccination comparison, the comparator vaccine (killed) must also have a similar potency. Alternatively, if PD50 data is not available, serological data is another highly correlative indicator of humoral immunity as it relates to protective vaccination response; results from assays measuring protection against generalized foot infection (PGP tests) where the preventive fraction can be calculated could also be used. In order to obtain this information from the selected commercial killed FMD vaccine manufacturer, some negotiations may be required. It was also recommended to include a naïve placebo group (sham-vaccinated with saline or phosphate buffered saline [PBS]). Enrollment of the naïve group could pose problems if the partner country will not agree to a group of unvaccinated animals for the study. This will depend in part on the status of FMD in the partner country, the stage/status of their FMDV control program, and possibly the region/district within the country in which the trial will take place. Inclusion of naïve treatment groups should be negotiated prior to selection of the partner country.

The working group noted that this study will most likely require hundreds of animals. However, the number of animals and experimental unit would ultimately be defined by the country and the FMD epidemiology profile in the country with the help of a statistician. The working group agreed that the "ideal" study would be performed entirely within one single herd and that one potential way to realize this "ideal" situation would be utilization of a dairy herd where the animals are likely to be maintained in larger groups, handled uniformly, and have uniform genetics. The drawback to using this type of herd would be the high likelihood that all animals would have previously been vaccinated, therefore reducing the potential for clinical disease, etc.

The working group suggested that one approach to minimize study design risk would be to purchase a large number of naïve animals, vaccinate in a FMDV-free area and then move the study animals to the final study site (at high risk for exposure to FMDV) for the study duration. The animals could be managed locally at the study site per "normal" practices (communal grazing). The study should be conducted for a period of 18 months and at the termination of the study, and pending regulatory concurrence, study animal ownership could be transferred to and distributed among all the individuals comprising the communal group. If there is no scientific evidence of FMDV exposure at the end of study month 12, strong consideration should be given to shipping study animals to a facility where experimental FMDV challenge can

be performed. It was noted that some African countries (e.g. Kenya) have government-owned farms with FMD naïve animals that are excluded from FMD vaccination campaigns. It was noted that utilizing this type of facility may be another means for achieving the "ideal" situation/setting for this study. Another method for sourcing naïve animals would be shipment from an FMD-free country or zone. Sourcing from an FMD region/zone that is in close proximity to the study site would minimize stress and help ensure similar breeds and resistance to local parasites/pathogens, etc.

Table 2: Animal Enrollment Criteria based on Previous FMD Vaccination/Infection Status

	NSP	SP-ELISA	SVN	Animal Origin	Animal Status	Recommendation
1	Negative	Negative	Negative	Endemic Region	No evidence of current infection or vaccination; if young animal, likely naïve; older animals may or may not be naïve as some animals may be exposed to FMD but after a certain amount of time have returned to serologically negative status	Enroll
				FMD-free zone	Animal is naïve	Enroll
	Negative	Negative	Positive	Endemic Region	May have been infected or vaccinated in the past; not currently infected	Enroll if needed
2	Negative	Positive	Positive	FMD-endemic zone	May have been infected or vaccinated in past; not currently infected	Enroll if needed
3	Positive	Positive	Positive	FMD-endemic zone	Previously infected, possibly vaccinated with crude vaccine	Do not enroll*

4	Positive	Negative	Positive	FMD-endemic zone	Previously infected; not vaccinated	Do not enroll unless other suitable animals cannot be located

*NOTE: Original workshop outcome was "enroll if needed" for category 3; however, upon further consideration, it was determined that enrollment of these animals may significantly complicate interpretation of results and the enrollment status was revised to "do not enroll".

Prior to admission into the study, it will be imperative to know (via laboratory testing and clinical history) the status of every animal. Prospective study animals should be tested for FMD exposure and FMD vaccination status using the NSP ELISA, SP ELISA, and serum virus neutralization (SVN) assay and subsequently categorized in Table 2. After testing, animals should be randomized and assigned to a treatment group. Before study admission the animals should also be evaluated for general health status, and depending on location of study, should be evaluated for other endemic diseases such as brucellosis, contagious bovine pleuropneumonia (CBPP), bovine viral diarrhea virus (BVDV), etc. Overtly/acutely ill animals should be excluded from the study. Criteria for definition of a healthy animal (one that will be included in the study) should be clearly defined in the study protocol. Prior to the onset of the study and during study design, investigators must determine whether or not this will be a "closed" or "open" study. Factors that will help this will most likely be the availability of animals and the environment that is chosen for the study site (commercial or village-type production system, see comments in paragraph above).

EXPERIMENTAL DESIGN FOR A VACCINE FIELD TRIAL IN AN FMD ENDEMIC COUNTRY

1. The experimental design should be developed and modeled based upon what is known about the epidemiology of FMD (and likely level of exposure) in the selected partner country.
2. The study should be performed as a double-blinded study
3. Perform study where there are no swine or in an area where exposure to swine is minimal to facilitate interpretation of results (consistent exposure across study groups)
4. Define primary outcomes for the study
    a. Demonstration of absence of clinical disease is the primary outcome
    b. Demonstration of absence of infection also defined as important outcome
    c. Develop a defined protocol for scoring clinical observations
5. Develop a defined protocol for scoring clinical observations

a. Clinical observations should be made at the individual animal level (not the herd level) and should occur daily by the animal handlers/workers trained in observational methods.
b. Scoring should be quantitative so as not to overwhelm the field staff.
c. Develop a protocol stringent enough to make a presumptive clinical diagnosis.

6. Consider using a penside antigen test (lateral flow or LAMP) to *rule in* animals with clinical signs.
    a. Samples from animals showing clinical signs should be sent for laboratory confirmation, but the use of a penside assay would provide immediate verification (rule in: if positive) that the animal had been exposed and was infected (given that the assay detects the circulating strains).
7. Develop a training module and data collection sheets for the standardized observational scoring component of this study.
8. Formal observations should be performed by the research team (a veterinarian and other research team members) on a prescribed schedule (Days 0, 1-3 and then weekly following vaccination) as well as on demand (when observation suggests an outbreak and/or other disease occurrence).
9. Prior to initiation of the study, sampling schemes and concept of operations should be well-defined.
    a. In order to demonstrate absence of infection, samples should be taken at defined time points, irrespective of clinical presentation.
        i. Samples should be sent to a laboratory capable of performing VI, RT-PCR, SP ELISA and NSP ELISA.
    b. In addition to absence of clinical disease and infection, another important measurement will be the number of carriers in an exposed/infected herd.
        i. Probang samples will be required for evaluation of this outcome.
            1. Probangs should be taken at Day 0 (for a baseline) and again at least at 3 subsequent time points.
            2. Each probang sample should be tested by RT-PCR.
10. The comparison killed conventional vaccine should be of high potency, NSP-free, and contain a known PD50 of at least 6.
11. A non-vaccinated group should be incorporated into the study
    a. This will help give an approximation of the time of exposure to FMDV
    b. Lesion aging could be utilized as a method to determine approximate exposure time
        i. The European Commission for the Control of Foot and Mouth Disease (EUFMD)/Pirbright has a manual that is utilized to age lesions for FMD (http://www.fao.org/fileadmin/user_upload/eufmd/docs/training/Kenya

ManualMarch2014Final.pdf), however, if this is utilized, field staff will need to be trained.

1. It was noted that this would be a very subjective method of determining exposure.

12. Enroll a naïve group in the study in addition to other treatment groups
    a. Enrollment of the naïve group could pose problems if the partner country will not agree to a group of unvaccinated animals for the study.
        i. This will depend in part on the status of FMD in the partner country, the stage/status of their FMDV control program, and possibly the region/district within the country in which the trial will take place.
        ii. This should be negotiated prior to selection of the partner country.
        iii. Note: Naïve animals that have not been vaccinated are considered both "naïve and unvaccinated" (group 1 from Table 2 above). This is the preference for the control group in this study. However, animals that have been exposed, recovered and not vaccinated (groups 2, 3, or 4 from Table 2 above) could also be considered "unvaccinated but not naïve" animals.
13. The working group suggested that one approach to minimize study design risk would be to purchase a large number of naïve animals, vaccinate in a FMDV-free area and then move the study animals to the final study site (at high risk for exposure to FMDV) for the study duration.
    a. The animals could be managed locally at the study site per "normal" practices (communal grazing).
    b. The study should be conducted for a period of 18 months and at the termination of the study, and pending regulatory concurrence, study animal ownership could be transferred to and distributed among all the individuals comprising the communal group.
    c. If there is no scientific evidence of FMDV exposure at the end of study month 12, strong consideration should be given to shipping study animals to a facility where experimental FMDV challenge can be performed.
        i. This would be challenging if a BSL3-Ag facility is required as there are few of these types of facilities available in FMD-endemic countries
14. Study Animals
    a. Inclusion/Exclusion Criteria
        i. Baseline data on animals should be collected prior to placement in treatment groups:
            1. Previous vaccination history
            2. Previous exposure history (FMD)

3. Previous lab data (e.g. NSP positive)
4. Individual animal data required (not herd level)
5. NSP, SP ELISA and SVN test using FMDV representative of vaccine strains

ii. General health status on day of vaccination (absence of other infections, immunosuppression, etc.)

iii. Age should be considered and factored into the randomization plan
1. Give preference to animals < 4 months of age

b. Study animals should be purchased and owned by the study sponsor; given to the local community or producer after the study

c. Evaluate the feasibility of purchasing animals in the U.S. and shipping to an endemic country
i. This would help ensure starting with an FMD-naïve herd; however, care has to be exercised in transporting animals to a new environment where they do not have any natural immunity to other circulating diseases that may be present.
ii. As an alternative, ideally, cattle could be purchased in FMDV-free area/zone and transported to study site.

d. Identification, Management & Treatment Groups
i. Animals should be uniquely identified with a tag (or other individual animal ID) as a part of the study
ii. Allotment/Randomization/Treatment Groups
1. Two Approaches
a. Randomly assign treatment within the unit or randomly assign the units and give them individual treatments (more expensive)
b. Treatment groups assigned within a unit
i. Ensures that groups within the unit have the same contact risk
ii. Drawback of this method is that herd immunity may protect the control group or the less effective vaccine group
iii. Group vaccinated with hAd5-FMD conditionally licensed vaccine
1. Subgroup I (FMD naïve)
2. Subgroup II (FMD history by vaccination and/or exposure)

iv. Group vaccinated with NSP-free commercial killed vaccine with a "known" PD 50 (PD 50 of approx. 6)
    1. Subgroup I (FMD naïve)
    2. Subgroup II (FMD history by vaccination and/or exposure)
v. Completely naïve but receive saline (sham vaccination with saline/PBS) – note that absence of adjuvant in shams will result in loss of statistical power with respect to hAd5-FMD vaccine safety observations

iii. Veterinary Care and Intervention
    1. Consider testing for endemic diseases depending on where the animals are sourced (brucellosis, BVDV, CBPP, etc.).
iv. A herd health management plan should be put in place before the start of the study.

e. Epidemiological Unit
    i. Animals should come from a single herd or village of sufficient size (possibly dairy)
        1. Uniform handling, genetics and sufficient numbers likely
        2. Drawback is the likelihood that animals in a dairy have all been vaccinated
    ii. If performed in one "village" then each village should be considered as one "epidemiological" herd unit
        1. Within the village small animal holders that are in close proximity will have similar animal husbandry and management practices
        2. For example, each herd unit may consist of 10-20 cattle

Group IV: Concept of operations and experimental design for evaluation of a DIVA diagnostic in an FMD endemic country

Moderated by Dr. Pam Hullinger

Summary

The primary goal/objectives of this part of the study are:

- To evaluate the performance of the soon to be licensed 3B ELISA (VMRD) and the Prionics PrioCHECK® FMDV NS ELISA with samples from naïve and vaccinated animals exposed to FMDV under field conditions, and

- Demonstrate DIVA capability of the 3B VMRD ELISA with the hAd5-FMD and high potency conventional FMD vaccines.

The intended purpose of the VMRD 3B FMD ELISA (from the U.S. perspective) is use as part of an active surveillance program during an FMD outbreak as well as in combination with the hAd5-FMD or high potency conventional FMD vaccines in a DIVA strategy to prove freedom from disease.

The working group discussed preliminary data that should be collected prior to a field trial with the VMRD 3B ELISA. The group recommended that a sensitivity assessment be completed with existing Pirbright reference panels and additional samples housed at the FMD World Reference Laboratory (WRL) at Pirbright. In addition, it was noted that a large negative cohort study (5,000 samples) had already been tested with v2.0 of the VMRD 3B ELISA kit. The negative cohort study performed with v2.0 will need to be repeated with the commercial, licensed version of the kit, once available. Specificity will be a key performance metric for use in the U.S. market*.

For the field study, the group noted that it would be important to pre-screen and know the status of animals included in the study. The group noted that it would be difficult to perform the diagnostic aspect of the study in a country where animals have been previously vaccinated (repetitively) with crude FMD vaccine(s). It will also be critical to determine a strategy for confirmation testing following a positive from either the VMRD or Prionics PrioCHECK® FMDV NS assays. The group proposed a diagnostic testing scheme in which the sample would first be retested with the same test to rule out user error and/or a false positive. If the sample was still positive, it would be re-tested with a secondary confirmatory test (the Virus Neutralization Test [VNT], Virus Infection-Associated Antigen [VIAA] agarose diffusion test, or a SP ELISA). Time points suggested for collection of samples in this study were Day 0 (day of vaccine), 4; 1, 2, 3 weeks; then 3, 6, 12, 18 months.

*NOTE: The VMRD 3B ELISA is currently being optimized. Once optimized, the next version will undergo testing with positive samples at the FMD WRL at Pirbright and at PIADC. Subsequently, a large negative cohort will need to be repeated within the National Animal Health Laboratory Network (NAHLN). These steps must be completed prior to field testing with this kit.

RECOMMENDATION/NEXT STEPS:

1. The licensed, commercialized version of the VMRD 3B FMD ELISA should be formally validated for diagnostic performance first by a U.S. based negative cohort study in

unvaccinated cattle (specificity) and using the FMD WRL and Panaftosa reference panels (sensitivity).

2. The VMRD 3B FMD ELISA's performance should be evaluated using samples from animals vaccinated with conventional high-potency FMD vaccine.
3. In view of the proposed use of the VMRD 3B FMD ELISA as a screening test for herd level surveillance during an FMD outbreak and proof of freedom from disease following a vaccination campaign, a clear strategy (decision tree) to discriminate true from false positives for the VMRD 3B FMD ELISA must be established.
4. In the context of the proposed study, the VMRD 3B FMD ELISA will be run in parallel with the Prionics PrioCHECK® FMDV NS assay to evaluate NSP antibodies in the context of both the hAd5 and conventional high-potency FMD vaccines; this might require a modified decision tree to resolve discordant results between the tests using an appropriate "gold standard".
5. If at all possible, the primary testing should be performed in-country, with confirmatory testing performed as necessary either in or out of country by an appropriate reference laboratory. In all cases, a separate aliquot of sera from all animals for all study points should be provided to the Foreign Animal Disease Diagnostic Laboratory at PIADC.
6. Prior to the onset of the study, confirmation is needed that the infrastructure is in place to support the field sample collection, identification, serum separation, refrigeration, and transfer of the samples to the designated location for testing and banking.
    a. This will most likely require some capacity building and training on standard operating procedures (SOPs).
7. The group also discussed and recommended consideration of the following logistical issues:
    a. Where will the samples be tested?
    b. Is there a desire to have the samples tested in more than one lab for reproducibility data?
    c. Provide in-country training for sample collection, identification, handling and transfer
    d. Provide in-country training and proficiency testing on the assays if testing is to be conducted in the country
    e. Determine resources and infrastructure for sample collection, transport, and testing
    f. Ensure cold chain is available for transport of samples

Options for a proposed methodology for the evaluation of discordant results to determine if they are indeed true positives are as follows:

- Re-run both assays

- More detailed evaluation of the data over time for both tests
- Re-evaluation and sampling of the animal(s) +/- testing probang samples
- Use individual structural protein-specific ELISAs appropriate for the outbreak strain
- Western blot analysis
- Saliva IgA ELISA

Summary Conclusions, Recommendations, and Next Steps

Over the course of the two and a half-day workshop, several overarching recommendations and discussion points were identified as common threads to help begin development and design of the international field trial. A resulting set of overarching recommendations, suggestions and next steps are summarized below by timeline. The summaries are broken down in to four major sections: Study Design, Partner Country Selection, Required Regulatory Processes, and Concept of Operations.

Study Design

This process should begin immediately and continue until execution of agreement with partner country. Initially, the design should be focused on enabling DHS to meet its primary objectives with the understanding that depending on the partner country selected, the design may need to be amended/augmented. After execution of agreement with the partner country, the detailed study implementation plan will be finalized and project will begin. NOTE: Flexibility should be built into the study design to allow for changes that may need to be accommodated upon final selection of partner country.

1. Develop a clear, detailed study design and protocol along with training manuals and SOPs;
    a. Solidify/finalize the goals and objectives for both the vaccine and diagnostic field trials;
    b. Define the desired/optimal performance metrics of the vaccine and diagnostic test to be assessed/measured by the field trial;
    c. Design detailed study;
        i. Study design to be developed in consultation with a statistician familiar with goals, objectives of the study as well as CVB requirements.
        ii. Study design reviewed by panel of national and international experts;
        iii. Study design reviewed by integrated product team (IPT);
    d. Determine training manuals and SOPs required for implementation of study;
        i. Develop training manuals and SOPs as required;
2. Finalize the set of requirements for partner country participation that will result in field trial goals and objectives being met; and
3. Finalize study design in collaboration with partner country.

Partner Country Selection

This process should initiate once a solid DRAFT study design has been developed that could be shared with prospective partner country officials. As noted above, once partner country agreement(s) have been executed, a detailed study implementation plan (to include a study

design and detailed workplan) can be finalized and required regulatory processes can begin.

1. Develop a "one pager" that clearly outlines the project purpose/objectives and potential incentives for collaborating countries;
    a. Determine objectives and incentives that are available for partner countries
2. Finalize the set of requirements for partner country participation that will result in field trial goals and objectives being met;
3. Assess status of intellectual property and potential for technology transition;
4. Develop and execute an EOI
    a. Before executing the EOI, notify U.S. CVO of intent to execute
    b. Develop EOI scoring system for down-selection of partner country;
5. Down-select 2-3 potential partner countries;
6. Perform site visits to each down-selected country
    a. Validate information provided in EOI response and obtain additional information as described in section "X" of this report to allow down-selection of partner country;
        i. See "Partner Country Selection: Recommendations/Next Steps" above for additional information
    b. Establish relationships/contacts in prospective partner country
    c. Identify an in-country, on-ground program supervisor/coordinator; and
7. Execute a written agreement with the partner country
    a. See "Partner Country Selection: Recommendations/Next Steps" above for information to be included in formal agreement.

Required Regulatory Processes/Approvals in the U.S. and Partner Country

Discussions with USDA CVB should begin immediately and continue throughout the design, implementation and data analysis phase. Many of the recommendations and next steps for regulatory processes in a partner country are defined in the "Partner Country Selection: Recommendations/Next Steps" section (above).

1. Begin conversations with partner country regulatory authorities regarding regulatory requirements for shipping experimental vaccine to the international partner;
    a. Dialog should begin immediately;
    b. Final decisions likely will not be available until partner country selected
    c. Engage USDA CVB in EOI preparation and site-visits to down-selected countries;
2. Determine treaty compliance, regulatory compliance and export controls for DHS-funded efforts;
3. Determine early how the study should be designed to coordinate the hAd5-FMD field trial with comparable conventional vaccine strains used in the region;

a. Perform *in silico* analysis up front to ensure that structurally the hAd5-FMD and comparable conventional vaccines to be evaluated match as close as possible to the currently used conventional vaccine strains used in the partner country so that the study isn't severely biased from the start;

4. Determine how inoculation requirements for the field study will align with local vaccine campaign strategies;
5. Define data required to support the objectives of the study. Design data collection templates; and
6. Determine which strains of the conventional vaccine to be evaluated are most appropriate and can be closely matched with the hAd5-FMD constructs (i.e. define which conventional vaccine is most appropriate for comparison).

Concept of Operations

1. Optimize, validate, and/or license the VMRD 3B FMD ELISA.
    a. Formally validate the VMRD 3B FMD ELISA for diagnostic performance by performing a U.S. based negative cohort study in unvaccinated cattle (specificity) and utilizing the FMD WRL and Panaftosa reference panels (sensitivity).
        i. This work should be performed with the final optimized version of the VMRD 3B FMD ELISA.
            1. Work is underway to generate the optimized kit.
2. Evaluate the VMRD 3B FMD ELISA's performance using samples from animals vaccinated with conventional high-potency FMD vaccine.
3. Develop a well-defined study design for field testing the VMRD 3B ELISA and comparing its performance to assays such as the Prionics 3ABC ELISA.
4. Develop a clear strategy (decision tree) to discriminate true from false positives for the VMRD 3B FMD ELISA.
5. Develop a modified decision tree to resolve discordant results between the VMRD 3B FMD ELISA and Prionics PrioCHECK® FMDV NS assay to evaluate NSP antibodies in the context of both the hAd5 and conventional high-potency FMD vaccines.
6. During the site visits and through the EOI, determine whether the infrastructure is in place to support the field sample collection, identification, serum separation, refrigeration, and transfer of samples to the designated location for testing and banking.
    a. Develop SOPs and training for field sample collection, identification, serum separation and transfer of samples to laboratory.

Workshop Outcomes

The workshop included a diverse group of participants, fostering productive discussions to help identify and prioritize the next steps for this project. In addition, the recommendations that arose as a part of this workshop will be utilized to formulate the workplan and experimental design as this project moves forward. The recommendations and conclusions from this meeting will be utilized to begin the process of formalizing concept of operations for implementation of this project, re-designing of timelines associated with project objectives, and defining the formal process by which to select a partner country.

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Acronyms

Acronym	Full Spelling
Ag	Antigen
APHIS	Animal and Plant Health Inspection Service
ASEAN	Association of Southeast Asian Nations
BVDV	Bovine Viral Diarrhea Virus
CBPP	Contagious Bovine Pleuropneumonia
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVB	Center for Veterinary Biologics
CVO	Chief Veterinary Officer
DHS	U.S. Department of Homeland Security
DIVA	Differentiate Infected from Vaccinated Animals
ELISA	Enzyme-linked Immunosorbent Assay
EOI	Expression of Interest
FAO	Food and Agriculture Organization of the United Nations
FMD	Foot and Mouth Disease
FONSI	Finding of No Significant Impact
GMT	Geometric Mean Titer
hAd5FMD	Human Adenovirus-5 based FMD vaccine candidate
HSARPA	Homeland Security Advanced Research Projects Agency
IBR	Infectious Bovine Rhinotracheitis
IPT	Integrated Product Team
IVP	*In Vitro* Product
LAMP	Loop-mediated Isothermal Amplification

NAFMDVB	North American Foot and Mouth Disease Vaccine Bank
NAHLN	National Animal Health Laboratory Network
NEPA	National Environmental Protection Act
NIES	National Import/Export Service
NSP	Non-structural Protein
OIE	World Organisation for Animal Health
OUP	Office of University Programs
PANVAC	Pan African Veterinary Vaccine Centre
PBS	Phosphate Buffered Saline
PCP	Progressive Control Pathway
PIADC	Plum Island Animal Disease Center
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
S&T	Science and Technology Directorate
SAARC	South Asian Association for Regional Cooperation
SIF	Summary Information Format
SP	Structural Protein
STAS	Science, Technology and Analysis Services
SVN	Serum Virus Neutralization
USDA	U.S. Department of Agriculture
VI	Virus Isolation
VIAA	Virus Infection-Associated Antigen
WRL	World Reference Laboratory

Appendix A: Workshop Participants

Name	Representing
Bruce Akey	Texas A&M Veterinary Medical Diagnostic Laboratory
Tammy Beckham	Institute for Infectious Animal Diseases
Melissa Berquist	Institute for Infectious Animal Diseases
David Brake	Department of Homeland Security
Kizzy Bundy	Department of Homeland Security
Michael Carter	USDA APHIS VS
Bruce Carter	Center for Veterinary Biologics, USDA APHIS VS STAS
Alfonso Clavijo	Institute for Infectious Animal Diseases
Matthew Coats	Department of Homeland Security
Michelle Colby	Department of Homeland Security
Ottorino Cosivi	PANAFTOSA
Michael David	USDA APHIS VS
Kris De Clercq	CODA-CERVA/OIE
Morella De Rosa	USDA
Amy Delgado	Center for Epidemiology and Animal Health
Hernando Duque	USDA APHIS VS STAS NVSL FADDL NAFMDVB
Ibrahim Eldaghayes	National Center of Animal Health, Libya
Larry Elsken	Merial/EDGE Veterinary Vaccines Consulting Group
Conrad Estrada (via webinar)	APHIS International Services
Mallory Gaines	National Cattlemen's Beef Association
Jessica Green	Center of Excellence for Emerging and Zoonotic Animal Diseases
Siddha Hover	Department of Homeland Security
Pam Hullinger	Lawrence Livermore National Laboratory
Jamie Jonker	National Milk Producers Federation
Barbara Kamicker	Plum Island Animal Disease Center
Don King	The Pirbright Institute
Mike Langford	Plum Island Animal Disease Center
Nadav Livni	Veterinary Services, Israel
John Mahoney	National Milk Producers Federation
Gregory Mayr	APHIS NVSL FADDL DSS
Doug Meckes	Department of Homeland Security
Soheir Hassan Abdel Kader Mohamed	Ministry of Agriculture, Cairo, Egypt
John Neilan	Plum Island Animal Disease Center
Nick Nwankpa	African Union Pan African Veterinary Vaccine Centre
Christopher O'Donnell	Department of Homeland Security
Cesár Orozco (via webinar)	USDA APHIS IS - Bolivia
Füat Özyörük	FMD Institute, Turkey
Elizabeth Parker	Food and Agriculture Organization of the United Nations

Lianne Parr	Department of Homeland Security
Nguyen Thanh Phuong	Center for Veterinary Diagnostics, Vietnam
Juergen Richt	Center of Excellence for Emerging and Zoonotic Animal Diseases
Abraham Sangula	State Department of Livestock, Ministry of Agriculture Livestock and Fisheries, Kenya
Darrel Styles	USDA APHIS VS
Mark Teachman	USDA APHIS VS
Rachel Whisenant	Institute for Infectious Animal Diseases
Justin Widener	Merial
Nick Wills	Benchmark Biolabs, Inc.
Cristóbal Zepeda	USDA APHIS VS NIES International Standards Services
Kurt Zuelke	CSIRO, Australian Animal Health Laboratory

Appendix B: Meeting Agenda

Workshop Agenda
June 10-12th, 2014
Washington, DC

Tuesday, June 10, 2014 | Renaissance Washington, DC Dupont Circle Hotel, Mt. Vernon Room

8:30 – 9:00 a.m.	**Welcome, Introductions, Workshop Goals** | Dr. Tammy Beckham, Director, Institute for Infectious Animal Diseases (IIAD) and Dr. Juergen Richt, Director, Center of Excellence for Emerging and Zoonotic Diseases (CEEZAD)
9:00 – 9:45 a.m.	**DHS International FMD Vaccine Field Trial: Project Outline and Goals for the Workshop** | Dr. Michelle Colby, Branch Chief, Chemical and Biological Division, Homeland Security Advanced Research Projects Agency, United States Department of Homeland Security, Science and Technology Directorate (DHS-S&T-HSARPA-CBD)
9:45 – 10:00 a.m.	**Break**
10:00 – 10:45 a.m.	**Challenges and Opportunities for performing vaccine field trials in an endemic country** | Dr. Nwankpa Nick, Senior Veterinary Vaccine Officer, AU-PANVAC
10:45 – 11:20 a.m	**Regulatory Requirements and Considerations for Licensure of FMD Vaccines and Expectations for International Field Trials** | Dr. Bruce Carter, Senior Staff Veterinarian, Licensing and Policy Development, Center for Veterinary Biologics, United States Department of Agriculture, Animal and Plant Health Inspection Services Veterinary Services (USDA-APHIS-VS-CVB)
11:25 – 12:00 p.m.	**International Regulatory Requirements for FMD Vaccines** | Dr. Kris DeClerq, Scientist, CODA-CERVA, OIE Collaborating Centre for Validation, Quality Assessment and Quality Control of Diagnostic Assays and Vaccine for Vesicular Diseases in Europe and Vice-President, OIE Scientific Commission
12:00 – 12:45 p.m.	**Lunch**
12:45 – 2:50 p.m.	**Chief Veterinary Officer Presentations on Challenges and Opportunities for International Field Trials** • Dr. Soheir Hassan Abdelkader, Undersecretary of Preventative Medicine, General Organization of Veterinary Services, Egypt

	• Dr. Abraham Kiprotich Sangula, Director of Veterinary Services, Ministry of Agriculture, Livestock, and Fisheries, Kenya • Dr. Nguyen Thanh Phuong, Deputy Manager and Head of Quality Assurance Laboratory, Center for Veterinary Diagnostics, Ministry of Agriculture and Rural Development, Vietnam • Dr. Nadav Livni, Veterinary Vaccine Dossier Accessor, Veterinary Services and Animal Health, Ministry of Agriculture and Rural Development, Israel • Dr. Ibrahim Eldaghayes, Committee Member, National Center of Animal Health, Ministry of Agriculture, Animal and Marine Wealth, Libya
2:50– 3:15 p.m.	**Presentation of Draft Experimental Design for International FMD Vaccine Field Trial** | Dr. Alfonso Clavijo, Senior Science Advisor, IIAD
3:15 – 3:30 p.m.	**Break**
3:30 – 3:45 p.m.	**Charge to Breakout Groups**| Dr. Tammy Beckham
3:45 – 5:15 p.m.	**Breakout Groups: Overarching Recommendations for International Field Trials** • **Moderator Group 1:** Experimental design for a vaccine field trial in an FMD endemic country (Moderator: Dr. David Brake, Scientific Consultant, Plum Island Animal Disease Center, DHS-S&T-HSARPA-CBD) • **Moderator Group 2:** Regulatory considerations and requirements to consider when performing a vaccine field trial in an FMD endemic country (Moderator: Dr. Larry Elsken, Consultant, Edge Veterinary Vaccines Consulting Group, LLC) • **Moderator Group 3:** Concept of operations and infrastructure requirements for performing a vaccine field trial in an FMD endemic country (Moderator: Dr. Elizabeth Parker, Animal Health Expert, Animal Production and Animal Health, Food and Agriculture Organization of the United Nations (FAO-AGAH)
5:00 – 5:30 p.m.	**Breakout Groups Report Out**
5:30 p.m.	**Summary and adjourn** | Dr. Tammy Beckham

Wednesday, June 11, 2014 | Renaissance Washington, DC Dupont Circle Hotel, Mt. Vernon Room

8:30 – 8:45 a.m.	**Welcome and Workshop Goals for the Day** | Dr. Tammy Beckham
8:45 – 9:30 a.m.	**Considerations/Requirements for performing diagnostic assay validation in an international setting: Perspective of the NAHLN MTWG** | Dr. Bruce Akey, Co-Lead, National Animal Health Laboratory Network Methods Technical Working Group (NAHLN-MTWG)
9:30 – 10:15 a.m.	**Performing Diagnostic Assay Field Trials in an International Setting** | Dr. Don King, Research Lead, Molecular Characterisation and Diagnostics Group. The Pirbright Institute, OIE Reference Laboratory for Foot and Mouth Disease
10:15 – 10:30 a.m.	**Break**
10:30 – 12:15 a.m.	**Breakout Groups: Protocol Design for International Field Trials** • Moderator Group 1: Experimental design for a vaccine field trial in an FMD endemic country (Moderator: Dr. David Brake) • Moderator Group 2: Regulatory considerations and requirements to consider when performing a vaccine field trial in an FMD endemic country (Moderator: Dr. Larry Elsken) • Moderator Group 3: Concept of operations and experimental design for evaluation of a DIVA diagnostic in an FMD endemic country (Moderator: Dr. Pam Hullinger, Veterinarian and Scientific Consultant, Lawrence Livermore National Laboratory)
12:15 – 1:30 p.m.	**Lunch**
1:30 – 2:30 p.m.	**Breakout Groups Report Out**
2:30 – 4:00 p.m.	**Large Group Discussion: Experimental Design, Concept of Operations and Regulatory Considerations for Performing a Vaccines and Diagnostics Field Trial in an FMD endemic country**
4:00 – 4:15 p.m.	**Break**
4:15 – 5:00 p.m.	**Identify Remaining Issues for Discussion on Day 3**
5:00 p.m.	**Summary and adjourn** | Dr. Tammy Beckham

Thursday, June 12, 2014 | Renaissance Washington, DC Dupont Circle Hotel, Mt. Vernon Room

8:30 – 8:45 a.m.	**Welcome and Charge to Breakout Groups**| Dr. Tammy Beckham
8:45 – 10:45 a.m.	**Summary/Roll-up Discussion on Outstanding Issues (return to Breakout Groups)**
10:45 – 11:00 a.m.	**Break**
11:00 – 12:15 p.m.	**Presentation of Experimental Design, Concept of Operations and Regulatory Considerations for Performing a Vaccines and Diagnostics Field Trial in an FMD endemic country (Moderator Report Outs)**
12:15 – 1:00 p.m.	**Summary and Action Items** | Dr. Tammy Beckham and Dr. Michelle Colby
1:00 p.m.	**Adjourn**

Workshop Objective: Establish recommendations for international field trials

- Identify potential field trial locations and partners
- Assess field trial processes, protocols, test plans, evaluation, and regulatory requirements
- Establish validation requirements for companion diagnostics
- Recommend concept of operations for testing

Appendix C: Presentation Abstracts

DHS International FMD Vaccine Field Trial: Project Outline and Goals for the Workshop

Dr. Michelle Colby, Department of Homeland Security, Science and Technology Directorate, Chemical and Biological Defense Branch, Washington DC, USA

Narrative: The Department of Homeland Security (DHS); Science & Technology (S&T) Directorate is tasked with researching and organizing the scientific, engineering and technological resources of the United States and leveraging these existing resources into technological tools to help protect the homeland. More specifically, as identified in Homeland Security Presidential Directive (HSPD) on Defense of United States Agriculture and Food (HSPD-9), which defines policy for defense of U.S. agriculture and food, identifies DHS as the lead agency to coordinate federal activities to "accelerate and expand development of current and new countermeasures against intentional introduction or natural occurrence of catastrophic animal, plant, or zoonotic diseases." DHS carries out this responsibility in close collaboration with its sector-specific agency partners. In the case of high-consequence livestock pathogens, these tools play a crucial role in the preventative, mitigation and recovery phases of an outbreak.

The Foreign Animal Disease (FAD) Vaccine, Diagnostic and Countermeasure program is currently developing a well-defined portfolio of Foot and Mouth Disease (FMD) vaccine candidates (serotype-specific not pan-specific) that are safe and efficacious against the FMD viruses (serotypes) currently known to be circulating outside the U.S. in FMD endemic countries. The project is also funding a 3ABC diagnostic test under U.S. regulatory development has been designed to detect all FMD viruses should perform well in any FMD endemic country. Additionally, the 3ABC ELISA test target antigens are absent from the vaccine formulations thus providing the ability to differentiate infected from vaccinated animals (DIVA).

The next step in validating the effectiveness of the vaccine is to conduct a cohort study under field conditions in an FMD endemic setting. However, operational deployment, testing & evaluation of Foot-and-Mouth Disease (FMD) vaccines (conventional or new biotechnology – based) and diagnostic assays (existing or new) in the United States (U.S.) is currently not a viable strategy given the FMD free status of the country. Demonstrating the effectiveness of these vaccines in an endemic environment will benefit both endemic countries by providing new tools for disease control and eradication programs and FMD free countries by providing new, DIVA compatible vaccine technologies that might be employed in an outbreak situation. This project will conduct international trials in endemic countries comparing traditional FMD

inactivated vaccine and lab-based ELISA diagnostic technologies to DHS S&T funded products including the newly developed FMD molecular vaccine and lab-based ELISA technologies.

Challenges and Opportunities for performing vaccine field trials in an endemic country

Dr. Nwankpa Nick, Senior Veterinary Vaccine Officer, AU-PANVAC

Introduction

Vaccines are the single most cost effective intervention in the control of most animal diseases. However before any veterinary vaccines can be approved for use in the prevention of diseases, their efficacy and safety of must be demonstrated by experiments under laboratory conditions and the verification of this efficacy and safety conducted under field conditions in target animals constitutes vaccine field trials. Vaccine field trial implementation involves three major aspects; Field Safety and Field efficacy trials; and animal Welfare considerations.

Vaccine field trials in Africa: role of AU-PANVAC

The Pan African Veterinary Vaccine Centre of the African Union (AU-PANVAC) is a technical Office of the African Union and the only organization mandated to ensure the Certification of all Veterinary vaccines produced or brought into Africa for use. The major role of AU-PANVAC in any vaccine trial in Africa is to guarantee the Purity, Safety and Efficacy of the trial vaccine i.e. to ensure that the vaccine is of good Quality.

Establishment of AU-PANVAC

The idea of an Independent Quality Control Centre was conceptualized between 1983 and 1986 to ensure quality of all rinderpest vaccine batches produced to support the Pan African Rinderpest Campaign (PARC). An FAO Technical Cooperation Project (TCP/RAF/6766 & TCP/RAF/6767) awarded to AU-IBAR to ensure Vaccine Quality Control between 1986 and 1993 resulted in the establishment of two Centers, one in Dakar (Senegal) for Central and Western Africa and the other in Debre Zeit (Ethiopia) for Eastern and Southern Africa. These two centers were eventually merged due to constraints in 1993 into one site at Debre Zeit (Ethiopia) as Pan African Veterinary Vaccine Centre.

However the contributions of these centers to the rinderpest campaign was well appreciated and recognized by various evaluation and review teams, consultants, beneficiary laboratories and governments who reported that PANVAC's activities resulted in a significant improvement in the quality of rinderpest vaccine produced in Africa thereby contributed to the success of PARC.

The final evaluation report of PARC and PACE stated that: "*The success of the Pan African Rinderpest Campaign (PARC) and the Pan African Programme for the Control of Epizootics*

(PACE) clearly demonstrated that no amount of vehicles, syringes, trained personnel, communication materials, would have eliminated Rinderpest if the vaccine batches used were of poor quality. The secondary and independent level of quality control assessment assured by PANVAC played a major role for this success and led, at the same time to a sustained improvement in the quality of vaccines against Rinderpest and Contagious Bovine Pleuropneumonia produced in Africa".

It was to strengthen these achievements in the interest of Africa that the 4th Conference of African Ministers responsible for Animal Resources recommended the elevation of PANVAC to a technical center of the OAU and that recommendation was approved in February 1998 by the 67th Ordinary OAU Council of Ministers. The Centre was officially launched as an AU Regional Office under the Department of Rural Economy and Agriculture of African Union Commission (AUC) in March 2004 with its headquarters at Debre Zeit (Ethiopia).

Presently the Mandate of AU-PANVAC is to promote the availability of safe, effective and affordable veterinary vaccines and diagnostic reagents; to facilitate the development and introduction of improved or new vaccine production technology into Africa; and to strengthen Africa's capacity building in veterinary vaccine development, production and quality assurance.

In order to implement its mandate, AU-PANVAC currently conducts QC on Veterinary vaccines and also maintains a repository of vaccines seed for distribution free of charge to vaccine production laboratories in Africa.

Collaboration with relevant centers in vaccine sciences

AU-PANVAC has collaborative partnerships with leading institutions in vaccine science (OIE, FAO, GALVmed, etc.). AU-PANVAC has been collaborating with the World Organization for Animal Health (OIE) since its inception and AU-PANVAC is presently an OIE World Reference Centre for Quality Control of Veterinary vaccines. It has also been involved in several projects with the OIE recent amongst which is a PPR Sub-grant project from the Bill and Melinda Gates Foundation. AU-PANVAC is presently a member of the working group on Veterinary Drug Registration and the FAO/AU-IBAR/OIE/IAEA Consultative Group on CBPP.

Other collaborations include the joint established of a Process Development Laboratory for vaccine process improvements and research by AU-PANVAC and the Global Alliance for Livestock Veterinary Medicine (GALVmed). Some of the work being implemented in this laboratory include the evaluation of the Xerovac technology for PPR vaccine production, the transfer of the Newcastle tablet vaccine technology to vaccine producing laboratories in Africa and the transfer of the Ben 1 CBPP vaccine to Africa. AU-PANVAC is also involved in collaborations with the KYEEMA Foundation and AusAID on the Control of Newcastle disease in

village chickens using the I-2 ND vaccine; and the on the sequestration of the Rinderpest virus amongst others.

Controlling FMD in Africa

Foot-and-mouth disease (FMD) a major impediment to intra-regional and international trade; and threatens food security and the livelihoods of people who depend on livestock. The disease is presently endemic in most parts of Africa, Asia, the Middle East and South America. Currently 3 out of the 7 global FMD virus pools are located in Africa.

Treatment is not indicated in the control and eradication of FMD as such alternative strategies such as stamping out, strict Movement control and vaccination are the only option. These strategies cannot however be effectively implemented in Africa due to Inadequate resources for stamping out and payment of compensations, difficulty in enforcing livestock movement control due to nomadism, apparent lack of economic benefit for huge investment in FMD control, lack of Political will by some Governments which have other priority problems, lack of appropriate veterinary service infrastructure in most regions, regional instability and civil strife and in some cases Corruption.

Challenges and opportunities of implementing vaccine field trials

Implementing Vaccine field trials in endemic countries such as may be found in Africa poses many challenges. Endemicity is usually associated with poor and small-holder farmers and convincing farmers with only a few animals which they consider as their only source of livelihood to participate in trials may be an issue. Other challenges include; Lack of adequate Veterinary infrastructure and capacity for the organization of a vaccine trial on a large scale, Inadequate capacity for monitoring, absence of animal movement control in most parts of Africa, Presence of different strains of circulating disease causing organism in different geographical locations, Rapid genetic evolution of the circulating organisms e.g. FMD virus, Presence of intercurrent infections which may introduce bias in the trials, difficulty in obtaining naïve animals to serve as controls, Lack of harmonized regulations in the different countries on the implementation of vaccine trails, Lack of Political will by some governments and Civil, religious and Political instabilities which is currently witness in many regions.

However, despite the challenges, the benefits and advantages of implementing vaccine trials in endemic countries are enormous. Endemicity would make it relatively easy to achieve natural exposure to infection in any trial since the infection is readily available. Other advantages include: stringent biosecurity measures may not be required as in the case of disease free countries, circulating strains of the causative organisms for study will be readily available, in most parts of Africa, decisions on the implementation of field trials are made by the

Government which makes it easy to ensure compliance by farmers, several stakeholders on animal disease prevention and control are already in the field so the communication infrastructure with the grass roots will still be in place.

Conclusion

Implementing vaccine field trials in Africa where diseases like FMD are endemic can be quite challenging; however, utilizing the communication channels already established by other stakeholders and involving partners already in the field will save resources, facilitate access to the field and eliminate suspicions and uncertainties associated with most trials by the farmers.

Regulatory Requirements for Licensure of FMDV Vaccines and Expectations for International Field Trials

Dr. Bruce Carter, United States Department of Agriculture, Animal and Plant Health Insepction Service, Veterinary Services, Center for Veterinary Biologics, Ames, IA

The USDA has issued 2 Product Permits for importation of FMDV vaccines, and 1 Product License for domestic manufacture of FMDV Vaccine, Serotype A24, Adenovirus Vector. Before a permit or license is issued a product must be shown to be pure, safe, potent, and effective. Regulatory rules and guidance are found in the Virus-Serum-Toxin Act, Title 9 of the Code of Federal Regulations, and Veterinary Services Memorandums (VSM) and Notices. Planning and preparation are critical to designing a study to generate data used to support a product license or for publication in a peer-reviewed scientific journal. Study design guidance is found in the following publications that are available to the public:

- VSM 800.200 Study Practices and Documentation
- VSM 800.202 Efficacy Studies
- VSM 800.204 Field Safety Studies
- VSM 800.301 Good Clinical Practices
- CVB-PEL Reviewer's Manual: Chapter 4.3 Protocols and Chapter 7.1 Statistics

Key elements for licensing Adenovirus vectored FMDV Vaccines include characterizing the Master Seed Viruses and Master Cell Stocks, conducting reversion to virulence studies, preparing a Summary Information Format (SIF) III-A for live vectored organisms, manufacturing pre-licensing serials per approved Outlines of Production, and conducting host animal safety and efficacy studies. Efficacy studies establish the minimum immunizing dose (titer of product used in efficacy study). Safety studies are conducted using product formulated at the efficacy Serial titer + 0.7 Log_{10} (assay variability) + 0.5 Log_{10} (or actual loss from the stability study).

A starting point for designing a pivotal study includes deciding what's most important (e.g. prevent clinical signs or prevent shed and spread), defining explicit focused objectives, and specifying a clinically relevant primary outcome. Critical design features include randomization, blinding and placebo control. Vaccinators and data collectors should not know what treatment an animal received and which other animals in the herd (e.g. pen, pasture) belong in the same group.

The USDA doesn't normally accept safety or efficacy data generated only in foreign studies but with FMDV vaccines the CVB will make an exception. Safety and efficacy studies should be

conducted according to well-designed protocols. Protocols to conduct studies in foreign countries should be discussed with the CVB, especially if studies are expected to generate data that may be used to support a product license. Per VSM 800.98, APHIS does not consider data resulting from a comparison study with a licensed product in its decision to issue a veterinary biological product license. APHIS does not review the results of comparative studies of such products published in scientific or trade journals.

International Regulatory Requirements for FMD Vaccines

Dr. Kris DeClerq, World Organisation for Animal Health Scientific Commission, Paris, France

One of the principal aims of the OIE is to safeguard world trade by publishing health standards for international trade in animals and animal products. OIE cooperates with its Member Countries and International organisations for harmonising the technical requirements for registration of veterinary medicinal products as there is a difference in their approach to ensuring the quality, safety and efficacy. Vaccines used for controlling foot-and-mouth disease (FMD) should comply with the general and specific international standards outlined in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. The specific requirements for FMD vaccines such as in-process control, inactivation and final product batch tests are described in detail in the FMD chapter of the Manual. In the general chapter on 'Principles of veterinary vaccine production' a specific paragraph is dedicated to safety and efficacy field tests and the related risk assessment. A practical approach for the field-testing of a new FMDV vaccine could be based on the note for guidance 'Field trials with veterinary vaccines' published by the Committee for Veterinary Medicinal Products of the European Medicines Agency (EMA).

CVO Roundtable: Challenges and Opportunities when Performing FMD Vaccine Field Trials (Egypt)

Dr. Soheir Hassan Abdelkader, General Organization of Veterinary Services, Egypt

Egypt is located in North Africa which represents a cross road between pools (FMD pools); it is already in pools 3 and 4.

FMD has been diagnosed in Egypt since the fifties.

National Control Programme started in 1970 against the O strain, using locally manufactured monovlent O mania vaccine.

In 2006 A African was diagnosed and a bivalent vaccine was used until a SAT2 was recorded. The current used vaccine is a trivalent oil adjuvant which is locally manufactured and composed of: O Panasian 2, A Iran 05 and SAT2.

In relation to PCP Egypt is (provisionally) in stage 2 and anticipate being officially in this stage by 2015.

The implemented action plan consists of: Regular massive vaccination twice / year, measuring immune levels after vaccination, active and passive surveillance and extension for all stakeholders.

Examples of sustained efforts for building capacity to improve Egypt's performance include the following:

1. Legislation: Law 13 for 2014 to deliver vaccine with fees.
2. Community Animal Health and Outreach (CAHO) teams: applying participatory disease surveillance (PDS) at the village level.
3. Epidemic surveillance Units (ESU): to be fully functional at the district level.
4. Cold chain improvement for vaccine transportation.
5. Data flow through variable database program either on Central or on Local levels.
6. Rapid primary field diagnosis by using mobile laboratories.

Egypt contracted with Merial international antigen bank for 5 years for 500000 doses each of Asian 1 and SAT 1 to be delivered within 5 days in the case of exposure to any FMDV.

CVO Roundtable: Challenges and Opportunities when performing FMD Vaccine Field Trials (Kenya)

Dr. Abraham Kiprotich Sangula, Ministry of Agriculture, Livestock, and Fisheries, Kenya

FMD is endemic in Kenya with serotypes O, A, SAT1 &2 reported in most of the 47 Counties of the Country from 2011-2013. Most of the reported outbreaks are in cattle populations in dairy farming areas while small ruminants are largely ignored.

In the past 8 months only serotypes O (EA2 topotype), SAT1 (NWZ topotype) & SAT2 (IV topotype) were reported.

Kenya produces FMD vaccines (aqueous) at the Government owned vaccine production laboratory (KEVEVAPI), which are used in outbreak responses and routine prevention as well as export to neighboring countries. The strains in use are: OK77/78 - EA1, AK5/80 – G1, SAT1T155/71 - NWZ, and SAT2K52/84 – IV).

The control of FMD in Kenya is defined by the national control strategy. FMD is a notifiable disease (Animal Diseases Act cap 364 - FMD rules, laws of Kenya). Any outbreak is reported to the veterinary authorities. Control actions include the imposition of quarantine and ring vaccination of cattle. FMD vaccines are widely accepted in Kenya. The coordination of actions is undertaken by both national (CVO/ DVS) and the County level by the County Director of Veterinary Services (CDVS). The vaccinations are undertaken by both the government and private veterinary staff.

The control program is supported by a National FMD laboratory at Embakasi with capacity for virus isolation, antigen & antibody detection, and genome detection. Post-vaccination serology is also routinely performed. The lab has capability to test vaccines under an experimental setting (BSL-2 facility) for potency test by cattle challenge. The lab has been in collaboration with the WRL – Pirbright, UK since inception in 1957.

National surveillance is supported by the Epidemiology and Economics Unit at the Veterinary headquarters.

The challenges faced by the control program include; the use of non-purified, aqueous vaccines conferring shorter immunity and DIVA challenge. Strain characterization and vaccine matching capacity also requires expansion. However, the country has some examples of successes in the past (pre-1980s) including FMD control programs (extensive and well funded vaccination program) and Rinderpest eradication program (regional approach and international support).

Currently, the country anticipates being fully in stage 2 of the FAO Progressive Control Pathway by June 2015. In support of this, the main improvements desired include: to increase production capacity and introduce oil adjuvant and purified vaccines of high potency and updated relevant strains.

The regulation of veterinary biologics in Kenya is by the DVS (CVO) /Pharmacy and Poisons Board. The DVS coordinates and participates in veterinary medicine field trials with the most recent being Rift Valley Fever Clone 13 vaccine trial (August 2011 to September 2012). The DVS would thus be interested in partnering on an international field trial with a new second generation vaccine (DIVA capable?) with the use of government/Institutional facilities for the trials giving better chances for successful trials.

CVO Roundtable: Challenges and Opportunities when performing FMD Vaccine Field Trials (Viet Nam)

Dr. Nguyen Thanh Phuong, Center for Veterinary Diagnostics, Ministry of Agriculture and Rural Development, Viet Nam

FMD samples submitted/tests performed/results obtained

FMD viruses continue to circulate in the provinces of Viet Nam, with the prevailing serotypes in 2012 to 2013 serotype O (PanAsia) and serotype A (Sea-97). Viet Nam recorded more than 35 outbreaks in 2012

The priority vaccines remain O Manisa, vaccine and the availability of supplementary strains such as O Manisa and O 3O39 from Merial should increase confidence that there are vaccines to cover the majority of known circulating O viruses.

From 2012 to 2013 Viet Nam sent samples from various province in Viet Nam routinely to WRLFMD where 86 FMD type O and type A viruses were isolated.

Vaccine matching studies carried out at WRLFMD demonstrated no match with the current vaccine strain for the region (O-Vit1/2012 and O-Vit12/2012 by VNT). However, antigenicity of FMDV 2013 is "significantly" different from FMDV 2012, and the vaccine O3039 and O Manisa still work well with FMDV 2013 isolates. Antigenicity of FMDV 2013 isolates is similar FMDV 2012 isolates and the vaccine A22 Iraq is still acceptable.

Vaccine matching studies for type O FMDV by VNT-WRL FMD						
Field Isolate:	**2dm VNT test ref:**	**O 3039**	**O 4625**	**O Manisa**	**O Taw98**	**O Tur 5/09**
O Vit 1/2012	Mean	0.20	0.18	0.07	0.48	0.12
O Vit 12/2012	Mean	0.33	0.23	0.08	0.51	0.18

Vaccine matching studies for type O FMDV by VNT-WRL FMD					
Field Isolate:	**Vaccines**				
	O 3039	**O Manisa**	**O Taw98**	OTur5/09	
				MSD pool	Boost pool
O Vit 11/2013 (mean)	>1.0	0.43	0.85	>0.74	>0.86
O Vit 18/2013 (mean)	>1.0	0.52	>0.98	0.47	>0.72

Vaccine matching studies for type A FMDV by VNT-WRL FMD				
Field Isolate:	**Vaccines**			
	A22 Irq	A Irn05	A May 97	A Tur06
A Vit 15/2012 (mean)	0.17	0.10	0.16	0.22
A Vit 16/2012 (mean)	0.25	0.11	0.14	0.24

Vaccine matching studies for type A FMDV by VNT-WRL FMD							
Field Isolate:	**Vaccines**						
	A Iran 2005	A May 97 VJ pool	A May 97* B980-4	A22 Irq	A sau 41/91	A Sau 95	A Tur06
A Vit 05/13	0.22	0.18	0.06	0.30	0.11	0.15	0.43
A Vit 66/13	0.27	0.22	0.10	0.32	0.20	0.13	0.40

In the case of VNT:

r_1 = ≥ 0.3. Suggests that there is a close relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection.

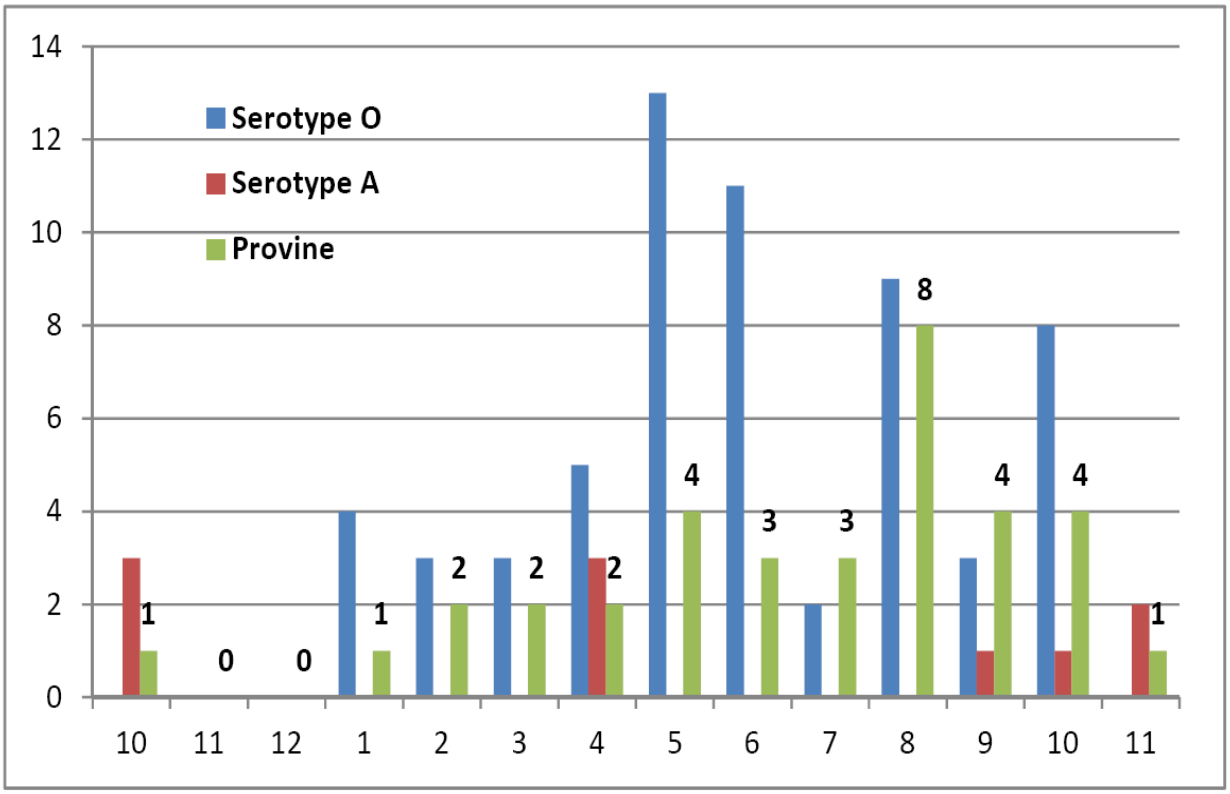
r_1 = < 0.3. Suggests that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect

Apart from the O virus circulating in Viet Nam, vaccine matching studies suggest that vaccines that are currently in use shown below should still protect against clinical disease when applied under systematic vaccination and revaccination schemes. However, this will now need very careful monitoring.

Overview of samples received and serotyping results

The Nation Center Veterinary Diagnosis (NCVD) and Regional Animal Health Office No 6 (RAHO6) laboratories received more than 96 samples in 2012 -2013 from provincial locations.

The proportion of the different serotypes detected in 2012-2013 is shown below, demonstrating more than 90% of the samples characterized in the two years were of the O serotype by ELISA detection antigen virus and real-time PCR. Also, our laboratory has been utilizing antibody-based detection assays such as Liquid phase ELISA (Pirbright) and 3ABC non-structure protein (Prionics kit), in addition to virus isolation and virus neutralization tests.



National Vaccine Program

Vaccination occurs 2 times a year. More than 247.000 doses are used in 2012-2013

Free routine vaccination for cattle and buffalo only (vaccine contains antigen of O 3039 and O Manisa). Vaccines for pigs are the same but the farmers have to pay for it themselves. National stocks for emergency vaccination contains a trivalenct vaccine: O (O Manisa + O 3039), A (A22 Irq + AMay 97) and Asia 1.

RAHO6 (Year)	**Cattle and Buffalo**		**Pig**	
	Population	Vaccination (doses)	Population	Vaccination (doses)
2012	886.271	809.227	3.549.007	2.940.432
To Sept 2013	964.949	442.742	4.480.930	1.026.108
Supplied by National Program		247.000		

Future plans

1. Trying to set up the SOP for vaccine matching test by using current vaccine (O 3039 and O Manisa) reference serum and homologous VNT titer which is supplied by WRL.
2. Do more VNT to the new isolates to monitor the efficacy of current vaccine to new isolates.
3. Speed up the application of sequencing for FMDV at RAHO6.

CVO Roundtable: Challenges and Opportunities when performing FMD Vaccine Field Trials (Israel)

Dr. Nadav Livni, Veterinary Services and Animal Health, Ministry of Agriculture and Rural Development, Israel

Israel's geographic location on the intersection between Africa and Asia, and the lack of diplomatic relations with most of its neighbors, are the reasons for the difficulties in eradicating the disease. In the last eight months only two outbreaks have been in Israel. The Kimron Veterinary institute, which is a FMD BSL-3 laboratory, is responsible for surveillance and diagnostics but not for challenges. Israel imports currently vaccine strains from Merial company, and their matching to the field strains is tested once a year or two years. The formulations are for cattle and pigs, and for sheep and goats. The Israel Field Veterinary Services has 6 district veterinary services, which are responsible for control and eradication. In Israel we vaccinate routinely and also when there is an outbreak. The vaccination is done only by government employees. When someone reports about suspicious clinical signs, a VNT compares to previous field strains is done by the FMD lab. Comparing to vaccine strains from commercial companies is done in Pirbright lab. We have successes in vaccination routine because there only few and small outbreaks. The uses of DIVA vaccine are: checking farms around the focus of the outbreak; checking farms of Israel's borders; checking farms according history of outbreaks. DIVA limitations of current vaccines: No NSP in the vaccines; Veterinary biologics are regulated by the Veterinary Services; No experience with genetically engineered vaccines. Israel has not been involved previously in U.S. government associated animal vaccination programs. Israel has the capability to participate in experimental field trail with a new second generation vaccine.

CVO Roundtable: Challenges and Opportunities when performing FMD Vaccine Field Trials (Libya)

Dr. Ibrahim Eldaghayes, National Center of Animal Health, Ministry of Agriculture, Animal and Marine Wealth, Libya

Introduction:

FMD is still a leading cause of loss of livestock economy in Libya. Outbreaks are still being reported from time to time around the year. Although, the disease has been controlled successfully in many parts of the world by regular vaccination of susceptible animals and slaughtering of infected animals, however, because of the highly contagious nature and rapid spread of the infection, no country has been considered safe, and the good example of that is the new outbreak of FMD in Tunisia; the country claimed to be free by vaccination since 1999!

Materials and Methods:

A monitoring system for FMD in Libya has been implemented and classified into three components:

- Component 1: investigations in FMD suspected outbreaks (active surveillance).
- Component 2: immune response of vaccinated animals.
- Component 3: serology (passive surveillance) to investigate the level of FMDV circulation (anti-NSP antibodies) and the serotypes presents (anti-SP serotype-specific antibodies).

Most of the lab work for the 3 components was carried out in IZSLER lab, Brescia, Italy. More recently the Veterinary Rapid Response Teams (RST) in Libya are using the Pen-Side tests for FMD diagnosis in the field. Pen-side tests were provided by EuFMD as part of the Component 2.3 of EuFMD workplan (REMESA).

Results:

For component 1: Of the three serotypes "O, A and SAT2" reported in Libya, type “O” has been found to be predominant over other types, with the detection of the new Indian strain O/ME-SA/Ind-2001 for the first time in Africa in September 2013. Initial diagnosis was carried out in Tripoli lab using IZSLER ELISA kits, confirmation and virus isolation was carried out in IZSLER lab in Brescia and further viral characterization and vaccine matching were carried out in Pirbright lab. For component 2 (2013): All small ruminants (Sheep and Goats) and Large ruminant (Cattle) used were immunized with high titer of antibodies against FMD after 30 days post vaccination.

For component 3 (2013): The prevalence of NSP antibodies in small ruminants and large ruminants was 15% and 18% respectively.

Discussion:

All 3 components are undergoing for this year 2014. However, for component 2, the immune response in vaccinated animals will be measured at 0, 2, 4 and 6 months after first vaccination, and some of the same animals will be revaccinated at 6 months post previous vaccination and the immune response will be measured at 2, 4 and 6 months after poster vaccination. Libya still in stage 1 according to the Progressive Control Pathway for FMD control (PCP-FMD). Lots of work and activities have been carried out in Libya in the last two years in order to control the disease; for example: support from Italy and an agreement was signed between Libya (Ministry of Agriculture) and Italy (IZSLER, Brescia), mass vaccination using killed purified Merial vaccine (once per year, and starting form this year 2014 cattle will be vaccinated twice per year), EuFMD workshops (to complete the Risk-Based Strategic Plan) and training of the Libyan vets in: Libya, Kenya (FMD Real-time training course supported by EuFMD) and Italy (4 vets trained in IZSLER lab in Brescia).

Considerations/Requirements for performing diagnostic assay validation in an international setting: Perspective of the NAHLN MTWG

Dr. Bruce Akey, Texas A&M Veterinary Medical Diagnostic Laboratory and Dr. Sarah Tomlinson, USDA - Animal and Plant Health Inspection Service - Veterinary Services

The National Animal Health Laboratory Network (NAHLN) is a partnership between Federal, State and university laboratories configured in a tiered structure based on level of capabilities and responsibilities. The Federal laboratories (USDA National Veterinary Services Laboratory and the Foreign Animal Disease Diagnostic Laboratory) are the national reference laboratories for the NAHLN. Various NAHLN labs are trained and approved to perform testing for one or more high consequence diseases including avian/swine influenza, Newcastle disease, foot and mouth disease, classical swine fever, African swine fever, bovine spongiform encephalopathy, scrapie, chronic wasting disease, vesicular stomatitis and pseudorabies. The NAHLN provides flexible capacity for laboratory support of routine and emergency animal disease diagnosis; use of standardized, rapid diagnostic techniques used at state, regional, and national levels; secure communication, alert, and reporting systems; modern equipment and experienced, certified personnel; national training, proficiency testing, and quality assurance (AAVLD, OIE, ISO, NAHLN); facilities that meet bio-containment and physical security requirements; regional and national animal health emergency training exercises. The NAHLN Coordinating Council, comprised of federal and state regulatory and laboratory representatives, provides a forum for discussion and input and makes recommendations to USDA APHIS and NIFA. The Council is supported by several working groups including a Methods Technical Working Group as well as information technology, and an exercises and drills group.

The NAHLN Methods Technical Working Group (MTWG) reviews method development and comparison proposals, assay dossiers and also provides scientific and practical implementation recommendations to USDA on the adoption and deployment of test methods in the NAHLN. This work is based on several key principles-- assays must be standardized and practical to deploy; determination of fitness for purpose and use; existing federal policies; and logistics of deployment. No modification of NAHLN protocols or reagents is permitted without review by the MTWG and agreement by the USDA.

All new methods proposed for deployment by the NAHLN are processed as follows:

- Initial Proposal to the MTWG – includes both general information (methodology type, purpose, development or comparison to existing protocol, commercial availability and challenges anticipated) and technical information (specific protocol, assay target, species and specimen validity, technology platform, controls).

- For a methods comparison effort, specific performance parameters must be measured including analytical sensitivity, repeatability, cross contamination, operator variability, diagnostic sample comparison
- New method validation follows guidelines compatible with those of the AAVLD and OIE and includes determination of analytical sensitivity and specificity, diagnostic sensitivity and specificity, repeatability, potential for cross contamination and both intra- and inter-laboratory reproducibility. This effort frequently includes a negative cohort study as well.
- Technical review by the MTWG of the validation or comparison data dossier.
- Recommendation from the MTWG to USDA on the advisability of adoption of the assay.
- Final determination by USDA as to the implementation of the assay in the NAHLN. This determination includes need for the assay, policy impacts, consequences of deployment, response to and reporting of assay results, and the logistics of deployment of the assay.

Performing Diagnostic Assay Field Trials in an International Setting

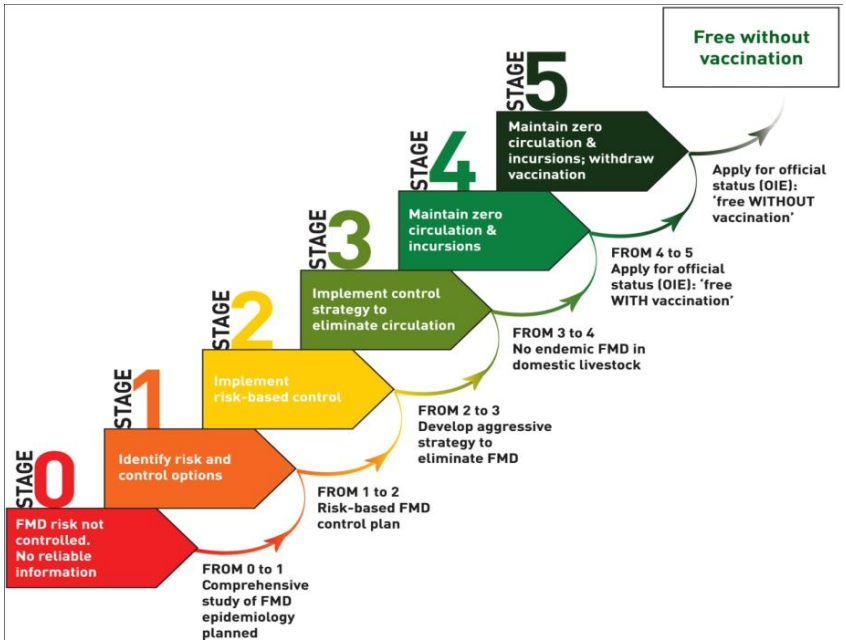
Dr. Donald P. King, The Pirbright Institute, United Kingdom

The Pirbright Institute hosts a number of reference laboratories for important livestock diseases such as foot-and-mouth disease (FMD). In order to monitor the changing global epidemiological patterns and risks, these laboratories undertake routine testing of field samples collected from countries where these diseases are endemic. This work is supported by applied and basic research projects, including on-going work to develop and evaluate new tools that might be used to provide rapid diagnostic results in the field. In this context, this presentation provided an overview of current field projects in Tanzania that are funded by BBSRC/DFID/Scottish Government, Wellcome Trust (SACIDS: http://www.sacids.org), IIAD/FAZD and the European Union (Rapidia-Field: http://rapidia.eu/). These projects are providing a challenging field environment to evaluate the robustness of new diagnostic tests for FMD such as PCR-based and novel isothermal molecular assays, as well as antigen-lateral flow devices. A number of key enabling activities have been important to ensure the success of these projects. These include: liaison with national authorities prior to recruitment of study sites, training of field personnel to collect, process and store samples, and parallel capacity building in the National reference Laboratory and a national veterinary university (Sokoine University of Agriculture, Morogoro).

Appendix D: Proposed Criteria for Country Participation

Criteria	Description	MUST HAVE	NICE-TO-HAVE
FAO recognition that country or targeted geographic location is currently at a specific PCP stage	Classifies country progress in FMD risk management (3 main components: – FMD control – Strengthening Veterinary Services – Prevention and control of other major diseases of livestock)	FAO recognition that country is currently in Stage 2	Currently in Stage 3 or expected to enter Stage 3
FMDV known to be circulating in cattle	WRLFMD or Regional FMD Lab confirmation of FMDV circulation	FMDV known to be present in past 6 months	FMDV known to be present in past 6 months
Finite number of known circulating FMDV strains in domestic livestock	Each strain classified by serotype, topotype and preferably lineage	At least one and no more than 4 FMDV strains	At least one and no more than 3 FMDV strains
Alignment to available AdFMD experimental vaccines	Degree to which circulating FMDV strains match vaccine strain (serotype, topotype, and lineage)	Matches at least two of the following WRLFMD vaccine targets: • O Manisa (O/ME-SEA) • O BFS • O/SEA • Asia 1 Shamir • A Iran 05 • A22 Iraq • SAT2 Saudi Arabia • A Eritrea • A Malaysia 97 • SAT2/Egypt/10 • SAT2/Saudi Arabia or Eritrea • SAT3 Zimbabwe	Matches three of the following WRLFMD vaccine targets: • O Manisa (O/ME-SEA) • O BFS • O/SEA • Asia 1 Shamir • A Iran 05 • A22 Iraq • SAT2 Saudi Arabia • A Eritrea • A Malaysia 97 • SAT2/Egypt/10 • SAT2/Saudi Arabia or Eritrea • SAT3 Zimbabwe
Relationship to FMD diagnostic reference lab	Ability to provide diagnostic samples for serotyping and genotyping	At least 1 year of continuous relationship with regional, national or WRLFMD reference lab	At least 3 years of continuous relationship with regional, national, or WRLFMD reference lab
Veterinary services infrastructure	Strength and sustainability	Per PCP Stage 2 definition	Per PCP Stage 3 definition
Good clinical	Ability to adhere to	Documentation that	Prior experience in

Criteria	Description	MUST HAVE	NICE-TO-HAVE
practices	standard GCP practices used of veterinary field trials	supports GCP practices	facilitating veterinary vaccine field trials using GCP
Country regulatory body	Established processes for approving field studies	Ability to provide in-country regulatory approval	Experience in approval of veterinary vaccine or diagnostic field studies
Stability	Ability to carry out the field study in an environment that is safe for human and animal participants	No active internal conflicts involving widespread human or animal violence for the past 2 years	No active internal conflicts involving widespread human or animal violence for the past 5 years
Relationship with FAO	Quality of interaction	Good and established for at least 1 year	Very Good and established for at least 3 years
Relationship with U.S. (*e.g.*, USDA FAS or other Ag related entity	Quality of interaction	Good and established for at least 1 year	Very Good and established for at least 3 years
Testing facilities for FMDV challenge	Ability to carry out controlled animal trials in an experimental setting	BSL-1 facility that can hold up to 15 cattle for up to 60 days with low probability of unintentional exposure to livestock pathogens	BSL-2 facilities that can hold up to 15 cattle for up to 60 days and track history in conducting BSL-2 studies
GMO	Tolerance to GMO field studies	Willingness to use a GMO vaccine	Prior experience in using GMO vaccines in the field



Stages of the FMD-PCP